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DRUGS, MEDICATIONS AND PERFORMANCE ALTERING SUBSTANCES: THEIR PERFORMANCE EFFECTS, DETECTION AND REGULATION: FROM 1800 TO KEENELAND, OCTOBER 12TH, 2005

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1/ Summary:

Racing has been testing for drugs and medications since about 1903: Racehorse testing is thus by far the longest established, broadest in scope and most sensitive drug testing performed on earth. Racehorse testing is also performed within an extremely stringent regulatory context, and my understanding is that many of our constitutional protections as US citizens are inoperative in the racing environment. Racehorse testing is also remarkable "clean" and the incidence of deliberate use of performance affecting substances seems to be very small indeed.

There are good reasons for all of the above: it is empirically clear that medication is highly likely to influence the performance of racing horses, although the scientific evidence for this is less than overwhelming.

In the mid nineteen eighties, the use of high potency drugs was not particularly well controlled. Following a directive from the Kentucky State Racing Commission, an interdisciplinary team at UK adapted ELISA testing to racing chemistry; this proprietary technology basically solved the problem of the abuse of high potency drugs in racing horses and these tests are now marketed worldwide out of Lexington.

ELISA testing also allows highly sensitive detection of trace amounts ("tail ends") of therapeutic medications, environmental and dietary substances. In the nineties, following another Racing Commission directive, the Gluck program pioneered the basic research that underpins the evolving and increasing use of "thresholds" in racing regulation.

Advances in testing are research driven. Once a medication is called, its use drops dramatically, to close to zero, but not quite zero; it appears that there are always people ready to try a medication that worked for them or for a colleague or rival in the past.

Overall, the rate at which medication violations are reported in racing is extremely small. For example, from 1995 – 1999 there were about 3 /100,000 samples for ARCI Class 1 violations when trace identifications of dietary and environmental substance are taken out of the mix. By Far the most commonly identifications reported are "traces" of therapeutic medications, dietary and environmental substances.

The ease with which "traces" of therapeutic medications, dietary and environmental substances can be detected using current testing technology is leading scientists and regulators away from the old "Zero Tolerance" approach, which many authorities now see as outdated, to regulatory limits or thresholds.

Future challenges include developing effective regulatory methods for the newer high technology products such as erythropoietin, and regulation of the classic low-tech approach of "milk-shaking".

2/ Background and Definitions:

There are at least 10 million known chemical substances and 4,000 or more prescription medications. Regulators in the United States, therefore, divide drugs and medications into two major groups:

The largest group comprises **"Performance-enhancing substances"**, whose presence in a horse is viewed with great regulatory concern. Testing for these substances usually proceeds at the highest level of sensitivity possible; so-called **"zero-tolerance"** testing. About 850 or so substances are classified by the Association of Racing Commissioners International (ARCI) Uniform Classification System for Foreign Substances as potentially performance enhancing in a five class system, the most complete listing of such substances available (http://www.arci.com/druglisting.pdf).

The second smaller group comprises the **"therapeutic medications"**. There are approximately 50 plus of these used therapeutically in horses in training (Table 1). Since about 2000, it has come to be accepted that we must set "limitations" on testing for therapeutic medications. These limitations are variously called **thresholds** or **reporting levels**, or **decision levels** (California) depending on the semantic preference of the jurisdiction.

Table 1: Therapeutic Medications Routinely Used and Identified as Necessary by the Veterinary Advisory Committee: (Racing Medication and Testing Consortium (RMTC) draft list of therapeutic medications, 2005)

1. Acepromazine
2. Albuterol
3. Aminocaproic Acid
4. Atropine
5. Beclomethasone
6. Betamethasone
7. Boldenone
8. Butorphanol
9. Cimetidine
10. Clenbuterol
11. Cromolyn
12. Dantrolene
13. Detomidine
14. Dexamethasone
15. Diazepam
16. DMSO
17. Dipyrone
18. Flunixin
19. Fluprednisolone
20. Fluphenazine
21. Furosemide
22. Glycopyrrolate
23. Guaifenesin
24. Hydroxyzine
25. Isoflupredone
26. Isoxsuprine
27. Ketoprofen
28. Lidocaine
29. Mepivacaine
30. Methocarbamol
31. Methylprednisolone
32. Nandrolone
33. Omeprazole
35. Phenylbutazone
36. Phenytoin
37. Prednisolone
38. Prednisone
39. Procaine Penicillin
40. Pyrilamine
41. Ranitidine
42. Reserpine
43. Stanozolol
44. Testosterone
45. Triamcinolone
46. Trichlomethiazide

3/ History:

Up to 100 years ago there was little concern about the use of medication in racing horses, particularly in North America. The 1800s had seen the purification of cocaine and morphine and availability of these substances made the acute stimulant medication of racing horses a reality. Around the turn-of-the-century (1890-1910), a number of American trainers went to Europe, taking with them the new American medications. As a group, they were so successful that they became known as the "Yankee Alchemists".

Fig. 1. Carl Vernet, France, early 1800's.

In the early 1900s the Honorable Mr. George Lambton, the leading English trainer of his time, grew tired of losing to the "Yankee Alchemists", as he soon grew tired of politely requesting the English Jockey Club to do "something" about the problem. He therefore purchased some American "dopes", and publicly announced that certain horses in certain races were going to be, well, shall we say, "medicated". These activities soon got the Jockey Club's attention, and in 1903 the medication of a racing horse was made an offense against the rules of racing in England. The record is silent as to how these rules were to be enforced, but the prescribed punishment was to be *"ruled off the turf"*, a punishment still in place in parts of the English speaking world.

Fig. 2

A trainer called Wishard, financed by "Betcha-A Million" Gates, reportedly brought stimulant medication to England.

Somewhat farther from home, a trainer by the name of James Keene was also having a very good run in Russia. This came to an abrupt halt one day, when Mr. Keene was met in the paddock by a Russian racing official, followed by Russian chemist, complete with a basket of frogs. Some saliva was taken from Mr. Keene's horse, and presumably force-fed to the frog, which then behaved in a most un-frog-like way. Mr. Keene's horse was duly declared "positive"; shortly thereafter, Mr. Keene left Russia and returned to Kentucky, where he founded a farm called Keeneland.

Classic analytical race testing as we know it started in France in the early 1900s; in 1935, Mr. William Woodward sent Dr. Catlett, a veterinarian, and Dr. Morgan, a chemist, from Florida to France to learn the French drug testing techniques. They returned to Florida and set up the first US drug testing lab; later the New York Racing Commission opened a racing chemistry laboratory on the10th floor of a building on Chambers Street in Manhattan. Mr. Robert Vessiney, likely working today as we speak at Truesdail Labs in Tustin, California, started there in 1941 under Dr. Charles Morgan; in 1947 the Association of Official Racing Chemists was formed.

Fig. 5

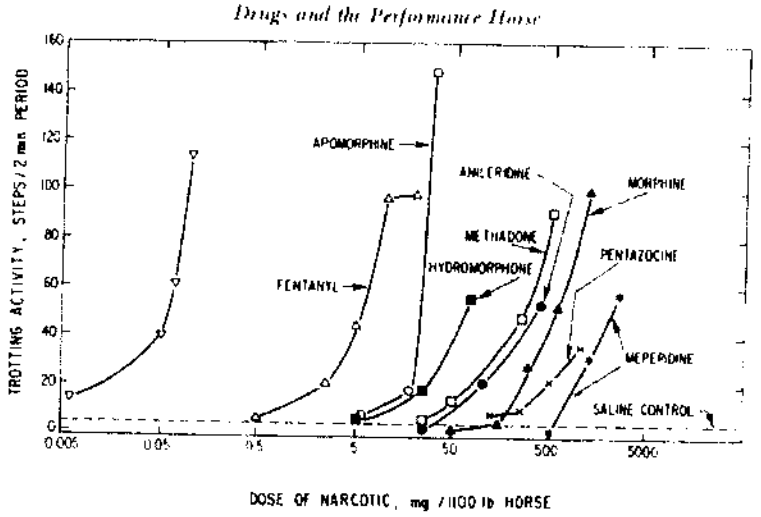


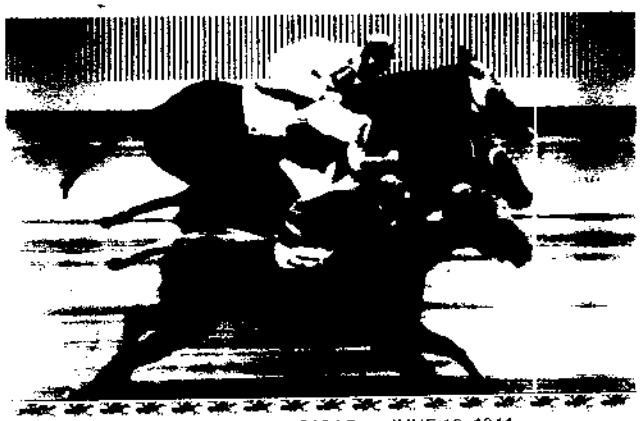
Figure 12-7 Trotting responses in horses after different narcotic drugs. Horses were injected IV with the indicated number of milligrams of each drug and the trotting response counted. The peak trotting response to each drug was then plotted against dose. An unnamed experimental drug was the most potent drug tested, being about 100 times more potent than fentanyl. Fentanyl was the next most potent drug, as 8 mg was enough to produce a peak response. After fentanyl, the next most potent drug was apomorphine, followed by hydromorphone, methadone, anileridine, morphine, pentazocine, and meperidine. This is the same order as their potency as narcotic analgesics in man. Note in particular the poor response to pentazocine (Lasix®) in this test.

As testing improved those individuals seeking an "opiate edge" began to use the more potent and thus more difficult to detect opiates. The unnamed but highly potent opiate at the far left of the above family of dose response curves is etorphine, or "elephant juice". Etorphine is one of the most potent opiates known and at the time that this figure was published in "Drugs and the Performance Horse", there was no test available that could detect it. This figure also shows, for one family of substances, the 10,000 fold range in dose/potency from the least potent opiate tested on the right, meperidine, at about a one gram/horse doses, to the highly potent etorphine on the far left, with 50 micrograms (50 millionths of a gram) producing an equivalent pharmacological effect to a gram or more of meperidine. And, of course, etorphine was, in round figures, about 10,000 time more difficult to detect that the old standbys of morphine and heroin one of whose slang names is/was "horse". This great increase in the potency of medications being used in horses set the stage for the development of ELISA Testing, as we will discuss.

Horses can also be medicated to win by relaxing them, and allowing the horse to run its best possible race. The widely used tranquilizer acepromazine, and any number of related or equivalent agents, have been used in this way.

Improving a horses "wind" by opening its airways through the use of bronchodilators may also improve the performance of horse, and especially one that is sub-clinically broncho-constricted. In this regard, at one time the best selling ELISA test was a particularly good bronchodilator test.

Fig. 6



THE CARTER HANDICAP — JUNE 10, 1944
AQUEDUCT RACETRACK — TRIPLE DEAD HEAT

Fig. 7

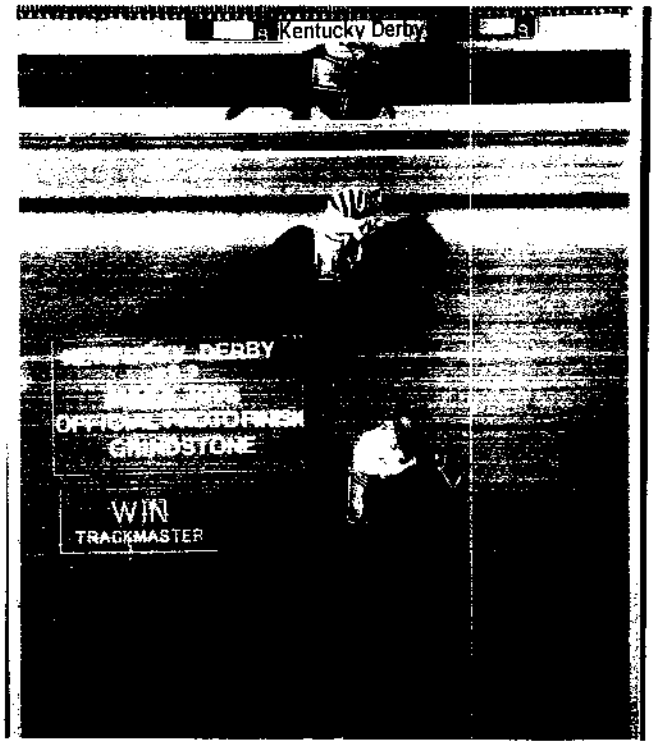


Fig. 3. *Mr. Robert Vessiney, Truesdail Labs, Tustin, CA, circa 2000. His working career, including this week, began in 1941 at the NY Commission Laboratory on Chamber St, Manhattan, and spans virtually the entire history of US racehorse testing, which started about 1935 in Florida and New York under Dr. Charles Morgan.*

4/ Can Drugs/Medications Influence the Outcome of a Race?

Drugs and medications can be used to influence the outcome of races in a number of ways. Acute stimulant medication is the administration of a stimulant substance to a horse shortly before post. Among the especially useful agents in this area are the opiates, which have long been used in racing horses, and also the amphetamine like stimulants, especially methylphenidate (Ritalin). All of these have been widely used, the opiates likely for hundreds of years, and presumably particularly so when testing for these agents was not available.

Fig. 4

Locomotor Responses to Fentanyl

Locomotor stimulation post-fentanyl administration

Dose and time response curves from 2 to 18 mg/horse

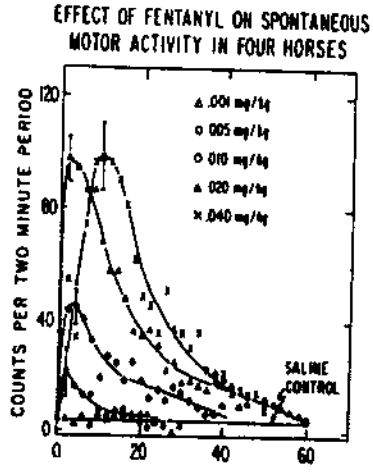
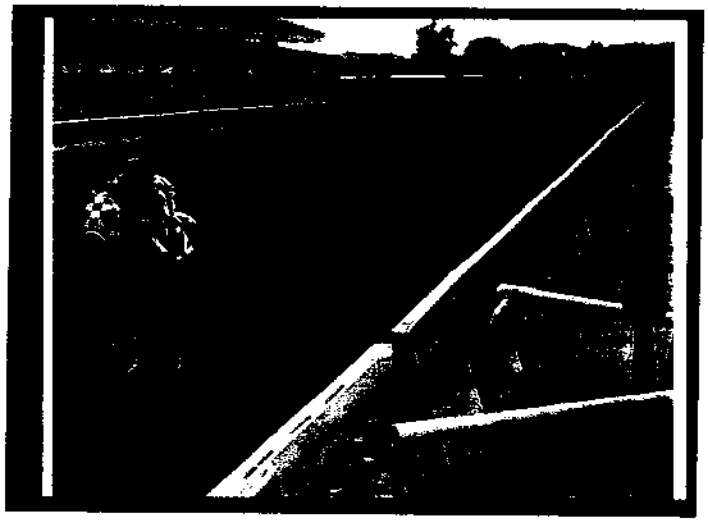


Fig. 8



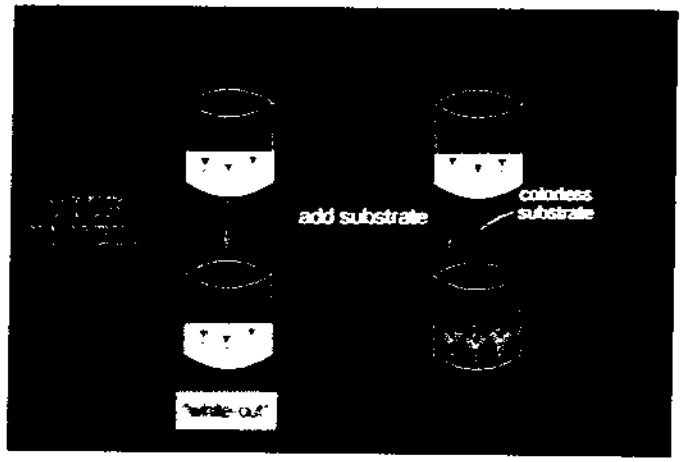
The difficulty with trying to scientifically demonstrate performance effects of drugs in small numbers of horses is that the drug needs to produce a positive performance effect of about the same magnitude as Secretariat's win at Belmont to meet the lowest level of statistical significance acceptable in science. This is a considerable experimental challenge; another way of looking at this is that successful horse trainers make far more subtle and discriminating judgments than most scientists, of which I think there is no doubt whatsoever.

Veterinarians certify horses as being sound in "wind and limb"; obviously medications that can affect these parameters have the potential to affect the both the presentation of a horse and also, presumably, the results of the ultimate performance analysis, the outcome of a race. By the mid-nineteen eighties the use of highly potent drugs and medications such as fentanyl (Sublimase) and etorphine had created a considerable problem for race testing.

5/ 1988: The Introduction of ELISA tTesting:

In the mid-1980s, race testing was for all practical purposes dependent on a primary screening technique called Thin Layer Chromatographic (TLC) screening. This technology was not particularly sensitive, and in the mid-1980s some horsemen were reportedly using high potency narcotics, stimulants, bronchodilators and tranquilizers with impunity. In 1985 we were requested (directed?) by the then Kentucky State Racing Commission to "fix this problem". The solution that we came up with, ELISA testing for high potency drugs and medications, is in place around the world today, and is evidenced here in Lexington by a thriving concern, Neogen Corp, on Nandino Boulevard, employing 100 people bringing in about US $30 million a year into Lexington (not all through ELISA tests/ www.neogen.com/forensicordering.htm).

Fig. 9



ELISA stands for **E**nyme **L**inked **I**mmuno**S**orbent **A**ssay, ELISA

Simply put, an ELISA test is a variant on the home pregnancy test technology. It requires a drop of urine; it can be performed relatively rapidly, it is/can be highly sensitive and can be read by eye. When ELISA testing was first introduced, the problem was to keep the technology from "putting down" too many trainers, especially in those jurisdictions that had frozen "back samples". Let me simply say that this was a trying time for me professionally, but matters eventually settled down and, as I indicated, ELISA testing is the backbone of drug screening worldwide today.

Fig. 10

This is a 96 well ELISA plate in which the full blue color has been developed. The clear wells on the left hand side are the positive controls containing calibration standards. All of the other wells represent ELISA "negative" urine samples. A track ELISA positive would show up as a clear well in the middle of the blue samples, a so called "whiteout", or an ELISA "positive".

An ELISA test will usually detect about 5ng/ml (or 5 parts per billion) of drug or drug

metabolite in the sample. Some tests are 10 fold more sensitive, detecting down to the high parts per trillion. To put these figures in perspective, one part per billion is one second in your life if you are 32 years old.

To put the matter of testing sensitivity in regulatory perspective, a sure prescription for regulatory friction/problems is a therapeutic medication (or a dietary or environmental substance) given at higher doses to horses, excreted efficiently in urine, and being tested for by an analyst with a highly sensitive ELISA test with no thresholds/decision levels in place.

Finally, we must always remember that an ELISA test simply binds to and "sees" one side/surface of the medication molecule. Therefore, while an ELISA "negative" is almost certainly a true negative, an ELISA test will interact with many substances other than the drug in question; As such, the rule with an ELISA "positive" is that it can always be, by definition, a "false positive". Which is, of course why, chemists perform Mass Spectral confirmations.

6/ Mass Spectral Confirmation:

While ELISA screening/testing is fast and highly sensitive, it is, as set forth above, far from specific. The second and absolutely critical stage step in the testing process is Mass Spectral confirmation. In this step, the molecule is isolated and its precise mass measured, and the molecule is also broken into a series of fragments. Both the mass and relative proportions of these fragments (the fragmentation pattern) are specific for the given drug, and are then matched with known controls/standards and run through the system. A full scan mass spectrum, with appropriate matching controls, is the gold standard in drug testing, and is considered definitive evidence for the presence of the substance in the sample in question. Independent replication of the primary findings in the "split" or 'referee" analysis usually neutralizes any challenges in the area of chemistry.

Fig. 11

Fig. 12

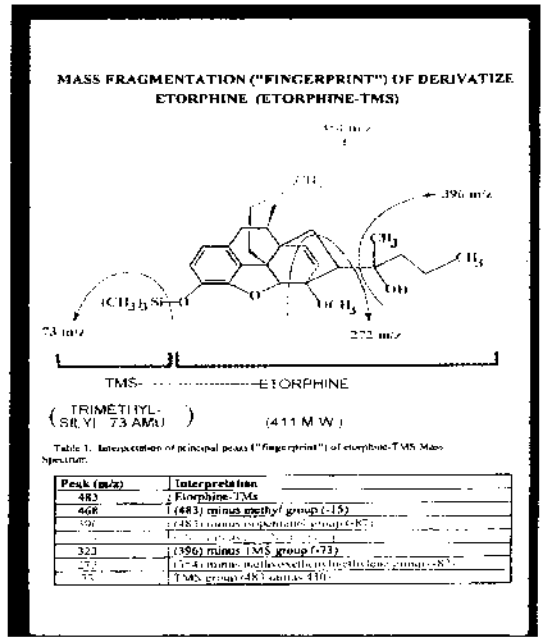
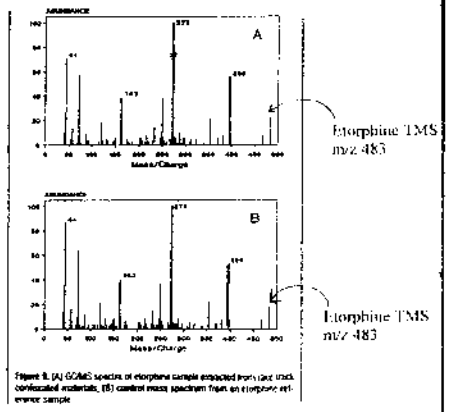


Fig. 13

MASS SPECTRAL CONFIRMATION OF ETORPHINE "ELEPHANT JUICE" IN A RACETRACK SAMPLE



Comparison of Mass Spectra of a post-race etorphine and an authentic standard. The lower figure shows the mass spectrum of an authentic etorphine laboratory standard. Note the molecular ion at mass 483, the base peak at mass 272 and the various other ions of the standard or control spectrum. Note the very close correspondence of the standard or control mass spectrum with the mass spectrum of the material recovered from the post-race sample, indicating that the material recovered is indistinguishable from authentic derivatized etorphorphine.

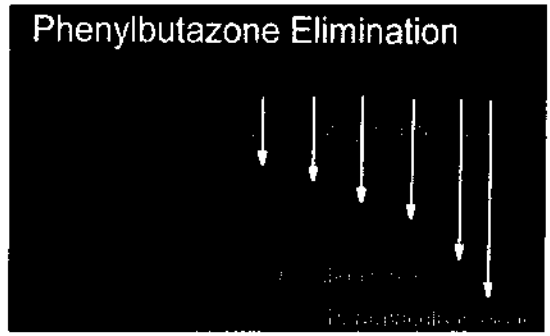
7/ "Zero Tolerance" Testing

"Zero Tolerance" testing is not testing down to "Zero" molecules, which no chemist can accomplish, but rather testing to the Limit of Detection (LOD) of the best available technology. While this may be an entirely appropriate approach for performance altering substances which have no place in racing, it is absolutely not considered appropriate for therapeutic medications. Therapeutic medications are substances used to maintain the health and welfare of horses, and to arbitrarily change the sensitivity of testing for these agents depending on either the whim of the chemist or the just now, today, availability of an improved technology is entirely inappropriate, as we will see from review of the basic mathematics of medication dosing and elimination.

8/ Medication Dosing and Elimination:

When you administer a dose of phenylbutazone to a horse, you administer more phenylbutazone molecules than there are stars in the known universe, that is about 6 followed by 21 zeros molecules. This is a very large number of molecules indeed.

Fig. 14



The horse will eliminate the bulk of this dose of phenylbutazone quite rapidly. If phenylbutazone in the horse has a 7.22 hour half-life, 50% of the drug will be eliminated by 7.22 hours post dosing, 75% by 14.5 hours post dosing, 87.5 by about 22 hours post dosing, and exactly 90% by 24 hours post- dosing. At the end of day 1, when 90% of the drug is eliminated, the pharmacology of the drug is gone, but you still have 6 followed by 20 zeros worth of phenylbutazone molecules in the body. Every day another 90% of the drug in the body will be eliminated, and other zero drops off, but if the chemist really wants to look, he or she can well find traces of the medication or its metabolites for 14 days post administration, a time post-administration detection that even the most conservative chemists and regulators generally do not wish to pursue an identification. However, the question now arises of when, precisely, should the chemist stop pursuing these traces?

9/ Thresholds, Including "No Effect Thresholds" (NETs):

The answer to this question is simple; the chemist should stop pursuing these traces precisely when he/she is told to stop. It is, however, slightly more complicated to determine the exact point at which the chemist should be told to cease and desist.

We approached this question experimentally in the Gluck Equine Research Center during the second half of the nineties. Simply put, we administered decreasing doses of local anesthetics to horses until we saw no local anesthetic effect, which gave us the No Effect Dose. Then we measured the concentrations of the drug, actually its metabolites, in the urine, and the concentrations we came up with are obviously not associated with any pharmacological effect. These concentrations then become "No Effect Thresholds" in urine for the specific therapeutic medication, and the chemist is advised not to test below these concentrations, plus an added safety factor.

Fig. 15

Fig. 16

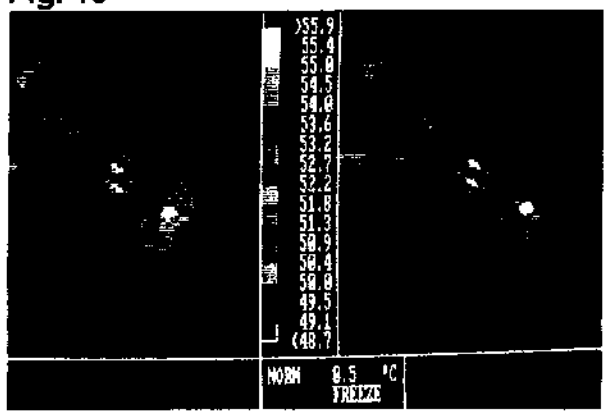
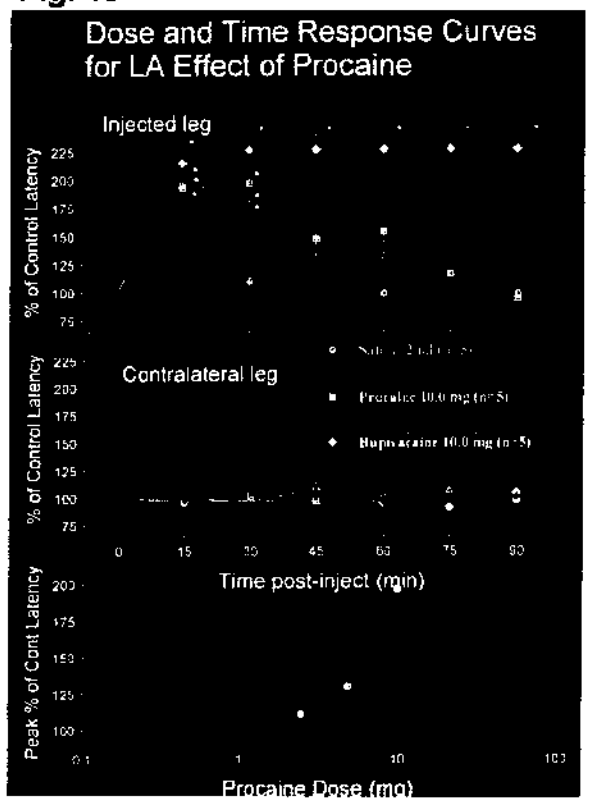


Fig. 18



We presented this scenario hypothetically in 94/95, and then started the actual research. We were immediately very vigorously attacked from conservative quarters, anonymously and libelously. In 96 one of these libelous letters surfaced signed by Mrs. Donna Ewing of the Illinois Hooved Animal Humane Society. The University shall we say, "encouraged" me to sue, which I did, and Mr. Davis was my attorney. While the suit was eventually dropped, it had the desired effect of silencing the complaining parties, who have not been heard from since. More to the point, we completed the research and published it in the refereed scientific literature. By the year 2000, the intellectual concept and more importantly, the actual word "thresholds" became more or less "safe" for an exceptionally courageous racing administrator to allow past his (or her) lips.

The concept approach of "Zero Tolerance" was to some extent officially voted out of favor and "off the regulatory island" in the opening paper of the International Conference of Racing Analysts and Veterinarians (ICRAV) 2000 at Cambridge, England. In this paper Professor Robert L. Smith addressed the concept of "Zero Tolerance", which he considered a *"fading illusion"*, and reviewed the events *"which are increasingly undermining the suitability of this approach"* In his words: *"The zero tolerance approach" ---- "is in essence an illusion in which the magician is the racing chemist".* and he continued that *"The Zero Tolerance approach is both philosophically and pragmatically unsound" --- "The goal for the future integrity of racing is to develop REPORTING LEVELS for therapeutic substances based upon rigorous analysis of their pharmacological and pharmacokinetic properties and using an appropriate model".*

10/ "Withdrawal Time Guidelines":

Let us now move from the entirely theoretical and illusionary concept of "Zero Tolerance" to practical horsemen's concerns. A "threshold" or a "reporting level" is a concentration value (say, for example 10 parts per billion in urine) that has, in the larger scheme of things, little actual reality for horsemen, since a horseman cannot see 10 parts per billion of anything in horse urine. What the horseman needs are clear transparent "withdrawal time guidelines": i.e., guidelines as to when he should stop administering the medication prior to post so that the blood or urine "reading" comes in below the threshold, whatever the particular threshold may be.

This question may actually be considerably more difficult to answer than the threshold determination. The only way to answer this question is again by actual experimental determination, followed by field application. The medication product in question must be specified, and the formulation, dose, route, and duration of administration specified. The medication must be administered to a significant number, hopefully at least 20-50, of Thoroughbred horses in training, and the blood or urinary concentrations of the parent medication or its principle urinary metabolite followed over time. The laboratory performing analyses should be appropriately (American Association of Laboratory Accreditation, A2LA) accredited, and have in place a validated quantitative method for the threshold substance (http://hbpa.org/resources/MedicationPolicy.pdf).

The data obtained must then be analyzed statistically, and hopefully fitted to a

recognizable mathematical distribution. One can then use this distribution to tell horsemen that if they administer the drug following X stipulation doses/days, and stop administration at Y hours prior to post, there will be a Z probability of exceeding the regulatory threshold. One of the things that everybody must understand is that if you administer a medication at any time close to post, there is always a finite mathematical probability of exceeding the threshold; all anybody can do is estimate as accurately as possible the probability of an overage, and make sure that the risk is a risk that the horseman can live with.

This finite probability of a therapeutic medication overage is most likely the reason that regulatory authorities are often reluctant to be associated with "withdrawal time" guidelines. While a 1/1000 risk of a "positive" may be an entirely acceptable risk for an individual horseman in a small number of horses, if the authority approves a given "withdrawal time" it assumes responsibility for all 10-20,000 or more samples tested in the jurisdiction, which increases the probability of problems 10-20,000 fold, or more if the authority tests more than 20,000 samples.

At the personal level, in the current state of play it is extremely difficult to give useful "withdrawal time information" advice. The number of factors which affect the withdrawal time is very large indeed, and in the absence of a defined threshold ("Zero Tolerance" testing) it can be little more that a guessing game. Whenever I get a "withdrawal time" estimate request, I try to make the uncertainties clear, and I always end with the statement that ***"there are no guarantees in life, and that most certainly includes withdrawal time estimates".***

The various factors that can affect "withdrawal times" are set forth in some detail in the National Horsemen's Benevolent and Protective Association, Inc. Proposed National Policy on Drug Testing and Therapeutic Medication. *J Eq Vet Sci* **23**(1): 4-5, 18-40, 2003. (http://hbpa.org/resources/MedicationPolicy.pdf)

11/ The Kentucky Medication Rule:

Thirty plus years ago, when the long-standing Kentucky medication rule was formulated, (even before I came to town), there were no thresholds or regulatory limits anywhere; indeed there were very few, if any, quantitative methods. Under these circumstances the Kentucky rule was clear, simple, effective and highly practical. You could not run on stimulants, depressants, local anesthetics, tranquilizers or narcotic analgesics, the classic performance altering substances. However, the use of substances that were perceived as therapeutic was permitted, with the goal of protecting the health and welfare of the horse. This Kentucky rule well fitted the regulatory technology then available, and indeed is, I understand, very close to the rule currently obtaining in human athletics. The fundamental rule has been in place for at least 30 years and, to the best of my knowledge, has served the horses and horsemen of Kentucky well.

12/ The Proposed Racing Medication Testing Consortium (RMTC) Rule:

It is a little curious that the proposed National RMTC rule has first chosen to take aim at various jurisdictions relatively liberal use of Non-Steroidal Anti-Inflammatories, (NSAIs), phenylbutazone and flunixin, on race day rules. In the first place, we should all clearly understand that phenylbutazone and flunixin are basically nothing, more or less, than horse aspirin. Aspirin is very rapidly eliminated by the horse, and is therefore not a particularly useful medication in horses. Phenylbutazone and flunixin are full brothers to aspirin, and produce more or less the same effect. In particular, in our hands phenylbutazone did not affect a horse's pain threshold, entirely consistent with our understanding of this medication and our every day experience with aspirin.

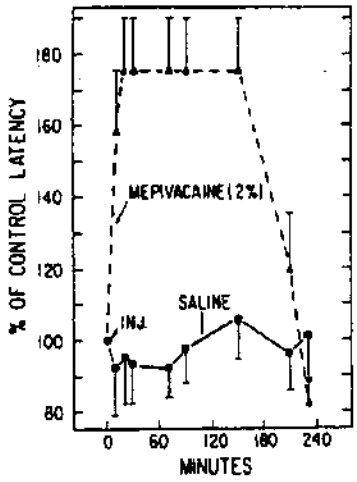


Fig. 2. Nerve blocking action of mepivacaine (2%) on hoof withdrawal reflex latency. Values are the mean post-treatment latencies (±S.E.M.) expressed as a percent of the mean pre-treatment (control) latency. Pre-saline latency = 8.4±0.6 s. Pre-mepivacaine latency = 6.3±0.4 s. n = 6.

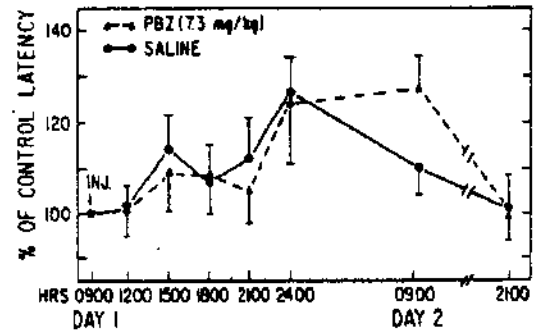


Fig. 4. Absence of analgesic effect of i.v. phenylbutazone (PBZ on hoof withdrawal reflex latency. Values represent mean post-treatment latency (±S.E.M.) expressed as a percent of the mean pre-treatment (control) latency. Pre-saline latency = 6.4 ±0.6 s. Pre-PBZ latency = 7.1±0.5 s. n = 8.

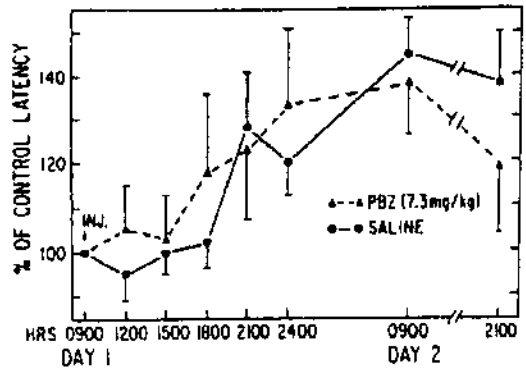


Fig. 3. Absence of analgesic effect of i.v. phenylbutazone (PBZ) on skin twitch reflex latency. Values represent mean post-treatment latency (±S.E.M.) expressed as a percent of the mean pre-treatment (control) latency. Pre-saline latency = 6.4±0.6 s. Pre-PBZ latency = 7.6±1.0 s. n = 8.

The above figures show the lack of effect of phenylbutazone on normal pain perception in the analgesia model that we used to characterize the local anesthetic or "pain-killing" effects of the local anesthetics such as mepivacaine included in this experimental sequence as a positive control.

The proposed RMTC rule adopts the mid-eighties phenylbutazone threshold of 5ug/ml in blood and adds two extra thresholds, one for flunixin of 20ng/ml and one for ketoprofen of 10ng/ml in blood. As we speak, California is looking carefully at the proposed RMTC flunixin threshold, and has recently extended the "phase in" period for this threshold, and may well be in the process of experimentally re-assessing this proposed threshold.

Such a re-evaluation would not be particularly surprising, because, to my knowledge, there is no definitive scientific study that approaches addressing the question of a 24-hour threshold of flunixin in the blood of a racing thoroughbred at the appropriate level of rigor required prior to its introduction as a national rule.

13/ Further Reading:

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3/ Tobin T, Mundy GD, Stanley SD, Sams RA, Crone D (eds): Testing for Therapeutic Medications, Environmental and Dietary Substances in Racing Horses, Proceedings of Workshop, Lexington, KY, 220 pages, 1995. [KY Ag Exp Sta #95-14-058]

4/ The Association of Racing Commissioners International (ARCI) Uniform Classification System for Foreign Substances: http://www.arci.com/druglisting.pdf

5/ Tobin T, Harkins JD, Sams RA: Testing for therapeutic medications: Analytical/ pharmacological relationships and the need for "limitations" on the sensitivity of testing for certain agents. *J Vet Pharm Therap*, 22:220-233. 1999. [KY Ag Exp Sta #98-14-134].

6/ Smith R.L "The Zero tolerance approach to doping control in horse racing: a fading illusion?": Proceedings of the 13th International Conference of Racing Analysts and Veterinarians (ICRAV) Cambridge, United Kingdom, p 9-14, 2000.

7/ Stirling K, Bellocq R, and Tobin T: National Horsemen's Benevolent and Protective Association, Inc. Proposed National Policy on Drug Testing and Therapeutic Medication. *J Eq Vet Sci* **23**(1): 4-5, 18-40, 2003. (http://hbpa.org/resources/MedicationPolicy.pdf)

8/ Neogen ELISA tests: www.neogen.com/forensicordering.htm

9/ Tobin T, Harkins JD, VanMeter PW, Fuller TA: The Mare Reproductive Loss Syndrome II: A Toxicokinetic/Clinical Analysis and a Proposed Pathogenesis; Septic Penetrating Setae. Intern. J. Appl. Res. Vet. Med., Vol 2 No 2 P 142 - 158, 2004.(www.jarvm.com/articles/Vol2Iss2/TOBINJARVMVol2No2.pdf)

Appendix #1

Association of Racing Commissioners International "Drug Positives"

8/16/2004 Thru 8/16/2005

RCI Drug Postive Rulings From 8/1/2004 Thru 8/1/2005

CountOfDRUGNAME	DRUGNAME
4	ACEPROMAZINE
1	ACETYLSALICYLIC ACID (ASPIRIN)
10	ALBUTEROL
4	AMINOREX
4	AMPHETAMINE
1	BENZOCAINE
9	BENZOYLECGONINE
1	BETAMETHASONE
1	BOLDENONE
1	BUMETANIDE
1	BUSPIRONE
1	BUTORPHANOL
16	CAFFEINE
3	CAFFEINE; THEOPHYLLINE
1	CAFTHEOBTHEOP
1	CARPROFEN
1	CELECOXIB
3	CIMETIDINE
26	CLENBUTEROL
6	CROMOLYN
1	DANTROLENE
1	DESMETHYLPYRILAMINE
1	DESMETHYLPYRLAMINE
1	DETOMIDINE
45	DEXAMETHASONE
1	DEXTRORPHAN
14	DICLOFENAC
20	DIMETHYLSULFOXIDE
1	DIPHENHYDRAMINE
1	DIPRENOPHINE
3	DORMOSEDAN
2	EPHEDRINE
1	ERGONOVINE
3	EXCESS TCO2
1	FEXOFENADINE
1	FLUMETHASONE
85	FLUNIXIN
4	FLUNIXIN/PHENYLBUTAZONE
1	FLUPHENAZINE
25	FUROSEMIDE
3	GUAIFENESIN
6	GUANABENZ
1	HALOPERIDOL
2	HYDROCORTISONE AND MEVIPICAINE
1	HYDROMORPHONE
1	HYDROXYDANTROLENE

CountOfDRUGNAME	DRUGNAME
2	HYDROXYDETOMIDINE
2	HYDROXYETHYL PROMAZINE SULFOXIDE
1	HYDROXYLIDOCAINE
1	HYDROXYMEPIVACAINE
1	IPRATROPIUM
4	IPRATROPIUM BROMIDE
1	ISOFLUPREDONE
2	ISOXSUPRINE
6	KETOPROFEN
18	KETOROLAC
10	LASIX
2	LIDOCAINE
3	MEPIVACAINE
7	METHAMPHETAMINE
24	METHOCARBAMOL
16	METHYLPREDNISOLONE
2	MORPHINE
9	NAPROXEN
1	NAPROXEN POSITIVE
2	NAPROXENTHE
1	NAQUASONE
1	NORPSEUDOEPHEDRINE DESMETHYLPYRILAMINE
1	O-DESMETHYLPYRILAMINE AND NORPSEUDOEPHEDRINE
2	PENTAZOCINE
2	PENTOXYFYLLINE
1	PERINDOPRIL
1	PHENYLBUTAZONE
4	PHENYOXYPHEN
1	PIRBUTEROL
3	POLYETHYLENEGLYCOL
15	PROCAINE
2	PROPANTHELINE
1	PROPRANOLOL
1	PSEUDOEPHEDRINE
1	PSEUDOEPHEDRINE And NORPSEUDOEPHEDRINE
1	PSEUDOEPHEDRINE AND NORPSEUDOEPHEDRINE AND DESMETHYLPYRILAMI
2	PYRILAMINE
8	RANITIDINE
1	SALICYLIC ACID
4	SALIX
1	TERBUTALINE POSTV
5	THEOPHYLLINE
47	TOTAL CARBON DIOXIDE
3	TRIAMCINOLONE
4	TRICHLORMETHIAZIDE
4	TRIMETHOPRIM
4	TRIPELENNAMINE
23	UNKNOWN
1	VENTIPULMIN SYRUP

Appendix #2

What can an attorney do?

Perhaps, it seems, quite a bit.

From: Terry Bjork
Date: Tue, 09 Aug 2005 09:18:00 -0500
Subject: Illinois Racing Board slaps Drysdale with suspension

In a racing development that some (well, me) thought would never come, Neil Drysdale is finally going to do some suspension time for the drugs positive in Flying Dash after the 2002 Hawthorne Derby. Which was run in May that year.

As those of you who were not in diapers at the time may recall, Flying Dash won the race, but then tested positive for clenbuterol and acepromazine. Drysdale immediately disputed the findings, saying (or lawyer saying) that Flying Dash would have been passed out in his stall from that level of acepromazine, and there must have been a mixup of test samples (though presumably any other horse would also have been passed out, thus nowhere near the test barn), and stuff like that. The usual.

Well, the dispute dragged on and on, with Mr. Drysdale and his lawyer Mr. Papiano (sp?) beating the IRB to death with discovery motions and other legal wrangling and who knows what, as it was all kept pretty hush hush. Meanwhile, the IRB demanded the purse money back, and it didn't come, so they suspended the license of Flying Dash's owner Mr. Sekiguchi (?), and then it did come back and they reinstated Mr. Sekiguchi's license, and the money went into mothballs in the horsemen's purse account, and the case dragged on and on, and somewhere in there Flying Dash died, and it was almost cleared up last November any day now, really they promised, but ... not quite!

Well, now in August of 2005, 3-1/2 years after the fact, comes word that the last piece of the puzzle is in place for the purse to finally be distributed. The IRB and Mr. Drysdale have finally hit on a solution to end the stalemate. The original penalty that was handed down in this case was 45 days suspended and a $2500 fine. But of course there has been inflation since then, so the new penalty is 7 days and no fine (at least according to this source). And conveniently, the 7 days suspension corresponds to the So Cal dark week in December, between the end of the Hollywood meet and the beginning of Santa Anita, so as to inconvenience Mr. Drysdale as little as possible.

"Mr. Drysdale - hold out your hand!"

slap!

Let that be a warning to evildoers everywhere - don't you be trying to drug those horses in Illinois!

News is at the very bottom of this article:

http://www.drf.com/news/article/67495.html

PS - No news on how much Mr. Drysdale or the taxpayers of Illinois spent in lawyer fees to resolve this.

Appendix #3

An Overview of Foreign Substance and Medication Violations and Penalties, 1995 to 1999

Proceedings of the 13th International Conference of Racing Analysts and Veterinarians

Cambridge, United Kingdom

2000

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MEDICATION VIOLATIONS AND PENALTIES FOR RCI CLASS 1, 2 AND 3 FOREIGN SUBSTANCES: A PRELIMINARY REPORT

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Abstract

This report summarises and analyses the Association of Racing Commissioners International (ARCI) Class 1, 2 and 3 foreign substances identifications, suspensions and penalties reported which were levied from 1995 to 1999 in California, Canada, Florida, Kentucky, Louisiana, Maryland, New York and Ohio.

The introduction of new tests was a factor in the increased frequency of individual identifications: 56 for clenbuterol (RCI Class 3) and 23 for metaraminol (RCI Class 1). Environmental contaminants were also highly represented, 16 identifications for morphine (RCI Class 1), 15 for benzoylecgonine (RCI Class 1), and 21 for caffeine (RCI Class 2). The third most commonly identified group was therapeutic medications including 28 identifications for lidocaine and 8 for mepivacaine, both RCI Class 2 agents. Among the RCI Class 3 agents, there were 35 identifications for promazine, 30 for pyrilamine, 28 for albuterol and 22 for procaine.

These identifications are based on an estimated one million samples tested. On this basis, the 62 Class 1 identifications represent a 'call-rate' of about 6/100,000 samples. If the potential environmental contaminants, morphine and benzoylecgonine, are eliminated, this rate is cut approximately in half. The rate for Class 1 identifications in Thoroughbred racing is lower than the 1/100,000 sample frequency found in Standardbreds and Quarter Horses. By any standards, this is a low identification rate.

Similarly, the overall identification rate for Class 2 agents is about 8/100,000 samples tested, and for Class 3 agents it is 2/10,000 samples tested. The overall identification rate for all Class 1, 2 and 3 agents is about 1/2,500 samples tested. These are very low rates, and many 'calls' apparently represent residual traces of therapeutic medications.

The data presented here show that horseracing, and especially Thoroughbred racing, has a low incidence of deliberate misuse of Class 1, 2 or 3 agents. Additionally, there appear to be significant differences between the rates for Thoroughbred, Harness and Quarter Horse racing.

Review of the fine and penalty data shows that the sanctions applied are less stringent than those presented in the RCI guidelines. However, in view of the relative rarity of Class 1, 2 or 3 identifications, and the fact that many reports involve environmental contaminants or 'traces' of therapeutic medications, the penalties applied may well be appropriate.

Introduction

The 'Uniform Classification Guidelines for Foreign Substances and Recommended Penalties and Model Rules' (Anon 2000) of the Association of Racing Commissioners International was first adopted in August, 1991 in response to the McKinsey Report (McKinsey *et al.* 1991). These guidelines were developed 'to assist racing stewards, hearing officers and racing commissioners in evaluating the seriousness of alleged violations of medication and prohibited substance rules in racing jurisdictions'. Each agent is placed in a category ranging from Class 1 (drugs with the highest potential to affect performance) to Class 5 (drugs with the least potential to affect performance). Assignment of an agent to a particular class is based on: 1) the pharmacology of the drug; 2) drug use patterns; and 3) suitability (therapeutic value) of the drug. Class 1 agents include opiates, opium derivatives, synthetic

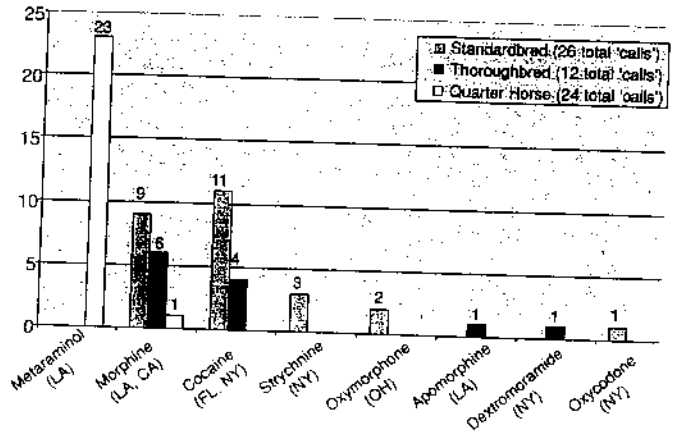
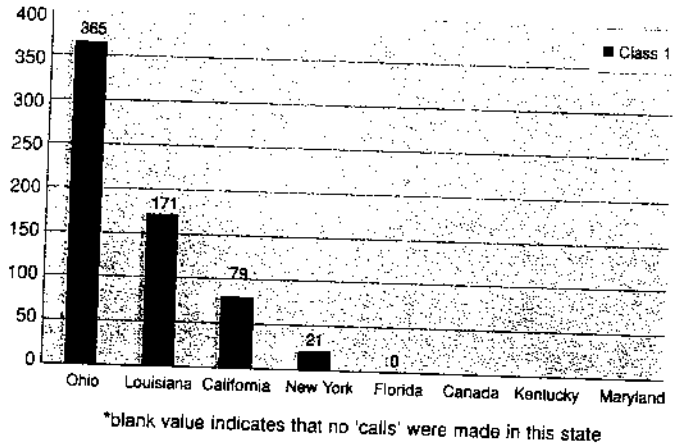
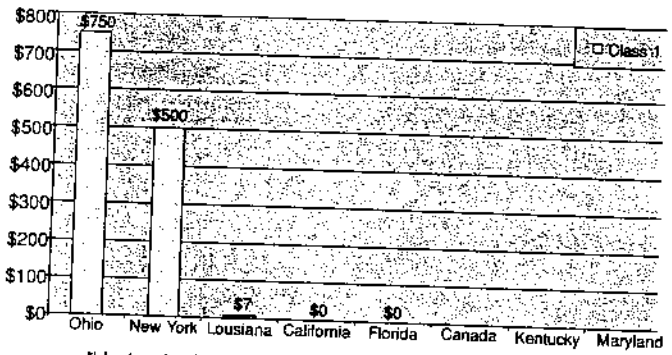


Fig 1a: Violations for RCI Class 1 agents in the major racing jurisdictions from 1995–1999.



*blank value indicates that no 'calls' were made in this state

Fig 1b: Mean suspension days for RCI Class 1 violations in the major racing jurisdictions from 1995–1999.



*blank value indicates that no 'calls' were made in this state

Fig 1c. Mean fines for RCI Class 1 violations in the major racing jurisdictions from 1995–1999.

opioids and psychoactive drugs, amphetamines and amphetamine-like drugs as well as related drugs, including but not limited to apomorphine, nikethamide, mazindol, pemoline, and pentylenetetrazol. These drugs have stimulant and depressant actions that are likely to affect the performance of the racehorse without any therapeutic effects. Class 2 agents have a high potential to affect performance, but less than the Class 1 agents. Class 2 agents include psychotropics, certain nervous system and cardiovascular system stimulants, depressants and neuromuscular blocking agents. Local anaesthetics that are injected are also placed in this class because of their increased potential to be abused as nerve blocking agents. Class 3 agents include bronchodilators and other drugs with primary effects on the autonomic nervous system, anti-histamines with sedative properties and some diuretics. They may or may not have acceptable therapeutic use in racehorses. Class 3 agents have less potential to affect performance than Class 2 agents.

The ARCI also developed penalty recommendations for each class of drugs. For a

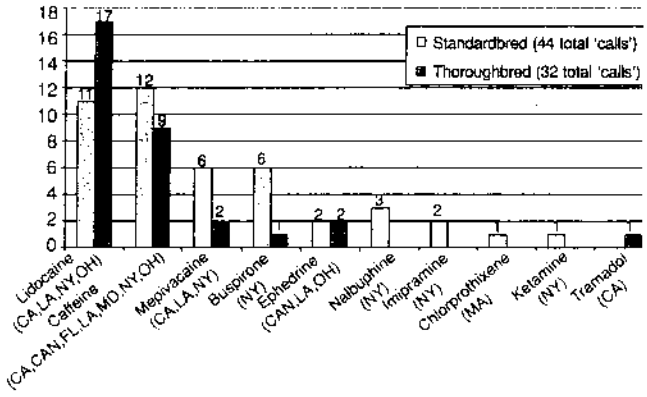
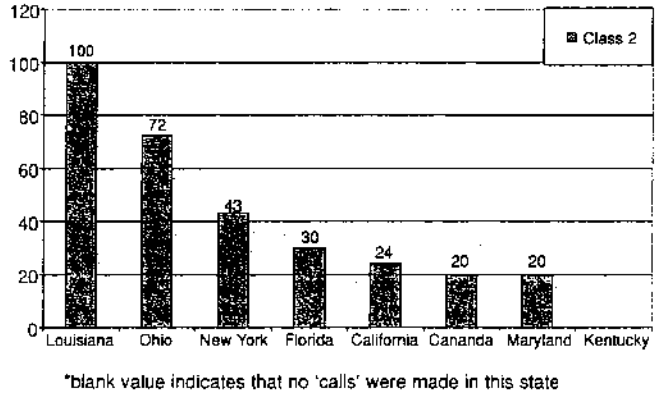
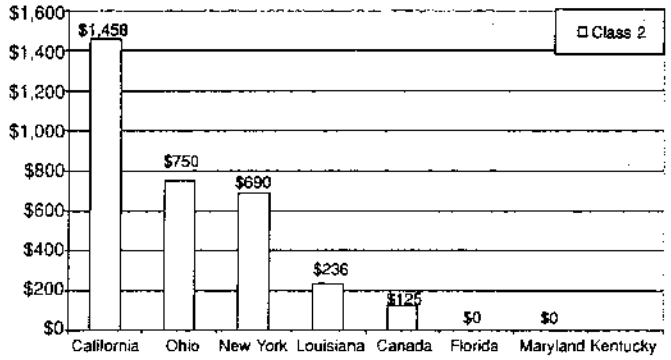


Fig 2a: Violations for RCI Class 2 agents in the major racing jurisdictions from 1995–1999.



*blank value indicates that no 'calls' were made in this state

Fig 2b: Mean suspension days for RCI Class 2 violations for the major racing jurisdictions from 1995–1999.



*blank value indicates that no 'calls' were made in this state

Fig 2c: Mean fines for RCI Class 2 violations in the major racing jurisdictions from 1995–1999.

Class 1 violation, the recommended penalty is a 1–5 year suspension and a $5,000 fine with a loss of purse. For a Class 2 violation, the recommended penalty is 6 months to one year suspension and a $1,500–$2,500 fine with a loss of purse. For a class 3 violation, the penalty recommended is 2–6 months suspension and up to $1,500 fine with a loss of purse.

These guidelines were designed to be a part of a National Medication Policy to bring uniformity to penalties and suspension days for offenders in various jurisdictions. In this communication we review all RCI Class 1, 2 and 3 medication violations reported by California (CA), Canada (CAN), Florida (FL), Kentucky (KY), Louisiana (LA), Maryland (MD), New York (NY) and Ohio (OH). The analysis was limited to RCI Class 1, 2 and 3 agents, and the number of violations per thousand tests were calculated or estimated for Thoroughbred, Standardbred and Quarter Horse races in each state.

Materials and Methods

These data were retrieved from the ARCI database. This is extremely extensive and, on occasions, the

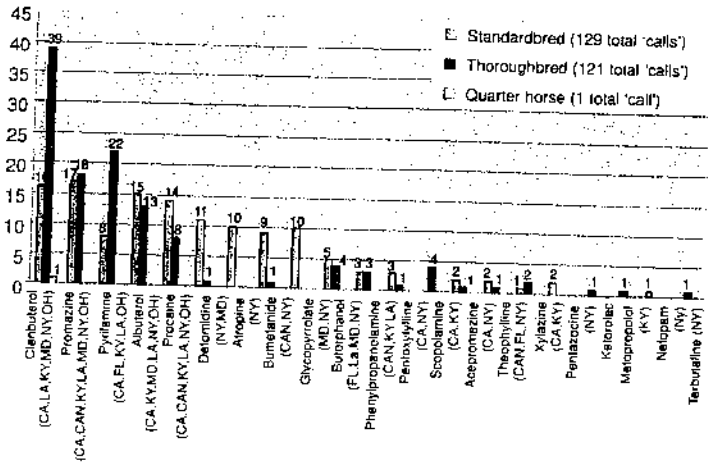


Fig 3a: Violations for RCI Class 3 agents in the major racing jurisdictions from 1995–1999.

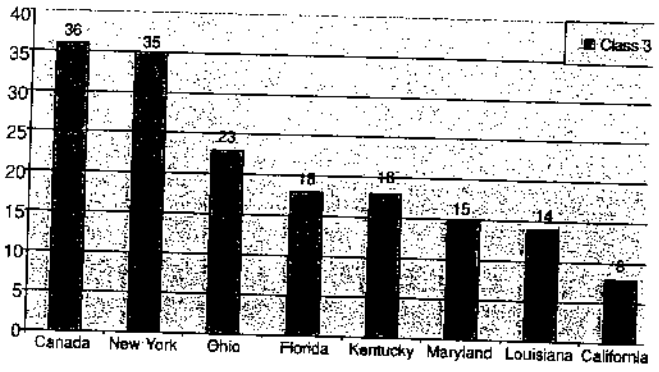


Fig 3b: Mean suspension days for RCI Class 3 violations in the major racing jurisdictions from 1995–1999.

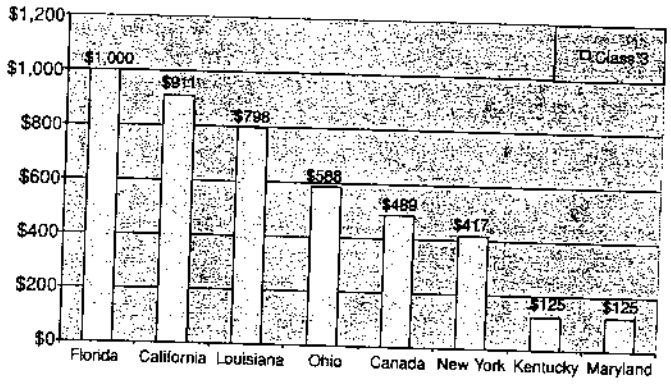


Fig 3c: Mean fines for RCI Class 3 violations in the major racing jurisdictions from 1995–1999.

data available were either ambiguous or at odds with other information. In such cases, direct communication with the ARCI or Commissions involved was undertaken to resolve ambiguities. A search was performed for each chosen jurisdiction identifying the number of 'calls' of Class 1, 2 and 3 agents from 1995–1999. Each search listed the particular foreign substance involved, the breed (Thoroughbred, Standardbred or Quarter Horse), amount of fine and the number of suspension days levied. The results are presented in Figures 1 to 3.

Results

RCI Class 1 agents

Figure 1a shows the RCI class 1 foreign substance 'calls' from 1995 to 1999 in the major racing jurisdictions. The total number of Class 1 'calls' in these jurisdictions for this 5-year period was 62. Of these, 12 were from Thoroughbred racing, 26 were from Standardbred racing and 24 were from Quarter Horse racing.

Of the 24 Quarter Horse identifications, 23 were for metaraminol in Louisiana. These identifications occurred over a short period of time and were associated with the introduction of a new test for metaraminol. This unusually large number of identifications presumably reflects the frequency of use of this agent in the Quarter Horse population, and the time between introduction of the method and reporting of the first 'identification'. To some extent, this 'burst' of identifications is a unique and onetime event.

Review of the remaining Class 1 'calls' shows that 15 were for cocaine, presumably as benzoylecgonine, and another 16 were for morphine, to yield a total number of 'calls' for both of these agents of 31, exactly 50% of all Class 1 identifications. Given that both agents can potentially occur as a result of environmental contamination, it appears that at least some of these Class 1 identifications may be due to circumstances outside the control of the trainer.

The authors estimated that about 200,000 total samples were tested and this yielded a 'call' rate for Class 1 agents of about 6/100,000 samples. If identification of morphine and benzoylecgonine (cocaine) is disregarded, the call rate is cut to about 3/100,000 samples. If the large number of metaraminol identifications in Quarter Horse racing in Louisiana is also removed, the call rate for RCI Class 1 agents in Thoroughbred and Standardbred racing drops to less than 1 in 100,000.

Figure 1b presents the mean suspension days associated with RCI Class 1 identifications. Offenders for RCI Class 1 violations were suspended in Ohio for an average of 365 days, Louisiana for 171 days, California for 79 days and New York for 21 days. Among all the states that suspended trainers for RCI Class 1 violations, Ohio clearly has the most rigorous policy.

Figure 1c presents the mean fines associated with Class 1 identifications. Ohio levied an average fine of $750 for a Class 1 violation, New York was second and Louisiana third, with an apparent average fine per violation of $7. Presumably, this very modest figure reflects a single fine spread over the 23 violations for metaraminol.

These penalties are much lower than the ARCI recommended penalties of 1–5 years suspension and/or a $5,000 fine for Class 1 violations.

RCI Class 2 agents

Figure 2a summarises the RCI Class 2 'calls' from 1995 to 1999. The most commonly identified agent was lidocaine, with 28 identifications distributed between Harness and Thoroughbred racing. This is consistent with what we know of the pharmacology and disposition of lidocaine. It is a local anaesthetic with an appropriate use in horses in training and it yields a relatively high urinary concentration of its major metabolite, the glucuronide of hydroxylidocaine, and is readily detectable in post race samples. Additionally, lidocaine is commonly added to topical over-the-counter antibiotic preparations to reduce the pain associated with local inflammatory responses (Harkins *et al.* 1998b). Given these circumstances, it is not surprising that lidocaine is the most commonly identified RCI Class 2 agent in North America and, as such, it was targeted as a research priority by the Kentucky Equine Drug Counsel research programme. (Harkins *et al.* 1998; Tobin *et al.* 2000; Dirikolu *et al.* 2001)

The next most commonly identified agent, reported 21 times, was caffeine with identifications distributed across the continent. Again, this is not surprising because caffeine is the most commonly used psychoactive agent and is a common environmental contaminant (Harkins *et al.* 1998a).

The third most commonly reported agent was mepivacaine, with 8 identifications, predominantly in harness racing. Because mepivacaine is an important agent in the diagnosis of lameness in horses, and is a legitimate therapeutic medication, these identifications may well be residues of medications. It is important to note that of the 3 Class 2 agents identified most commonly, 2 are legitimate therapeutic agents and the other is a common environmental contaminant (Harkins *et al.* 1999; Woods *et al.* 2000).

Apart from these 3 agents, identifications of Class 2 agents were sporadic and somewhat localised. Harness racing in New York appeared to be particularly well represented, yielding 6 of 7 buspirones, 3 of 3 nalbuphines, 2 of 2 imipramines and one ketamine. These identifications reflect both frequent use and effective testing for these agents.

Assuming that these identifications were made in about 1,000,000 samples over 5 years, the raw identification rate for Class 2 'calls' is about 8/100,000 samples analysed. Review of the New York data also suggests a large preponderance of identifications in Harness racing, compared with Thoroughbred racing. Inspection of the buspirone, imipramine, ketamine and nalbuphine identification rates show that, of 13 identifications reported in New York racing, 12 were made in Harness racing and only one in Thoroughbred racing.

Figure 2b presents data on mean suspension days related to Class 2 agents. Louisiana had the highest mean number of suspension days (100), whereas Canada and Maryland were lowest with 20 days.

Mean fines for Class 2 agents are presented in Fgure 2c, which shows that the largest, about $1,500, was levied in California, and the lowest were levied by Florida and Maryland ($0). The RCI recommendations are 6–12 months suspension and a $1,500–$2,500 fine.

RCI Class 3 agents

Figure 3a presents the 251 Class 3 identifications by state and breed. The most commonly identified agent was clenbuterol (56 identifications), for which a new and highly sensitive test was introduced in May of 1998. Review of the data shows that virtually all of these identifications occurred after May 1998 and presumably were associated with the introduction of the new and more sensitive technology, which can detect clenbuterol for 28 days or longer after the last administration. On the other hand, no clenbuterol identifications were reported in Canada during this time, although approximately one third of the total number of samples tested were from Canada.

The second most commonly identified agent was promazine which is a tranquiliser used in racehorses. While promazine, for which a highly sensitive ELISA test exists, has not been studied in detail, it is less potent than clenbuterol and is administered at relatively high doses. As such, this relatively high rate is consistent with what we know about the therapeutic use of this agent and its relative ease of detection (Yang *et al.* 1988).

The next most commonly identified agent was pyrilamine, which is common and for which a highly sensitive ELISA test exists. Pharmacokinetic data show that pyrilamine administered by any route remains detectable in the urine for 7 days or longer. (Woods *et al.* 2001) Again, the relatively high call rate is consistent with what is known about the therapeutic use of this agent and its relative ease of detection.

The next most commonly identified agent, albuterol, is a therapeutic bronchodilator used widely in racehorses. Throughout the survey, California had in place a threshold level for albuterol of 1 ng/ml in urine. Despite this, there were reports of albuterol identifications in California. It is also interesting to note that no identifications of albuterol were reported in Canada during this period.

Procaine was the next most commonly reported identification. Given the widespread therapeutic use of procaine penicillin and its ease of detection in post administration samples, this moderate rate of identification was encouraging. At one time procaine, presumably from procaine penicillin, was one of the most commonly reported post race identifications (Harkins *et al.* 1996).

These data yielded an identification rate of about 2/10,000 samples tested for Class 3 agents.

Figure 3b shows that, for Class 3 agents, the highest mean number of suspension days were 36 in Canada, 35 in New York, 23 in Ohio, 18 in Kentucky and Florida, down to 8 days in California. Figure 3c shows that the mean values for fines for Class 3 infractions range from $1,000 in Florida to $125 in Kentucky and Maryland.

Discussion

The major variable influencing the rate of drug identifications was the introduction of new or more sensitive tests. During this survey, a more sensitive test (clenbuterol) and a new test (metaraminol) were introduced and the number of identifications for these agents increased dramatically.

The second factor influencing the identification rate was the possible role of environmental contamination. Approximately half of the Class 1 identifications were for morphine and cocaine, agents considered by some to be environmental contaminants. Caffeine is also considered an environmental contaminant and is highly represented in the Class 2 identifications. An ability to distinguish between environmental contamination and misuse of these agents would be very helpful to the racing industry.

A third factor influencing the identification rate appears to be venue. When the metaraminol test was introduced in Louisiana, it detected abuse of this drug in Quarter Horse racing, but not in Thoroughbred or Standardbred racing. Similarly, the data from New York seemed to suggest a much greater rate of medication misuse among Harness horses than among Thoroughbred horses, and the reasons for this difference are not clear.

For those who are familiar with the therapeutic medications used in horses in training, the disposition characteristics and the sensitivity of testing, there are few surprises in the pattern of identifications or findings of residues of therapeutic medications. When therapeutic agents are administered at relatively large doses, excreted in urine at relatively high concentrations and for long periods, and are sensitive to ELISA testing, these agents will clearly be among those most frequently identified.

Among drug testing programmes, horseracing has the longest established, most broad-based and most highly sensitive system in existence. Among the 500 RCI Class 1, 2 and 3 agents tested during the period 1995–1999, 39 different substances were detected a total of 389 times. Based on these figures, the overall identification rate in these jurisdictions was about 1/2,500 samples tested.

Additionally, the identification rate in Thoroughbred racing was substantially lower than in Harness or Quarter Horse racing.

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Appendix #4

THE NITTY-GRITTY DDETAILS OF THE FIELD AND MEDICATION REGULATION

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Viewpoint

New National Horsemen's Benevolent and Protective Association Proposed Medication Rules

In this issue, you will find a copy of the 2002 draft of the National Horsemen's Benevolent and Protective Association (HBPA) Proposed National Policy on Drug Testing and Therapeutic Medication (pages 18-40). This proposed policy represents a major revision and extension of the 2001 policy, first presented about 1 year ago.

The National HBPA and some of its local affiliates have supported research on regulatory procedures for therapeutic medications for close to 10 years. The scientific contributions opened in the form of a major international workshop Testing for Therapeutic Medications, Environmental and Dietary Substances in Racing Horses held at the Gluck Equine Research Center in 1994. This workshop reviewed and endorsed the long-standing Canadian approach of limited sensitivity testing for therapeutic medications and environmental and dietary substances.

After this workshop, the National HBPA and some local HBPAs supported research designed to establish the scientific validity of thresholds for therapeutic medications. As part of this project, the HBPA also supported research programs in synthetic chemistry, a program that now provides analytic standards to racing chemists worldwide.

The HBPA also supported research to establish the validity of the thresholds approach to therapeutic medication regulation. The thresholds approach is now well established scientifically, as set forth in an invited scientific review entitled "Testing for Therapeutic Medications: Analytical/Pharmacological Relationships and Limitations on the Sensitivity of Testing for Certain Agents."[1]

At the same time this scientific groundwork was being laid, a number of states adopted the thresholds approach, starting with California in 1995. Following this lead, the state of Ohio in 1999 formally adopted no fewer than 31 thresholds for therapeutic medications and dietary and environmental substances. In the fall of 2001, the American Association Equine Practitioners organized an Equine Medication Summit with the goal of standardizing medication testing throughout the United States. Contributing to this process, the National HBPA drafted its first Proposed National Policy on Drug Testing and Therapeutic Medication. This document was the first detailed national policy ever proposed and served as an initial template for approaches to identifying a national policy in this area. This policy was presented then, and this draft is presented now, as a living document that will change as knowledge concerning therapeutic medications and their regulation increases.

The current HBPA proposed national policy represents a major expansion of the first draft of this document. The policy now covers 30 Association of Racing Commisssioners International (ARCI) class 2, 3, and 4 therapeutic medications, and no fewer than 6 dietary or environmental substances are identified.

Other major deficiencies of the field are also specifically addressed. Terms are defined, the analytic standards made available courtesy of HBPA-supported research are identified, a comprehensive listing of worldwide thresholds for therapeutic medications is presented, and detailed analyses of the factors affecting "detection times" and "withdrawal time guidelines" are also presented. All of this material is referenced, so that if necessary, the original data sources can be identified.

This document makes clear that in equine medication control, the devil is in the details. This document overviews current practices throughout North America, identifies the best and most practical approaches, and presents specific regulatory details for each therapeutic medication. This proposed medication policy is, quite simply, the most detailed, broad-based, and comprehensive review of equine medication control in North America and how this problem is currently being handled.

Caution: This Proposed National Policy on Drug Testing and Therapeutic Medication, and the attached appendices, should not be taken for anything other than a "proposed policy" with supporting documentation. Much of the regulatory information set forth in the supporting documentation is, like this proposed policy itself, subject to change. The information contained in

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doi: 1053/jevs.2003.6

this document should never be taken as an authoritative guide with respect to drug testing and medication regulation in any individual jurisdiction. Horsemen and other industry professionals should always consult with their veterinary advisors and/or the appropriate regulatory authorities when seeking information or guidance with regard to the specific regulations or regulatory procedures in place in any individual jurisdiction at any given time.

On behalf of the National and local HBPA affiliates who have made this research and this document possible, we remain,

Kent H. Stirling
Chairman, National HBPA Medication Committee Research

Remi Bellocq
Executive Director, National HBPA

Thomas Tobin, MVB, PhD, DABT
Professor, Gluck Equine Research Center
University of Kentucky

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National Horsemen's Benevolent & Protective Association, Inc

Proposed National Policy on Drug Testing and Therapeutic Medication

December 3, 2002

First published October 18, 2001

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Appendices

1. EXECUTIVE SUMMARY

The National Horsemen's Benevolent & Protective Association (National HBPA) herein presents its 2002 updated National Policy on Drug Testing and Therapeutic Medication for Association of Racing Commissioners International (ARCI) class 1, 2, 3, and 4 substances. This document defines the relevant terms and sets forth the regulatory need and scientific basis for:

1.1 ZERO TOLERANCE TESTING for performance-altering substances that have no legitimate use in horses in training or racing. This ZERO TOLERANCE policy also applies to prohibited practices, including but not limited to administration of milkshakes, erythropoietins, growth hormones, or unregulated shockwave therapy.

1.2 THRESHOLDS/REGULATORY LIMITS for substances recognized by racing jurisdictions and/or the American Association of Equine Practitioners (AAEP) as therapeutic medications for the horse. The thresholds/regulatory limits herein are based on published scientific research and/or thresholds/regulatory limits adopted by one or more racing jurisdictions.

1.3 THRESHOLDS/REGULATORY LIMITS for the following therapeutic medications: acepromazine, albuterol, bupivacaine, butorphanol, clenbuterol, dantrolene, dexamethasone, flumethasone, flunixin, furosemide, glycopyrrolate, hydrocortisone, isoflupredone, isoxsuprine, ketoprofen, lidocaine, meclofenamic acid, mepivacaine, methocarbamol, methylprednisolone, naproxen, pentazocine, phenylbutazone, prednisolone, prednisone, procaine, promazine, pyrilamine, and terbutaline.

1.4 THRESHOLDS/REGULATORY LIMITS for dietary or environmental substances that are also ARCI substances, namely atropine, benzoylecgonine, caffeine, morphine glucuronides, salicylic acid/salicylates and theobromine.

1.5 SALIX (LASIX) CONTROL: Application of these thresholds/regulatory limits for substances in urine requires that Salix (furosemide, Lasix) administration be controlled such that urinary dilution does not interfere with testing.

1.6 WITHDRAWAL TIME GUIDELINES: The need for practical withdrawal time guidelines keyed to the relevant specific thresholds/regulatory limits set forth herein is explicitly recognized. Research to establish the best possible scientific basis for such withdrawal time guidelines should be a high priority.

1.7 BLOOD TESTING provides a significantly superior scientific basis for the regulation of therapeutic medication. All testing laboratories should have LC-MS or LC-MS-MS instrumentation to optimize regulatory practices through application of blood testing.

1.8 STANDARDS are proposed for administrative procedures, laboratory accreditation, the reporting of chemical identifications and their quantitative determination, independent analysis, and review, with an emphasis on the importance of expert professional review.

1.9 RESEARCH: The development of new therapeutic medications and analytical technologies means that the specifics of this policy will evolve with time.

2. PREAMBLE

2.1 SCOPE OF THE POLICY: The National HBPA herein presents its National Policy on Drug Testing and Therapeutic Medication for ARCI class 1, 2, 3, and 4 substances.

2.2 GOAL OF THE POLICY: The goal of this policy is to harmonize medication policies and their regulation across the United States. In approaching this goal, the National HBPA has chosen to build on established regulatory precedent. Established regulatory precedent includes thresholds or regulatory limits, as set forth in this and the previous draft of this document. This policy now also explicitly sets forth the need for withdrawal time guidelines keyed to the regulatory thresholds, as set forth in Section 12.2 and Appendix I.

2.3 REGULATORY PRECEDENTS FOR THE POLICY: In presenting this document, the National HBPA recognizes and endorses the approaches first set forth in the long-established Canadian policy of limited sensitivity testing for therapeutic medications, the McKinsey Report (1991),[1] the National Thoroughbred Racing Association Racing Integrity and Drug Testing Task Force report (May 2002),[2] and communications from the Racing Medication and Drug Testing Consortium. Beyond this, however, this document draws freely on terms, definitions, and specific thresholds/limits/decision levels/regulatory limits (hereinafter "thresholds/regulatory limits") already in place in North American racing jurisdictions, including Arizona, Arkansas, California, Colorado, Delaware, Florida, Idaho, Illinois, Indiana, Iowa, Kansas, Kentucky, Louisiana, Maryland, Michigan, Minnesota, Montana, Nebraska, New Hampshire, New Jersey, New Mexico, Ohio, Oklahoma, Oregon, Pennsylvania, Texas, Virginia, Washington, West Virginia, Wyoming, Canada, and other national and international jurisdictions.

2.4 TECHNICAL AND SCIENTIFIC BASIS FOR THE POLICY: As set forth in this document, standardized national medication rules cannot be put in place without access to appropriate analytical standards, validated analytical methods, and appropriate research bases. In this regard, the National and local HBPAs, in cooperation with other groups, have supported research on the synthesis of analytical standards, the development of vali-

dated analytical methods, and the development of appropriate research bases for many of the listed therapeutic medications. This research base is summarized in the attached scientific review (Appendix IX) and the scientific papers that are referenced throughout the text and listed in Appendix VIII.

2.5 ADMINISTRATIVE BASIS FOR THE POLICY: Horses are commonly entered to race at 48 hours prior to post. Where possible, the therapeutic medication policies presented here have been structured, or on revising should be structured, so as to minimize interference with the process of entering horses to race while preserving the health and welfare of the horse.

2.6 DEFINITIONS: Central to any regulatory or scientific process is the precise definition of terms. This document, therefore, defines the relevant regulatory and scientific terms and sets forth the regulatory need and the best available scientific basis for this policy (superscript letters throughout text refer to the definitions presented in Appendix II).

3. ZERO TOLERANCE TESTING POLICY ON PROHIBITED PRACTICES AND PERFORMANCE-ALTERING SUBSTANCES

3.1 ZERO TOLERANCE for prohibited practices, including but not limited to administration of milkshakes, erythropoietins, growth hormones, or unregulated shock-wave therapy.

3.2 ZERO TOLERANCE TESTING[A] for performance-altering substances[B] that have no legitimate use in horses in training or racing; for these substances, any quantity detected is violative.

3.3 ZERO TOLERANCE TESTING means, in practice, utilizing the most sensitive testing procedures available that encompass the full scope and sensitivity of modern analytical methods.

3.4 ZERO TOLERANCE TESTING, therefore, includes the fullest possible range of highly sensitive ELISA tests and instrumental and other screening[C] and confirmation[D] methods.

3.5 ZERO TOLERANCE TESTING for performance-altering substances mandates vigorous research efforts to develop highly sensitive tests for performance-altering substances.

3.6 ZERO TOLERANCE TESTING for performance-altering substances, with the application of appropriate penalties, is unequivocally supported and endorsed by the National HBPA and all HBPA affiliates throughout North America.

3.7 Endorsement of this ZERO TOLERANCE TESTING approach is based on the assumption that all analytical results and proposed administrative actions shall be reviewed by appropriate experts. Within the limits of available knowledge and technology, innocent explanations of the practices or substances in question shall have been rigorously examined prior to consideration of any regulatory action.

4. TESTING FOR THERAPEUTIC MEDICATIONS

4.1 Therapeutic medications[E,F] are necessary to preserve the health and welfare of horses. The National HBPA recognizes that horses in training, like all athletes, may at times require the administration of certain therapeutic medications to preserve their health.

4.2 The National HBPA specifically recognizes the role of the AAEP in identifying substances as therapeutic medications (Appendix III). The National HBPA further recognizes, encourages, and supports the AAEP's role in defining appropriate standardized therapeutic dosage regimens[G] of these therapeutic medications with the primary goal of preserving the health of horses. These standardized therapeutic dosage regimens will also serve to guide analytical chemists, pharmacologists, regulators, and other industry professionals across the nation.

4.3 Zero tolerance testing, as established and set forth above for performance-altering substances, is inappropriate for use in the regulation of therapeutic medication. Zero tolerance testing can lead to the detection of insignificant trace concentrations[H] of therapeutic medications long after their therapeutic effects are over. Additionally, zero tolerance testing continually increases in sensitivity as analytical methods improve. As such, zero tolerance testing is, by definition, inappropriate for application to testing for therapeutic medications.

5. THE PROBLEM: LACK OF NATIONAL STANDARDS

5.1 In the absence of national standards, zero tolerance testing for ineffective traces of therapeutic medications or dietary or environmental substances/contaminants[I] is a significant problem that causes damage to the sport of racing in the following ways.

5.2 First, and foremost, it damages the health and welfare of horses through prohibition of the administration of therapeutic medications, thereby interfering with proper and humane preservation of the health of racing horses.

5.3 Second, it damages the reputation of racing through media stories that are inaccurate or incomplete and that unfairly and unnecessarily harm public confidence in the integrity of racing.

5.4 Third, it damages the reputations of individual trainers by associating them in the minds of owners and the racing public with supposedly improper medication practices.

5.5 Fourth, it causes damage to the reputations of affected owners and, by extension, all owners, thereby discouraging their participation in racing.

5.6 Fifth, individual regulators may utilize tests of differing sensitivities for therapeutic medications, resulting in industry-wide confusion and inequitable penalties, further exacerbating these problems.

6. THE SOLUTION: NATIONAL THRESHOLDS/REGULATORY LIMITS FOR THERAPEUTIC MEDICATIONS AND DIETARY AND ENVIRONMENTAL SUBSTANCES/CONTAMINANTS

6.1 The solution is for racing to adopt uniform national testing standards, in effect, national thresholds/regulatory limits[J] for therapeutic medications, based on published research and thresholds/regulatory limits already in place in Arizona, Arkansas, California, Colorado, Delaware, Florida, Idaho, Illinois, Indiana, Iowa, Kansas, Kentucky, Louisiana, Maryland, Michigan, Minnesota, Montana, Nebraska, New Hampshire, New Jersey, New Mexico, Ohio, Oklahoma, Oregon, Pennsylvania, Texas, Virginia, Washington, West Virginia, Wyoming, Canada, and other national and international racing jurisdictions.

6.2 As set forth below, the National HBPA has supported research in these areas and has contributed to the synthesis of a substantial number of specific equine medication metabolites and analytical standards[K] required for quantification of analyte concentrations in horse urine or plasma (Appendix IV). The National HBPA, therefore, proposes the following uniform national thresholds/regulatory limits for various ARCI class 1, 2, 3, and 4 substances.[3-7]

6.3 Withdrawal Time Guidelines[L]: Thresholds/regulatory limits are concentrations[M] of substances in biological fluids above which regulatory processes may be initiated. As a practical matter, however, horsemen need "withdrawal time guidelines" keyed[N] to the specific thresholds/regulatory limits set forth hereafter. Current availability of such information is very limited; this area is, therefore, a high priority for research.

7. NATIONAL THRESHOLDS/REGULATORY LIMITS FOR THERAPEUTIC MEDICATIONS

7.1 ARCI CLASS 2 THERAPEUTIC MEDICATIONS

Thresholds/regulatory limits in place in North America for three ARCI class 2 local anesthetics are presented below. All of these thresholds/regulatory limits are in urine and are well documented in published research supported in part by the National and several local Horsemen's Benevolent & Protective Associations (Appendix V). No withdrawal time guidelines for these local anesthetics keyed to these thresholds/regulatory limits are currently available. To prevent the improper use of synergistic combinations of local anesthetics ("cocktails"), these thresholds/regulatory limits will not apply if more than one pharmacologically-related ARCI class 2 local anesthetic is detected. Thresholds/regulatory limits for local anesthetics in blood are within current technical capabilities and would better serve the industry.

7.1.1 BUPIVACAINE (LOCAL ANESTHETIC).

Target Analyte[O]: 3-hydroxybupivacaine.
Threshold/Regulatory Limit: 5 ng/mL, from/in urine.

Ohio and Washington have adopted this threshold/regulatory limit for bupivacaine, an ARCI class 2 therapeutic medication. This threshold/regulatory limit is well supported by published research, and the target analyte, 3-hydroxybupivacaine, is commercially available.[6,8]

Withdrawal Time Guideline: To our knowledge, no withdrawal time guidelines keyed to a standardized therapeutic dosage of bupivacaine at the above threshold/regulatory limit are available at this time.

7.1.2 LIDOCAINE (LOCAL ANESTHETIC).

Target Analyte: 3-hydroxylidocaine.
Threshold/Regulatory Limit: 50 ng/mL, from/in urine.

Ohio and Washington have adopted this threshold/regulatory limit for lidocaine, an ARCI class 2 therapeutic medication. This threshold/regulatory limit is well supported by published research.[9,10] The target analyte, 3-hydroxylidocaine, is a major urinary metabolite of lidocaine in the horse and is commercially available.

Withdrawal Time Guideline: To our knowledge, no withdrawal time guidelines keyed to a standardized therapeutic dosage of lidocaine at the above threshold/regulatory limit are available at this time.

7.1.3 MEPIVACAINE (LOCAL ANESTHETIC).

Target Analyte: 3-hydroxymepivacaine.

Threshold/Regulatory Limit: 10 ng/mL, from/in urine.

California, Washington, and New Mexico have adopted this threshold/regulatory limit for mepivacaine, an ARCI class 2 therapeutic medication. This threshold/regulatory limit is well supported by published research.[11,12] The target analyte, 3-hydroxymepivacaine, is a major urinary metabolite of mepivacaine in the horse and is commercially available.

Withdrawal Time Guideline: To our knowledge, no withdrawal time guidelines keyed to a standardized therapeutic dosage of mepivacaine at the above threshold/regulatory limit are available at this time.

7.1.4 Five other ARCI class 2 therapeutic medications,

namely diazepam (sedative), fluphenazine (long-acting tranquilizer), hydroxyzine (anti-histaminic), ketamine (tranquilizer/anesthetic), and reserpine (long-acting tranquilizer) are recognized therapeutic medications (Appendix III) for which no published thresholds/regulatory limits or withdrawal time guidelines are currently available.

7.2 ARCI CLASS 3 THERAPEUTIC MEDICATIONS
Thresholds/regulatory limits in place in North America for ten ARCI class 3 therapeutic medications are presented below. With the exception of clenbuterol, all of these thresholds/regulatory limits are in urine. Also, with the exception of clenbuterol, no withdrawal time guidelines keyed to these thresholds/regulatory limits are available.

Recent research on blood testing supported in part by the National and several local Horsemen's Benevolent & Protective Associations has presented data suggesting a withdrawal time guideline of four days in blood serum for clenbuterol.[13] This research is apparently consistent with in-house research from Ohio, New York, and Pennsylvania. With regard to the other listed substances, withdrawal time guidelines keyed to the indicated thresholds/regulatory limits are needed for either the presented urinary thresholds/regulatory limits or their equivalent thresholds/regulatory limits in blood plasma or serum.

To prevent the improper use of synergistic combinations of ARCI class 3 therapeutic medications ("cocktails"), these thresholds/regulatory limits will not apply if more than one pharmacologically related ARCI class 3 therapeutic medication is detected.

7.2.1 ACEPROMAZINE (TRANQUILIZER).
Target Analyte: 2-(1-hydroxyethyl) promazine sulfoxide (HEPS).
Threshold/Regulatory Limit: 25 ng/mL, from/in urine.

California, New Mexico, Ohio, and Washington have adopted this threshold/regulatory limit for acepromazine, an ARCI class 3 therapeutic medication. The target analyte 2-(1-hydroxyethyl) promazine sulfoxide (HEPS) is a major urinary metabolite of acepromazine and is commercially available.[5,6]

Withdrawal Time Guideline: To our knowledge, no withdrawal time guidelines keyed to a standardized therapeutic dosage of acepromazine at the above threshold/regulatory limit are available at this time.

7.2.2 ALBUTEROL (BRONCHODILATOR).
Target Analyte: Albuterol.
Threshold/Regulatory Limit: 1 ng/mL, from/in urine.
California and New Mexico have adopted this threshold/regulatory limit for albuterol, an ARCI class 3 therapeutic medication. The threshold/regulatory limit for albuterol in one unidentified American jurisdiction is reportedly 2 ng/mL in urine.[2]

Withdrawal Time Guideline: To our knowledge, no withdrawal time guidelines keyed to a standardized therapeutic dosage of albuterol at the above threshold/regulatory limit are available at this time.

7.2.3 BUTORPHANOL (ANALGESIC).
Target Analyte: Butorphanol.
Threshold/Regulatory Limit: 10 ng/mL, from/in urine.

Ohio has adopted this threshold/regulatory limit for butorphanol, an ARCI class 3 therapeutic medication.

Withdrawal Time Guideline: To our knowledge, no withdrawal time guidelines keyed to a standardized therapeutic dosage of butorphanol at the above threshold/regulatory limit are available at this time.

7.2.4 CLENBUTEROL (BRONCHODILATOR).
Target Analyte: Clenbuterol.
Thresholds/Regulatory Limits: 10 pg/mL, from/in plasma/serum; 5 ng/mL, from/in urine.

The 10 pg/mL plasma/serum threshold/regulatory limit for clenbuterol, an ARCI class 3 therapeutic medication, is supported by published research[13] and in-house research (Ohio, New York) and is consistent with Canadian policy. The 5 ng/mL urinary threshold/regulatory limit is supported by research performed at the University of California, Davis, and is in place in California and Washington. The threshold/regulatory limit for clenbuterol in one unidentified American jurisdiction is reportedly 10 ng/mL in urine.[2]

Withdrawal Time Guideline: Data suggesting a 4-day withdrawal time and keyed to the 10 pg/mL plasma/serum threshold for clenbuterol at a dose of 0.8 µg/kg of Ventipulmin orally b.i.d. for 10 days are published in the scientific literature.[13] This research was supported in part by the National and several local Horsemen's Benevolent & Protective Associations.

7.2.5 GLYCOPYRROLATE (BRONCHODILATOR).
Target Analyte: Glycopyrrolate.

Threshold/Regulatory Limit: 5 ng/mL, from/in urine.
Ohio has adopted this threshold/regulatory limit for glycopyrrolate, an ARCI class 3 therapeutic medication. This threshold/regulatory limit is supported by published Canadian research.

Withdrawal Time Guideline: To our knowledge, no

withdrawal time guidelines keyed to a standardized therapeutic dosage of glycopyrrolate at the above threshold/regulatory limit are available at this time.

7.2.6 PENTAZOCINE (ANALGESIC).
Target Analyte: Pentazocine.
Threshold/Regulatory Limit: 50 ng/mL, from/in urine.

Ohio has adopted this threshold/regulatory limit for pentazocine, an ARCI class 3 therapeutic medication.

Withdrawal Time Guideline: To our knowledge, no withdrawal time guidelines keyed to a standardized therapeutic dosage of pentazocine at the above threshold/regulatory limit are available at this time.

7.2.7 PROCAINE (LOCAL ANESTHETIC).
Target Analyte: Procaine.
Threshold/Regulatory Limit: 50 ng/mL, from/in urine.

Ohio has adopted a 50 ng/mL threshold/regulatory limit for procaine, an ARCI class 3 therapeutic medication. This threshold/regulatory limit is well supported by published research.[14] Procaine penicillin is an important therapeutic medication in racing horses. Development of a blood/plasma threshold/regulatory limit for this substance would likely permit its more effective use closer to post than this currently in place urine threshold/regulatory limit. Currently in place blood/plasma thresholds/regulatory limits include 25 ng/mL in plasma in Canada and 20 ng/mL in plasma in Pennsylvania, with strict reporting requirements concerning the pre-race administration of procaine penicillin.[15]

Withdrawal Time Guideline: To our knowledge, no withdrawal time guidelines keyed to a standardized therapeutic dosage of procaine at the above urinary threshold/regulatory limit are available at this time.

7.2.8 PROMAZINE (TRANQUILIZER).
Target Analyte: 3-hydroxpromazine.
Threshold/Regulatory Limit: 50 ng/mL, from/in urine.

Ohio has adopted this threshold/regulatory limit for promazine, an ARCI class 3 therapeutic medication. The target analyte, 3-hydroxypromazine, is a major urinary metabolite of promazine in the horse and is commercially available.[5,6]

Withdrawal Time Guideline: To our knowledge, no withdrawal time guidelines keyed to a standardized therapeutic dosage of promazine at the above threshold/regulatory limit are available at this time.

7.2.9 PYRILAMINE (ANTIHISTAMINIC).
Target Analyte: O-desmethylpyrilamine.
Threshold/Regulatory Limit: 50 ng/mL, from/in urine.
Ohio has adopted a Thin Layer Chromatography threshold/regulatory limit for pyrilamine, an ARCI class 3 therapeutic medication, estimated at 50 ng/mL. The target analyte, O-desmethylpyrilamine, is a major urinary metabolite of pyrilamine in the horse and is commercially available.[5,6,16-18]

Withdrawal Time Guideline: To our knowledge, no withdrawal time guidelines keyed to a standardized therapeutic dosage of pyrilamine at the above threshold/regulatory limit are available at this time.

7.2.10 TERBUTALINE (BRONCHODILATOR).
Target Analyte: Terbutaline.
Threshold/Regulatory Limit: 10 ng/mL, from/in urine.

Ohio has adopted this threshold/regulatory limit for terbutaline, an ARCI class 3 therapeutic medication.

Withdrawal Time Guideline: To our knowledge, no withdrawal time guidelines keyed to a standardized therapeutic dosage of terbutaline at the above threshold/regulatory limit are available at this time.

7.2.11 Three other ARCI class 3 therapeutic medications, namely aminophylline (theophylline with ethylenediamine, a bronchodilator), detomidine (analgesic/sedative), and xylazine (analgesic/sedative) are recognized therapeutic medications (Appendix III) for which no published thresholds/regulatory limits or withdrawal time guidelines are currently available.

7.3 ARCI CLASS 4 THERAPEUTIC MEDICATIONS
ARCI class 4 substances have less ability to influence the performance of horses, and many are recognized therapeutic medications. Many are also readily detected and regulated in blood as well as urine.

Because these substances have been detectable for many years, most jurisdictions have long-established regulatory policies for them. Beyond this, it should be made clear that in certain jurisdictions some of these substances are accepted as therapeutic medications whose administration on race day is approved by rule or statute.

At least part of the reason that certain of these substances have been approved by rule, statute, or regulatory limit as race day medications is the considerable technical difficulty in establishing realistic "no race day medication" thresholds/regulatory limits along with the associated withdrawal time guidelines for these agents, as set forth in detail in 7.3.4: Flunixin, 7.3.13: Phenylbutazone, and Appendix I below.

This section of the medication policy recognizes these

long-established regulatory precedents for ARCI class 4 therapeutic medications and simply lists regulatory policies and thresholds/regulatory limits currently in place.

7.3.1 DANTROLENE (MUSCLE RELAXANT).
Target Analyte: Dantrolene.
Threshold/Regulatory Limit: 100 ng/mL, from/in plasma.

Ohio has adopted this threshold/regulatory limit for dantrolene, an ARCI class 4 therapeutic medication, and this threshold/regulatory limit is also under review in another state.

Withdrawal Time Guideline: To our knowledge, no withdrawal time guidelines keyed to a standardized therapeutic dosage of dantrolene at the above threshold/regulatory limit are available at this time.

7.3.2 DEXAMETHASONE (STEROIDAL ANTI-INFLAMMATORY).
Target Analyte: Dexamethasone.
Threshold/Regulatory Limit: 60 ng/mL, from/in urine.
Ohio has adopted this threshold/regulatory limit for dexamethasone, an ARCI class 4 therapeutic medication, and this threshold/regulatory limit is also under review in another state.

Withdrawal Time Guideline: To our knowledge, no withdrawal time guidelines keyed to a standardized therapeutic dosage of dexamethasone at the above threshold/regulatory limit are available at this time.

7.3.3 FLUMETHASONE (STEROIDAL ANTI-INFLAMMATORY).
Target Analyte: Flumethasone.
Threshold/Regulatory Limit: 10 ng/mL, from/in urine.

Ohio has adopted this threshold/regulatory limit for flumethasone, an ARCI class 4 therapeutic medication, and this threshold/regulatory limit is also under review in another state.

Withdrawal Time Guideline: To our knowledge, no withdrawal time guidelines keyed to a standardized therapeutic dosage of flumethasone at the above threshold/regulatory limit are available at this time.

7.3.4 FLUNIXIN (NONSTEROIDAL ANTI-INFLAMMATORY).
Target Analyte: Flunixin.
Threshold/Regulatory Limit: 1000/500/100/10 ng/mL, from/in plasma/serum.

New Mexico has adopted a 1000 ng/mL threshold/regulatory limit for flunixin, an ARCI class 4 therapeutic medication. California has adopted a 500 ng/mL threshold/regulatory limit for flunixin. Ohio and Idaho have adopted a 100 ng/mL threshold/regulatory limit for flunixin, and this threshold/regulatory limit is also under review in at least one other state. Pennsylvania has adopted a 10 ng/mL threshold/regulatory limit for flunixin. Pennsylvania guidelines state that "flunixin at 1.1 mg/kg administered IV or PO [orally] 24 hours prior to race day should not result in a violation."[19] This 100-fold range in thresholds/regulatory limits for flunixin suggests that the times prior to post that flunixin can be withdrawn in each of these jurisdictions may also be very different.

Withdrawal Time Guideline: To our knowledge, other than as set forth above for Pennsylvania, no withdrawal time guidelines keyed to a standardized therapeutic dosage of flunixin at the above thresholds/regulatory limits are available at this time.

7.3.5 HYDROCORTISONE (STEROIDAL ANTI-INFLAMMATORY).
Target Analyte: Hydrocortisone.
Threshold/Regulatory Limit: 1000 ng/mL, from/in urine.

Ohio has adopted this threshold/regulatory limit for hydrocortisone, an ARCI class 4 therapeutic medication.

Withdrawal Time Guideline: To our knowledge, no withdrawal time guidelines keyed to a standardized therapeutic dosage of hydrocortisone at the above threshold/regulatory limit are available at this time.

7.3.6 ISOFLUPREDONE (STEROIDAL ANTI-INFLAMMATORY).
Target Analyte: Isoflupredone.
Threshold/Regulatory Limit: 60 ng/mL, from/in urine.

Ohio has adopted this threshold/regulatory limit for isoflupredone, an ARCI class 4 therapeutic medication.

Withdrawal Time Guideline: To our knowledge, no withdrawal time guidelines keyed to a standardized therapeutic dosage of isoflupredone at the above threshold/regulatory limit are available at this time.

7.3.7 ISOXSUPRINE (VASODILATOR).
Target Analyte: Isoxsuprine.
Threshold/Regulatory Limit: 1000 ng/mL, from/in urine.

Ohio has adopted this threshold/regulatory limit for isoxsuprine, an ARCI class 4 therapeutic medication, and this threshold/regulatory limit is also under review in at least one other state. This threshold/regulatory limit is supported by Canadian research.[20]

Withdrawal Time Guideline: To our knowledge, no with-

drawal time guidelines keyed to a standardized therapeutic dosage of isoxsuprine at the above threshold/regulatory limit are available at this time.

7.3.8 KETOPROFEN (NONSTEROIDAL ANTI-INFLAMMATORY).
Target Analyte: Ketoprofen.
Thresholds/Regulatory Limits: 100/50 ng/mL, from/in plasma.

Ohio has adopted a 100 ng/mL threshold/regulatory limit for ketoprofen, an ARCI class 4 therapeutic medication, and this threshold/regulatory limit is also under review in another state. California has adopted a 50 ng/mL threshold/regulatory limit for ketoprofen.

Withdrawal Time Guideline: To our knowledge, no withdrawal time guidelines keyed to a standardized therapeutic dosage of ketoprofen at the above thresholds/regulatory limits are available at this time.

7.3.9 MECLOFENAMIC ACID (NONSTEROIDAL ANTI-INFLAMMATORY).
Target Analyte: Meclofenamic Acid.
Threshold/Regulatory Limit: 1000 ng/mL, from/in plasma.

Ohio and New Mexico have adopted this threshold/regulatory limit for meclofenamic acid, an ARCI class 4 therapeutic medication.

Withdrawal Time Guideline: To our knowledge, no withdrawal time guidelines keyed to a standardized therapeutic dosage of meclofenamic acid at the above threshold/regulatory limit are available at this time.

7.3.10 METHOCARBAMOL (MUSCLE RELAXANT).
Target Analyte: Methocarbamol.
Threshold/Regulatory Limit: 1000 ng/mL, from/in plasma.

Ohio has adopted this threshold/regulatory limit for methocarbamol, an ARCI class 4 therapeutic medication, and this threshold/regulatory limit is also under review in at least one other state.

Withdrawal Time Guideline: To our knowledge, no withdrawal time guidelines keyed to a standardized therapeutic dosage of methocarbamol at the above threshold/regulatory limit are available at this time.

7.3.11 METHYLPREDNISOLONE (STEROIDAL ANTI-INFLAMMATORY).
Target Analyte: Methylprednisolone.
Threshold/Regulatory Limit: 1000 ng/mL, from/in urine.

Ohio has adopted this threshold/regulatory limit for methylprednisolone, an ARCI class 4 therapeutic medication.

Withdrawal Time Guideline: To our knowledge, no withdrawal time guidelines keyed to a standardized therapeutic dosage of methylprednisolone at the above threshold/regulatory limit are available at this time.

7.3.12 NAPROXEN (NONSTEROIDAL ANTI-INFLAMMATORY).
Target Analyte: Naproxen.
Threshold/Regulatory Limit: 5000 ng/mL, from/in plasma/serum.

Idaho has adopted this threshold/regulatory limit for naproxen, an ARCI class 4 therapeutic medication, and this threshold/regulatory limit is also under review in at least one other state. This threshold/regulatory limit is supported by Canadian research.

Withdrawal Time Guideline: To our knowledge, no withdrawal time guidelines keyed to a standardized therapeutic dosage of naproxen at the above threshold/regulatory limit are available at this time.

7.3.13 PHENYLBUTAZONE (NONSTEROIDAL ANTI-INFLAMMATORY).
Target Analyte: Phenylbutazone.
Threshold/Regulatory Limit: 5000/3000/2600/2200/2000 ng/mL, from/in plasma/serum.

Arizona, California, Colorado, Florida, Idaho, Indiana, Kansas, Louisiana, Michigan, Montana, Nebraska, New Mexico, Ohio, Oklahoma, Oregon, Pennsylvania, Texas, Washington, West Virginia, and Wyoming have adopted a threshold/regulatory limit of 5000 ng/mL for phenylbutazone, an ARCI class 4 substance. Arkansas and Minnesota have adopted a threshold/regulatory limit of 3000 ng/mL for phenylbutazone. Delaware, Maryland, New Jersey, and Virginia have adopted a threshold/regulatory limit of 2600 ng/mL for phenylbutazone. Iowa has adopted a threshold/regulatory limit of 2200 ng/mL for phenylbutazone. Illinois has adopted a threshold/regulatory limit of 2000 ng/mL for phenylbutazone. Phenylbutazone is, by rule or law, a race-day medication in Kentucky and New Hampshire. According to the AAEP Guidelines for Drug Detection Times, "a detection time of 48 hours is likely if phenylbutazone has been administered in a multiple dosing regimen and the threshold is 5 µg/mL. Single intravenous doses of 2 grams of phenylbutazone produce plasma concentrations that are below the 5 µg/mL threshold by 24 hours after the dose."[21]

Withdrawal Time Guideline: To our knowledge, no withdrawal time guidelines keyed to a standardized ther-

apeutic dosage of phenylbutazone at any of the above thresholds/regulatory limits are available at this time. Most jurisdictions apparently consider their thresholds/regulatory limits to be consistent with a 24-hour rule.

7.3.14 PREDNISOLONE (STEROIDAL ANTI-INFLAMMATORY).
Target Analyte: Prednisolone.
Threshold/Regulatory Limit: 1000 ng/mL, from/in urine.

Ohio has adopted this threshold/regulatory limit for prednisolone, an ARCI class 4 therapeutic medication. Prednisolone is, by law, a race-day medication in Florida.

Withdrawal Time Guideline: To our knowledge, no withdrawal time guidelines keyed to a standardized therapeutic dosage of prednisolone at the above threshold/regulatory limit are available at this time.

7.3.15 PREDNISONE (STEROIDAL ANTI-INFLAMMATORY).
Target Analyte: Prednisone.
Threshold/Regulatory Limit: 100 ng/mL, from/in urine.

Ohio has adopted this threshold/regulatory limit for prednisone, an ARCI class 4 therapeutic medication, and this threshold/regulatory limit is also under review in at least one other state.

Withdrawal Time Guideline: To our knowledge, no withdrawal time guidelines keyed to a standardized therapeutic dosage of prednisone at the above threshold/regulatory limit are available at this time.

7.3.16 Nine other ARCI class 4 therapeutic medications, namely betamethasone (steroidal anti-inflammatory), dembrexine (mucolytic), dipyrone (muscle relaxant), guaifenesin (expectorant/muscle relaxant), ibuprofen (nonsteroidal anti-inflammatory), methylergonovine (vasoconstrictor), phenytoin (muscle relaxant), triamcinolone (steroidal anti-inflammatory), and trichlormethiazide (diuretic) are recognized therapeutic medications (Appendix III) for which no published thresholds/regulatory limits or withdrawal time guidelines are currently available.

8. POLICY ON FUROSEMIDE AND OTHER AGENTS USED TO PREVENT AND/OR TREAT EXERCISE-INDUCED PULMONARY HEMORRHAGE (EIPH)
Medications to reduce the incidence of Exercise-Induced Pulmonary Hemorrhage (EIPH) include furosemide (Salix), aminocaproic acid (Amicar), carbazochrome, Premarin, and tranexamic acid. No EIPH-related medication should be administered closer than 3 hours prior to post.

8.1 FUROSEMIDE Furosemide (as Salix) may be administered on race day for the prevention or alleviation (prophylaxis) of EIPH. Five states permit administration of furosemide up to 3 hours prior to post. The recommended dose of furosemide varies from 250 to 500 mg by single intravenous injection. Optimal regulatory control of the use of furosemide is by quantification of urinary specific gravity and serum furosemide concentrations. A violation of the furosemide rule may be deemed to have occurred if the urinary specific gravity is less than 1.010 and the serum concentration of furosemide is greater than 100 ng/mL. Care should be taken to ensure that regulatory samples are drawn from the opposite side on which Salix was administered (Appendix I, Section 7.2).

8.2 OTHER ADJUNCT MEDICATION FOR EIPH The use of certain approved adjunct bleeder and other adjunct medications in combination with Salix should be permitted, with appropriate information communicated to the betting public. The use of adjunct prophylactic medications such as aminocaproic acid (Amicar), carbazochrome, Premarin, and tranexamic acid should be permitted at the discretion of the treating veterinarian, as is the practice in a number of jurisdictions.

9. POLICY ON DIETARY AND ENVIRONMENTAL SUBSTANCES/CONTAMINANTS
For the purposes of this document, dietary and environmental substances/contaminants are ARCI substances that unavoidably become part of the food supply or environment of the horse. Environmental and/or dietary substances/contaminants that are also ARCI substances include atropine, cocaine/benzoylecgonine, caffeine, morphine/morphine glucuronides, salicylic acid/salicylates, and theobromine. A number of states have established thresholds/regulatory limits for the following environmental contaminants:

9.1 ATROPINE
Target Analyte: Atropine. Threshold/Regulatory Limit: 10 ng/mL from/in urine.

California and New Mexico have adopted this threshold/regulatory limit for atropine, an ARCI class 3 substance.

Withdrawal Time Guideline: No withdrawal time guidelines, since these are neither relevant nor applicable to dietary and environmental substances/contaminants.

9.2 BENZOYLECGONINE
Target Analyte: Benzoylecgonine.
Threshold/Regulatory Limit: 150 ng/mL, in urine.

Ohio and Louisiana have adopted this threshold/regulatory limit for benzoylecgonine, the major urinary metabolite of an ARCI class 1 substance and an environ-

mental contaminant.[22] This threshold/regulatory limit is also under review in more than one jurisdiction.
Withdrawal Time Guideline: No withdrawal time guidelines, since these are neither relevant nor applicable to dietary and environmental substances/contaminants.

9.3 CAFFEINE
Target Analyte: Caffeine. Threshold/Regulatory Limit: 100 ng/mL in urine.

Ohio and Washington have adopted this threshold/regulatory limit for caffeine, an ARCI class 2 substance and a common environmental contaminant. This threshold/regulatory limit is well supported by published research[23] and is apparently in place in three other unidentified American jurisdictions. This threshold/regulatory limit is also under review in more than one jurisdiction.

Withdrawal Time Guideline: No withdrawal time guidelines, since these are neither relevant nor applicable to dietary and environmental substances/contaminants.

9.4 MORPHINE GLUCURONIDES
Target Analyte: Morphine.
Threshold/Regulatory Limit: 100 ng/mL, in urine.

Three thresholds/regulatory limits for morphine glucuronides, the major urinary metabolites of an ARCI class 1 substance, a not uncommon addition to human foodstuffs as poppy seeds and also a potential environmental contaminant, are in place in the United States. The threshold/regulatory limit in one unidentified American jurisdiction is 100 ng/mL,[2] and it is also under review in another. In Louisiana, it is 75 ng/mL; a slightly lower (50 ng/mL) limit is in place in Ohio. This threshold/regulatory limit is also under review in more than one jurisdiction. These thresholds/regulatory limits are well supported by more recent research from the Horseracing Forensic Laboratory (HFL) in England,[24] which shows urinary concentrations of 110 ng/mL after administration to horses of 2-g doses of poppy seeds containing 3 mg of morphine per dose. These thresholds/regulatory limits are dramatically lower than the 2000 ng/mL "cut-off" in place in human workplace medication testing.[19,24,25]

Withdrawal Time Guideline: No withdrawal time guidelines, since these are neither relevant nor applicable to dietary and environmental substances/contaminants.

9.5 SALICYLIC ACID/SALICYLATES
Target Analyte: Salicylic Acid.
Threshold/Regulatory Limit: 750,000 ng/mL, from/in urine.

Ohio, Texas, California, Washington and New Mexico have adopted this threshold/regulatory limit for salicylic acid, an ARCI class 4 substance. This is also the generally accepted international threshold/regulatory limit for salicylates.

Withdrawal Time Guideline: No withdrawal time guidelines, since these are neither relevant nor applicable to dietary and environmental substances/contaminants.

9.6 THEOBROMINE
Target Analyte: Theobromine.
Threshold/Regulatory Limit: 2000 ng/mL, from/in urine.

Ohio and Texas have adopted this well-established international threshold/regulatory limit for theobromine, an ARCI class 4 substance. This is also the generally accepted international threshold/regulatory limit for theobromine.

Withdrawal Time Guideline: No withdrawal time guidelines, since these are neither relevant nor applicable to dietary and environmental substances/contaminants.

9.7 Scopolamine is an example of an ARCI class 3 dietary and/or environmental substance/contaminant for which a threshold/regulatory limit is required.

10. POLICY ON TESTING LABORATORIES, ADMINISTRATIVE PROCEDURES, AND ANALYTICAL FINDINGS

10.1 The National HBPA policy on testing laboratories[P] is consistent with those of ARCI and the North American Pari-Mutuel Regulators Association (NAPRA) in that all testing laboratories shall be accredited to American Association for Laboratory Accreditation (A2LA) standards, or International Standards Organization (ISO)/International Electrotechnical Commission (IEC) 17025 standards, or their equivalent, as set forth in Appendix VI.

10.2 All administrative procedures associated with medication violations should remain confidential until completion of the entire administrative process.

10.3 These administrative procedures shall include a split sample rule following the principles set forth in the ARCI and NAPRA Model Rules.[26,27]

10.4 For all analytical findings for target analytes with thresholds/regulatory limits, the regulatory process shall include determination of the concentration of analytes in the test sample by a validated, peer-reviewed method[Q] or, failing that, the best available method.

10.5 If the primary laboratory reports the presence of a target analyte at a concentration greater than the threshold/regulatory limit, then the trainer or the trainer's designated representative shall have the opportunity to designate any laboratory accredited to A2LA or ISO/IEC 17025 standards as set forth in 10.1 above as

his or her "split sample" or "reference" laboratory to obtain a quantitative[R] determination of the analyte. He/she shall be free to request any additional testing of the sample, including genetic testing, as may be required to assist in his or her defense and/or the authorities in their review of the circumstances giving rise to the chemical identification in question.

10.6 All quantitative results/reports shall include a statistical estimate of the MEASUREMENT UNCERTAINTY.[S] No target analyte shall be reported unless the lower limit of the 95% CONFIDENCE LIMIT[T] for the measured concentration of the target analyte is greater than the threshold/regulatory limit.

11. POLICY ON EXPERT PROFESSIONAL REVIEW

11.1 The National HBPA hereby endorses and supports the 1995 recommendation of the ARCI that "all chemical findings in official test samples be subjected to a documented review process by a veterinary pharmacologist prior to any regulatory action."

11.2 The National HBPA endorses the use of an independent Equine Medical Director (EMD), as set forth by the California Horse Racing Board. The EMD should oversee implementation of the guidelines established above and promote research aimed at identifying thresholds/regulatory limits for therapeutic medications, dietary and environmental substances/contaminants. The EMD should also contribute to the development of withdrawal time guidelines for therapeutic medications and educate the racing community at large on matters affecting preservation of the health and welfare of horses.

12. FURTHER RESEARCH

12.1 BLOOD TESTING The National HBPA recognizes that blood, as a regulatory sample, yields data that are, in forensic terms, much more confidently interpretable than urinary data. The National HBPA also notes that recent advances in analytical chemistry, specifically LC-MS and LC-MS-MS technology, increasingly make possible the quantitative confirmation of therapeutic medications in blood plasma and serum samples.

The National HBPA, therefore, recommends that all testing laboratories have in place LC-MS or LC-MS-MS testing technology to optimize regulatory practices for horse racing and to better preserve the health and welfare of horses.

Application of LC-MS and LC-MS-MS testing technology will allow racing chemists to confirm and quantify an increasing number of ARCI class 2, 3, and 4 therapeutic medications in blood, thereby avoiding many of the problems associated with urine testing.

Urine testing does not allow confident interpretation of the pharmacological significance of quantitative data from urine because of the very large inherent variability in urinary concentrations of therapeutic medications and/or their metabolites (Appendix I, Section 4).

Quantitative blood data can be much more confidently interpreted than urinary data. The advantage for horses, horsemen, and the industry at large is that urinary findings may be found to be without significance based on negative or subthreshold quantitative data from the blood sample, a very significant regulatory advance.[13]

A further problem with urine testing has been that the analytes detected in urine are often unique metabolites of the medication in question. Analytical standards of these metabolites can be difficult to obtain, of uncertain chemical stability, and challenging to quantify, all of which lead to significant technical problems and difficulties with quantitative urine testing.

On the other hand, the analyte detected in a blood test is almost always the parent medication. Advantages of this technique are that suitable standards are virtually always available, these standards are generally stable, and it is almost always easier to accurately recover and quantify parent medications in blood than the more complex and poorly characterized metabolites of unknown stability identified in or recovered from urine. This is a problem that has been specifically addressed by research supported by the National and local HBPAs (Appendix V).

Additionally, to our knowledge, Salix administration does not interfere with the detection or quantification of any medication in blood plasma or serum, again leading to more equitable regulation of therapeutic medication.

A further problem with urine testing is that some substances are slow to accumulate in urine and thus may be nondetectable shortly after their administration. This deficit in urine testing could be exploited through the administration of performance-altering substances close to post. Blood testing suffers from no such limitations and can be a very reliable method of detecting the administration of performance-altering substances close to post.

In summary, because it avoids the many technical problems associated with urine testing, blood or serum-based testing provides a significantly superior scientific basis for the regulation of therapeutic medication. As such, blood-based testing has the potential to significantly benefit horses, horsemen, and the industry at large.

On this basis, the National HBPA recommends and strongly supports the accelerated implementation of LC-

MS or LC-MS-MS blood testing technology for therapeutic medications, with the goal of avoiding the many regulatory uncertainties inherent in urine testing.

12.2 WITHDRAWAL TIME GUIDELINES As set forth in this National Policy on Drug Testing and Therapeutic Medication, thresholds/regulatory limits are a critical regulatory tool; thresholds/regulatory limits, however, are not practically usable by most industry professionals. What industry professionals need are withdrawal time guidelines keyed to the specific thresholds/regulatory limits in place in the jurisdiction.

A withdrawal time guideline is a suggested period before an event during which administration of a medication should cease in order to minimize the probability of exceeding the threshold/regulatory limit for the substance.

All withdrawal time guidelines are "best estimates." Adherence to a withdrawal time guideline merely serves to reduce the risk of inadvertently exceeding the threshold/regulatory limit; it never guarantees that exceeding the regulatory limit will not occur.

A more detailed definition of withdrawal time guidelines and their limitations is set forth under Appendix II: Definitions. A listing of "Factors Affecting Withdrawal Times" is set forth in Appendix I.

To our knowledge, the only scientifically well-established withdrawal time guidelines keyed to a standardized therapeutic dosage and a specific regulatory limit currently in place are those for clenbuterol in serum and flunixin in serum Pennsylvania (7.3.4).[13]

In summary, the development of withdrawal time guidelines keyed to each specific in place threshold/regulatory limit and the appropriate standardized dosage regimen for each therapeutic medication is a high research priority.

12.3 The National HBPA recognizes that the specifics of forensic testing and therapeutic medication and the sensitivity and scope of analytical methods change with time. Nothing in this policy shall be interpreted to preclude its modification in the light of increasing knowledge about the detection, actions, effects, and uses of performance-altering substances and the capability of identifying therapeutic medications or dietary or environmental substances/contaminants in horses in training or racing.

APPENDIX I: FACTORS AFFECTING WITHDRAWAL TIMES

It is important to allow an adequate withdrawal time between administration of a therapeutic medication and competition. Withdrawal times, however, are affected by a large number of poorly characterized or understood factors. Any guideline, therefore, is unlikely to be inclusive of all the possible variations that can affect a withdrawal time in any individual horse.

The following, in approximate order of their importance, is a list of factors that influence withdrawal times.

1. Dose. Medications administered at gram doses (2 to 10 g/horse) are much more likely to be detectable for longer periods than medications administered at low milligram doses (5 mg or less/horse).
Precaution: Be aware of the actual quantity, in grams, milligrams, or micrograms per administration, of the medications you administer.

2. Sensitivity of the testing process. Increasing the sensitivity of a test by 100-fold or more is likely to greatly extend (perhaps triple) the withdrawal time.
Precaution: If an ELISA test for an agent has been developed/introduced, a general rule is to at least double the withdrawal time that was used prior to development/introduction of the ELISA test.

3. Local testing procedures. Testing methods are not standardized, so what constitutes a violation in one jurisdiction may not necessarily constitute a violation in another. For example, Canada has limited sensitivity testing for therapeutic medications and certain Canadian "detection times"[U] are shorter than the "detection times" for the same medications in the United States.
Precaution: Because the Canadian authorities have limited the sensitivity of their tests for many medications, all Canadian detection times should be treated with caution outside of Canada.
Note: The setting of a threshold/regulatory limit immediately standardizes testing for that medication in all jurisdictions adhering to that threshold/regulatory limit. Setting a threshold/regulatory limit immediately requires the laboratory to put into place specific analytical procedures that allow it to quantify medication concentrations at the level of the threshold/regulatory limit.

4. Urine pH and volume. The pH of the urine (whether the urine is acidic or alkaline) that the horse produces post race can be a major factor (potentially 100-fold or greater) in determining urinary medication or medication metabolite concentrations and, therefore, the withdrawal time. While this factor is outside the control of the horseman, it may play an important role in determining the withdrawal time and/or the significance of a urinary finding. Urine may also be concentrated or diluted, depending on the state of hydration of the horse or the presence of diuretics, which can also affect detection and withdrawal times.

Note: This potentially very large (100-fold or greater) variability in the urinary concentrations of therapeutic medications makes blood testing a much more equitable forensic procedure than urine testing.

5. Route of administration. Oral administration can greatly prolong withdrawal times. It may take up to 5 days for pills or tablets to pass through the intestinal tract of a horse; a pill or tablet that breaks down slowly in the intestinal tract can potentially release medication into a horse's system for 5 days.

Precaution: Avoid oral administrations close to post. Therapeutic medications that are administered close to post should, where appropriate, be administered intravenously.

6. Frequency of medication use. Repeated or long-term administrations of some medications, especially repeated oral administrations, can greatly extend withdrawal times. Good examples of such medications include isoxsuprine and the acepromazine family of tranquilizers.

Precaution: Where possible, avoid repeated or prolonged schedules of administration.

Note: The potential effect of repeated administrations on detection times/withdrawal times is the reason that withdrawal time guidelines must be keyed to the regulatory threshold, the formulation used, the daily dose, and the number of days for which the medication is administered (see AAEP comments on phenylbutazone detection times, 7.3.13). All of these are veterinary matters and, as such, should be specified by appropriately trained and experienced veterinarians.

7. Contamination.

7.1 Contamination of the horse's environment. Any stall that a horse inhabits during a course of therapy becomes contaminated with the medication in question. This has been shown to occur even if the medication is administered parenterally (other than orally). Contamination is obviously much more likely to occur if the medication is administered orally or in the feed at relatively large doses. Isoxsuprine, for example, is notorious in this regard, but this effect holds at some level for all therapeutic medications.[28-30]

Precaution: Care should be taken with orally administered medication to ensure that the stall does not become contaminated or that other horses in the stable do not become exposed to the medication. Move a treated horse to a fresh stall during the withdrawal period prior to competition to eliminate the possibility of stall or environmental contamination extending the withdrawal time.

7.2 Contamination of the sample prior to collection. Research with furosemide has unequivocally demonstrated the necessity of drawing the test blood sample on the contralateral side from the site of administration. This is because inadvertent extravascular administration of even miniscule volumes of therapeutic medications has the potential to release medication from these extravascular sites into the jugular vein, giving rise to spuriously high readings from the injection site vein.[31]

Precaution: With the increasing emphasis on blood testing, every effort should be made to ensure that blood samples drawn for regulatory purposes are drawn from the opposite side of the horse on which the administrations were made.

7.3 Postcollection contamination. Postcollection contamination can occur during the collection of urine samples. It usually occurs with prescription medications or substances otherwise present in the detention barn. When it occurs, the principal protection for the horseman is the absence of metabolized forms of the medication in the urine sample; the absence of such metabolites may be prima facie evidence that such postcollection contamination occurred, as it indicates that the substance did not pass through the horse's system prior to collection.

Note: In the event of postcollection contamination, the blood sample may be expected to be negative, a further advantage of blood testing.

8. Time of last meal. If medications are administered orally, recent food intake is likely to reduce the peak blood concentration attained and delay the time at which peak blood concentration is reached, as food may interfere with absorption of the medication into the bloodstream.

9. Release times of the medication preparation. Sustained-release preparations[V] for either oral or intramuscular use may be specifically formulated to delay release of the medication into the horse's system, thereby extending withdrawal times.

Precaution: Where possible, avoid sustained-release preparations.

10. Medication formulation. For any dosage form other than simple intravenous (IV) administration, variations in the formulation of a medication may result in substantially different withdrawal times. These variations can be quite significant among different oral formulations.

Precaution: Never assume that seemingly similar products from different manufacturers will have the same withdrawal times.

11. Other factors. Individual variation between animals (eg, amount of body fat), the breed and gender of the horse, coadministration of other medications, the health of the horse, and the amount of stress that the horse is subjected to are some additional factors that may affect withdrawal times.

For more detailed information, consult your veterinarian

and the appropriate regulatory body for your particular sport and jurisdiction. See also the AAEP's *Guidelines for Drug Detection Times*, Vols 1-3 (American Association of Equine Practitioners, 1999, 2000, 2001), as well as *Equine Drugs and Vaccines: A Guide for Owners and Trainers* by Eleanor M. Kellon, VMD (Breakthrough Publications, 1995) and Drugs and the Performance Horse by Thomas Tobin (Springfield, Ill: Charles C. Thomas; 1981) or relevant publications that may be available in the scientific literature.

APPENDIX II: DEFINITIONS

A. ZERO TOLERANCE TESTING: For the purposes of this document, zero tolerance testing shall mean utilization of the most sensitive and rigorous testing procedures possible for performance-altering substances, encompassing the full scope and sensitivity of modern analytical technology. As such, the analytical limit defined by zero tolerance testing is simply the "Limit of Detection" (LOD) of the most sensitive testing technique available. Zero tolerance testing, therefore, continually increases in sensitivity as analytical methods improve.

B. PERFORMANCE-ALTERING SUBSTANCE: For the purposes of this document, a performance-altering substance shall be any ARCI class 1, 2, 3, or 4 substance not identified as a therapeutic medication by an American racing authority or the AAEP or any substance with no accepted therapeutic use in horses in training or racing.

C. SCREENING TEST: For the purposes of this document, a screening test is a preliminary test that is used to rapidly evaluate whether a sample may or may not contain a prohibited substance. By definition, a screening test is merely suggestive and does not constitute definitive evidence of the presence of the prohibited substance. Thin Layer Chromatography (TLC) and Enzyme-Linked ImmunoSorbent Assay (ELISA) tests are classic examples of screening tests. By definition, a screening test yields a "presumptive" identification, which may or may not be correct.

D. CONFIRMATORY TEST: For the purposes of this document, a confirmatory test is a definitive chemical test performed under rigorously controlled conditions that unequivocally establishes the presence of the identified substance in the sample in question. Confirmatory tests are optimally independent of and operate on different chemical principles from the screening test. Mass spectrometry is the current basis for most of the confirmatory tests used in equine forensic science. By definition, a confirmatory test is extremely good evidence for the presence of the reported substance.

E. THERAPEUTIC: For the purposes of this document, therapeutic means "serving to cure or heal or to preserve health." It is derived from the Greek word therapeuein, meaning to nurse (Webster's Dictionary, 1995).

F. THERAPEUTIC MEDICATION: For the purposes of this document, a therapeutic medication shall be any ARCI class 2, 3, or 4 substance recognized as a therapeutic medication by an American racing jurisdiction or the AAEP and/or any substance "administered by or under the supervision of a veterinarian that supports the health, welfare, and fitness of horses during training and racing or facilitates their safe and humane handling during routine procedures" (draft AAEP definition of therapeutic medication, communicated November 11, 2002).

G. STANDARDIZED THERAPEUTIC DOSAGE REGIMEN: For the purposes of this document, a standardized therapeutic dosage regimen refers to a defined formulation of a therapeutic medication, administered at a defined daily dose for a defined number of days. These criteria are defined so as to reflect optimal therapeutic use of the medication in veterinary practice. These defined therapeutic dosage regimens will serve to guide analytical chemists, pharmacologists, regulators, and other industry professionals across the nation.

H. TRACE CONCENTRATION: For the purposes of this document, a trace concentration is defined as a pharmacologically insignificant concentration of the substance in question in the biological fluid.[5] The term "trace" is well established in the field and is the term used in the pivotal ARCI resolutions in this area, adopted in Oklahoma in April 1995.[26]

I. DIETARY OR ENVIRONMENTAL SUBSTANCES/ CONTAMINANTS: For the purposes of this document, a dietary or environmental substance/contaminant shall be any ARCI class 1, 2, 3, or 4 substance that is or may become part of the food supply and/or environment of horses.

J. THRESHOLD/REGULATORY LIMIT: For the purposes of this document, a threshold/regulatory limit (or "decision level"/"cut-off"/"reporting level") is any defined concentration of an analyte in a biological fluid that relates to a regulatory event. Concentrations greater than the threshold/regulatory limit may initiate regulatory action; concentrations below the threshold/regulatory limit are of no regulatory interest. The terms "threshold/regulatory limit," "cut-off," "limitation on the sensitivity of testing," "reporting level," and "decision level" are, for all practical purposes, equivalent in scientific and regulatory terms.[5] "Threshold" is the historically established term in this area (Appendix IX). A current list of world thresholds/regulatory limits is presented in Appendix VII.

K. ANALYTICAL STANDARDS: For the purposes of this document, an analytical standard is a certified chemically pure sample of a medication or medication metabolite used by an analyst as a reference in order to reliably and reproducibly identify and quantify medications and medication metabolites in a forensic sample (Appendix IV).

L. WITHDRAWAL TIME GUIDELINES: For the purposes of this document, a withdrawal time is a suggested period before an event to cease administration of a medication so as to minimize the risk of post-race detection of a residue of the medication. When establishing a withdrawal time, veterinarians must take numerous factors into account, including but not restricted to the longest known "detection times" for the medication, the dose used, the form in which the medication was/is administered, the route of administration, the duration of treatment, the sensitivity of testing/known detection time, the chemical and pharmacokinetic characteristics of the medication, the appropriate level of risk, and any unique characteristics of the horse or the event in which the horse is participating.

Withdrawal time estimates are almost always significantly longer than the longest reported detection time for the medication and can vary from jurisdiction to jurisdiction depending on the testing methodology and/or the specific thresholds/regulatory limits employed by the laboratory or the authority.

Withdrawal times should be based on consideration of these and other factors and are best recommended by practicing veterinarians who have a unique knowledge of the physiological characteristics of the horse in question and also their accumulated professional experience with regard to the jurisdiction, medication, and horse in question.

Based on the above considerations, it is clear that any withdrawal time recommendation carries with it a finite possibility of error. The probability of a residue being detected increases in direct proportion to the number of times that a given withdrawal time guideline is applied.

M. CONCENTRATION ("LEVEL"): In forensic science, a concentration is the weight, generally expressed as micrograms, nanograms, or picograms, of the substance in question dissolved in a unit volume, usually 1 mL of plasma/serum or urine.

A microgram is one millionth of a gram. A concentration of 1 microgram (mcg, µg) per milliliter, represents a concentration of one part per million (ppm). For example, a common regulatory threshold for phenylbutazone is 5 mcg per mL (5 µg/mL) in plasma/serum (7.3.13).

A nanogram is one billionth of a gram. A concentration of 1 nanogram (ng) per milliliter represents a concentration of 1 part per billion (ppb). For example, a common regulatory threshold for furosemide is 100 nanograms per mL (100 ng/mL) in plasma/serum (8.2).

(To relate one part per billion to everyday life, one part per billion represents one second in your life if you are 32 years of age.)

A picogram is one trillionth of a gram. A concentration of 1 picogram (pg) per milliliter represents a concentration of 1 part per trillion. For example, the proposed plasma/serum threshold for clenbuterol is 10 picograms per mL (10 pg/mL) of plasma/serum (7.2.4).

Obviously, following the point of reference established above, one part per trillion represents one second in your life if you are 32,000 years of age.

While "concentration" is the correct scientific term, some technical journals (clinical journals) and most lay publications speak of blood or urinary "levels," which are equivalent to blood or urinary "concentrations."

N. KEYED: For the purposes of this document, with reference to a withdrawal time guideline, the term "keyed" means that the guideline is based on research that specifies: 1, the formulation used; 2, the dose and route of administration; 3, the duration of administration; 4, the measured rate of decline of the concentration of the target analyte in the forensic sample being analyzed; 5, the relevant threshold/regulatory limit; and 6, the best estimate of the uncertainty associated with any withdrawal time guideline presented. (See 7.2.4, clenbuterol, for an example of a keyed withdrawal time guideline.)

O. TARGET ANALYTE: For the purposes of this document, the target analyte refers to the specific analyte detected and, where appropriate, quantified in the forensic sample. The target analyte may be the parent material or medication administered to the horse or a metabolite or portion of a metabolite of the material identified in or recovered from the forensic sample. Unless otherwise specified, the target analyte is the analyte on which regulatory action is based and, for the purposes of thresholds/regulatory limits, the target analyte is the only analyte quantified.

P. TESTING LABORATORY: For the purposes of this document, a testing laboratory is a laboratory employed by or under contract to a racing authority that meets the criteria set forth by NSFTC, A2LA, or ISO/IEC 17025, as presented in Appendix VI.

Q. VALIDATED METHOD: For the purposes of this document, a validated method is a qualitative or quantitative analytical method that has been rigorously characterized and tested, in more than one laboratory, so that it reliably performs as described in the Standard Operating Procedure[W] (SOP).

R. QUANTITATIVE TEST: For the purposes of this document, a quantitative test is a test that both unequivocally identifies and defines the concentration of the prohibited substance in the test sample.

S. MEASUREMENT UNCERTAINTY: For the purposes of this document, the result of any measurement of the

concentration of a substance is only an estimate of the true value. Therefore, the result is complete only when accompanied by a quantitative statement of its uncertainty (eg, a confidence interval) as established by appropriate statistical methods.

T. 95% CONFIDENCE LIMIT: For the purposes of this document, the 95% confidence interval is a range of concentration values within which 95% of all measurements will fall. In order for a "positive" to be called, the lower limit of the 95% confidence interval for a determined concentration must be greater than the threshold/regulatory limit.

U. DETECTION TIME: For the purposes of this document, a detection time is an officially or scientifically reported period of time after administration during which a medication, or a metabolite thereof, has been detected in the blood, urine, or other body fluid of a horse.

Detection times are almost always based on results obtained in experimental situations with small numbers of horses that are not actually racing. These limitations must be kept in mind when extrapolating from reported detection times to actual withdrawal time guidelines.

Good sources of detection time information include the *AAEP Guidelines for Drug Detection Times*, and the Canadian, Australian, and European guides to detection times summarized in *An Overview of the Effective World Rules on Therapeutic Medications*, available from the Gluck Equine Research Center.[32]

V. SUSTAINED-RELEASE PREPARATIONS: Many therapeutic medications are formulated as sustained-release or controlled-release preparations. These formulations are typically administered intramuscularly, and the therapeutic medication is then slowly released from the formulation.

Slow release of the medication serves the very useful purpose of prolonging its therapeutic effect. It also, however, prolongs the detection time of the medication and other substances used in the formulation.

Procaine penicillin is a typical sustained-release formulation, administered intramuscularly, in which the prolonged release of procaine, a substance used in the formulation, becomes a regulatory problem for horseracing.

W. STANDARD OPERATING PROCEDURE: For the purposes of this document, a Standard Operating Procedure (SOP) is a complete description of an analytical method or procedure that enables its confident replication in the hands of an appropriately trained and equipped individual.

APPENDIX III: AMERICAN ASSOCIATION OF EQUINE PRACTITIONERS' THERAPEUTIC MEDICATIONS LIST, 1995

Note: An American Association of Equine Practitioners "Therapeutic Medication Committee" under the chairmanship of Dr. Rick Arthur has been at work updating this therapeutic medication list for some time. As well as updating the actual medication list, the AAEP also needs to extend this list of therapeutic medications to include defined dosage schedules, as set forth under item 7 in Appendix II: Definitions. As set forth throughout this document and explicitly set forth under item 7 in Appendix II, these are absolute prerequisites for standardized testing. In the absence of defined medication schedules and specified thresholds/regulatory limits, withdrawal time guidelines for horsemen, veterinarians, and the racing industry at large cannot be developed (see AAEP comments on phenylbutazone detection times, 7.3.13).

Name	ARCI Class	Name	ARCI Class
Diazepam	2	Dipyrone	4
Fluphenazine	2	Flumethasone	4
Hydroxyzine	2	Flunixin	4
Ketamine	2	Guaifenesin	4
Lidocaine	2	Hydrocortisone (Cortisol)	4
Mepivacaine	2	Ibuprofen	4
Reserpine	2	Isoflupredone (Fluoroprednisolone)	4
Acepromazine	3	Isoxsuprine	4
Albuterol	3	Ketoprofen	4
Aminophylline	3	Meclofenamic Acid	4
Atropine	3	Methocarbamol	4
Butorphanol	3	Methylergonovine	4
Clenbuterol	3	Methylprednisolone	4
Detomidine	3	Nandrolone	4
Glycopyrrolate	3	Naproxen	4
Pentazocine	3	Pentoxifylline	4
Procaine	3	Phenytoin	4
Promazine	3	Prednisolone	4
Pyrilamine	3	Stanozolol	4
Terbutaline	3	Testosterone	4
Xylazine	3	Thiosalicylate	4
Acetylsalicylic Acid	4	Triamcinolone	4
Aminocaproic Acid	4	Trichlormethiazide	4
Betamethasone	4	Cimetidine	5
Boldenone	4	Cromolyn	5
Dantrolene	4	Dimethylsulfoxide	5
Dembrexol (Dembrexine)	4	Dimethylsulphone	5
Dexamethasone	4	Ranitidine	5

This table was generated by circulating a list of several hundred medications to AAEP members and asking them to indicate which agents they routinely used in their practice. The data were collected and reviewed by the AAEP and presented for publication as Appendix G in the Proceedings of the "Testing for Therapeutic Medications, and Environmental and Dietary Substances in Racing Horses," pp. 191-192, 1995, Lexington, KY.[3,5]

APPENDIX IV: EQUINE MEDICATION AND MEDICATION METABOLITE STANDARDS SYNTHESIZED

As set forth throughout this document, most urinary identifications of therapeutic medications are based on the detection of specific urinary metabolites of the medication, herein specified as the target analyte. Until recently, few if any of these target analytes were available to equine forensic scientists. Starting in 1995, and supported by the National and local Horsemen's Benevolent & Protective Associations, the Kentucky Equine Drug Council, and the University of Kentucky, a chemical synthesis program has been instituted to make these target analytes/standards/metabolites available to the racing industry.

The left hand column of the table below lists the parent therapeutic medication, while the right hand column lists the metabolite/target analyte as the specific chemical name of the target analyte/standard.

	Parent therapeutic	Chemical name of medication target analyte/standard
1	Acepromazine	2-(1-hydroxyethyl) promazine sulfoxide
2	Acepromazine	(1-hydroxyethyl) promazine (uncrystallized)
3	Acepromazine	Acepromazine sulfoxide
4	Amitraz	d6-N-2,4-Dimethylphenyl-N'-methylformamidine
5	Bupivacaine	3-hydroxybupivacaine
6	Chlorpromazine	7-hydroxychlorpromazine
7	Clenbuterol	1-(4-Amino-3,5-Dichlorophenyl) ethane-1,2-diol
8	Clenbuterol	2-(2-)4-Amino-3,5-2-Dichlorophenyl) Hydroxyethylamino]-2-Methyl-Propan-1-Ol
9	Clenbuterol	Clenbuterol-D9
10	Colterol and Bitolterol	3-O-Methylcolterol
11	Fluphenazine	7-hydroxyfluphenazine
12	Furosemide	Furosemide-D5
13	Guanabenz	Hydroxyguanabenz
14	Lidocaine	3-hydroxylidocaine
15	Lidocaine	4-hydroxylidocaine
16	Mazindol	2-(2-Aminoethyl)-3-(4-chlorophenyl)-3-hydroxy-2,3-dihydro-Isoindol-1-one
17	Mepivacaine	3-hydroxymepivacaine
18	Mepivacaine	4-hydroxymepivacaine
19	Phenylbutazone	Phenylbutazone-D9
20	Procaine	Procaine-D10
21	Promazine	3-hydroxypromazine
22	Promethazine	Promethazine sulfoxide
23	Propanolol	4-hydroxypropanolol
24	Propiomazine	2-(1-hydroxypropyl) promethazine sulfoxide
25	Propionylpromazine	2-(1-hydroxypropyl) promazine sulfoxide
26	Pyrilamine	O-desmethylpyrilamine
27	Ropivacaine	3-hydroxyropivacaine
28	Ropivacaine	4-hydroxyropivacaine
29	Selegiline	Desmethylselegiline
30	Tramadol	Desmethyltramadol
31	Tripelennamine	3-OH-Tripelennamine

APPENDIX V: NATIONAL AND LOCAL HORSEMEN'S BENEVOLENT AND PROTECTIVE ASSOCIATIONS THAT HAVE SUPPORTED EQUINE MEDICATION RESEARCH

National HBPA
National Horse Center
Building B Suite 2
4063 Iron Works Parkway
Lexington, KY 40511-8905

Canada HBPA
609 West Hastings Street, Suite 888
Vancouver, BC
V6B 4W4

Florida HBPA
Calder Race Course
PO Box 1800
Opa-Locka, FL 33055

Nebraska HBPA
6406 South 150th Street
Omaha, NE 68137

Kentucky HBPA
PO Box 9317
Louisville, KY 40209

Ontario HBPA
135 Queen's Plate Drive, Suite 370
Rexdale, Ontario
M9W 6V1

Charles Town HBPA
PO Box 581
Charles Town, WV 25414

Ohio HBPA
3684 Park Street
Grove City, OH 43123

Arkansas HBPA
PO Box 1670
Hot Springs, AR 71902

Michigan HBPA
4800 South Harvey
Muskegon, MI 49444-9762

Pennsylvania HBPA
PO Box 88
Grantville, PA 17028

Alabama HBPA
1523 Hidden Hills
Hartsdale, AL 35640

Total support approaching $500,000 since 1994.

APPENDIX VI: LABORATORY STANDARDS*

In order to receive accreditation under National Forensic Science Technology Center (NFSTC), American Association for Laboratory Accreditation (A2LA), or International Standards Organization (ISO)/International Electrotechnical Commission (IEC) 17025, laboratories must meet a series of minimum requirements. These standards include the following:

The laboratory must have a suitably qualified technical leader having either a 4-year baccalaureate with college credit courses in chemistry, pharmacology and toxicology, or related subjects, course work in statistics, and 5 years of experience as an analytical chemist in a laboratory analyzing substances in body fluids, including experience in giving evidence, or a graduate degree with college credit courses in chemistry, pharmacology and toxicology, or related subjects, course work in statistics, and 2 years of experience as an analytical chemist in a laboratory analyzing substances in body fluids, including experience in giving evidence.

The laboratory must demonstrate that it has effective systems in place to manage information collection, analysis, and dissemination.

The laboratory shall maintain a list of all analysts, the tests they are authorized to perform, and the reports they are authorized to sign.

All authorized analysts must have successfully completed a competency test before being allowed to perform unsupervised analyses and sign reports.

The laboratory must prepare a list of critical reagents, which are those materials utilized in analyses which can determine the accuracy of testing and the nonfunctioning of which would result in significant loss of sample. All critical reagents must be shown to be of suitable quality before being released for routine use.
The laboratory must be able to establish and maintain the forensic integrity of samples.

Samples must be received, identified, have their receipt recorded, and be stored under conditions which protect them from loss, contamination, and deleterious change. All analytical data, including quality control data, manual data transfers, calculations, chain of custody records, and conclusions must be verified by another authorized analyst.

All equipment and laboratory apparatus, the performance of which could affect the quality of test results, must be calibrated and maintained at appropriate intervals. The calibration status of all equipment must be clearly noted on or by that equipment.

The laboratory must have measures to ensure that the incidence of false-negative results is kept to a minimum.

**Courtesy of the National Forensic Science Technology Center, 2002.*

	Medication	Concentration	Fluid	Jurisdiction	Ref #
1	Acepromazine	25 ng/mL	urine	Ohio	1
	Acepromazine	25 ng/mL	urine	California	2
	Acepromazine	25 ng/mL	urine	Washington	3
	Acepromazine	25 ng/mL	urine	New Mexico	4
2	Albuterol	1 ng/mL	urine	California	2
	Albuterol	1 ng/mL	urine	New Mexico	1
3	Arsenic	200 ng/mL	urine	Texas	5
	Arsenic	300 ng/mL	urine	International	6
4	Atropine	10 ng/mL	urine	California	2
	Atropine	10 ng/mL	urine	New Mexico	4
5	Benzocaine	50 ng/mL	urine	California	2
	Benzocaine	50 ng/mL	urine	Washington	3
	Benzocaine	50 ng/mL	urine	New Mexico	4
6	BZE* (Benzoylecgonine)	50 ng/mL	urine	Unattributed	7
	BZE (Benzoylecgonine)	150 ng/mL	urine	Ohio	1
	BZE (Benzoylecgonine)	150 ng/mL	urine	Louisiana	8
7	Betamethasone	60 ng/mL	urine	Ohio	1
8	Bupivacaine	5 ng/mL	urine	Ohio	1
	Bupivacaine	5 ng/mL	urine	Washington	3
9	Butorphanol	10 ng/mL	urine	Ohio	1
10	Caffeine	250 ng/mL	serum	Canada	9
	Caffeine	1,000 ng/mL	urine	Canada	9
	Caffeine	10 ng/mL	plasma	Hong Kong	1
	Caffeine	10 ng/mL	urine	Jockey Club of Brasileiro	11
	Caffeine	30 ng/mL	urine	Hong Kong	1
	Caffeine	100 ng/mL	urine	Ohio (see 7.2.4)	1
	Caffeine	100 ng/mL	urine	Louisiana	8
	Caffeine	100 ng/mL	urine	Washington	9
11	Carbon Dioxide	37 mmol/mL	plasma	International	6
12	Clenbuterol	1 ng/mL	urine	Ohio	1
	Clenbuterol	5 ng/mL	urine	Washington	3
	Clenbuterol	5 ng/mL	urine	California	1
13	Dantrolene	100 ng/mL	plasma	Ohio	1
14	Dexamethasone	60 ng/mL	urine	Ohio	1
15	Dimethylsulfoxide	10,000 ng/mL	urine	Ohio	1
	Dimethylsulfoxide	5,000 ng/mL	urine	International	6
	Dimethylsulfoxide	1,000 ng/mL	plasma	International	6
16	Dipyrone	1,000 ng/mL	plasma	Jockey Club of Brasileiro	11
17	Flumethasone	10 ng/mL	urine	Ohio	1
18	Flunixin	1,000 ng/mL	plasma	New Mexico	4
	Flunixin	500 ng/mL	plasma	California	2, 2a
	Flunixin	100 ng/mL	plasma	Idaho	13
	Flunixin	100 ng/mL	plasma	Ohio	1
	Flunixin	10 ng/mL	plasma	Pennsylvania	12
	Flunixin	40 ng/mL	urine	Sweden	3
19	Furosemide	50 ng/mL	plasma	Oklahoma	10
	Furosemide	100 ng/mL	plasma	Others	7
	Furosemide	100 ng/mL	plasma	Jockey Club of Brasileiro	11
	Furosemide	60 ng/mL	plasma	Illinois	14
	Furosemide	100 ng/mL	plasma	Texas	5
20	Glycopyrrolate	5 ng/mL	urine	Ohio	1
21	Hydrocortisone	1,000 ng/mL	urine	Ohio	1
	Hydrocortisone	1,000 ng/mL	urine	International	6
22	Imipramine	20 ng/mL	plasma	Jockey Club of Brasileiro	11
23	Indomethacin	50 ng/mL	plasma	Jockey Club of Brasileiro	11
24	Isoflupredone	60 ng/mL	urine	Ohio	1
25	Isoxsuprine	1,000 ng/mL	urine	Ohio	1
26	Ketoprofen	100 ng/mL	plasma	Ohio	1
	Ketoprofen	50 ng/mL	plasma	California	2
27	Lidocaine	25 ng/mL	plasma	Jockey Club of Brasileiro	11
	Lidocaine	50 ng/mL	urine	Ohio	1
	Lidocaine	50 ng/mL	urine	Washington	3
	Lidocaine	25 ng/mL	urine	Louisiana	8
28	Meclofenamic Acid	1,000 ng/mL	plasma	Ohio	1
	Meclofenamic Acid	1,000 ng/mL	plasma	New Mexico	4
	Meclofenamic Acid	2,500 ng/mL	plasma	USA Equestrian	15
	Meclofenamic Acid	1,000 ng/mL	blood	Idaho	13
29	Mephenesin	200 ng/mL	plasma	Jockey Club of Brasileiro	11

Continued on next page

APPENDIX VII: *Continued*

	Medication	Concentration	Fluid	Jurisdiction	Ref #
30	Mepivacaine	5 ng/mL	urine	Ohio	1
	Mepivacaine	10 ng/mL	urine	California	2
	Mepivacaine	10 ng/mL	urine	Washington	3
	Mepivacaine	10 ng/mL	urine	New Mexico	4
31	Methocarbamol	1,000 ng/mL	plasma	Ohio	1
32	Methoxytramine	4,000 ng/mL	urine	International	6
33	Methylprednisolone	1,000 ng/mL	urine	Ohio	1
34	Morphine	50 ng/mL	urine	Ohio	1
	Morphine	75 ng/mL	urine	Louisiana	8
	Morphine	110 ng/mL	urine	HFL	16
35	Naproxen	5,000 ng/mL	blood	Idaho	13
36	Oxyphenbutazone	5,000 ng/mL	plasma	North America (ARCI)	17
	Oxyphenbutazone	5,000 ng/mL	plasma	Ohio	1
	Oxyphenbutazone	5,000 ng/mL	plasma	Louisiana	8
	Oxyphenbutazone	5,000 ng/mL	blood	Idaho	13
	Oxyphenbutazone	165,000 ng/mL	urine	Louisiana	8
37	Pentazocine	50 ng/mL	urine	Ohio	1
38	Phenylbutazone	5,000 ng/mL	plasma	North America (ARCI)	17
	Phenylbutazone	700 ng/mL	plasma	Jockey Club of Brasileiro	11
	Phenylbutazone	5,000 ng/mL	plasma	Louisiana	8
	Phenylbutazone	5,000 ng/mL	plasma	Texas	5
	Phenylbutazone	5,000 ng/mL	plasma	California	2
	Phenylbutazone	5,000 ng/mL	plasma	Pennsylvania	12
	Phenylbutazone	5,000 ng/mL	plasma	New Mexico	4
	Phenylbutazone	5,000 ng/mL	blood	Idaho	13
	Phenylbutazone	165,000 ng/mL	urine	Louisiana	8
	Phenylbutazone	165,000 ng/mL	urine	Idaho	13
39	Prednisolone	1,000 ng/mL	urine	Ohio	1
40	Prednisone	100 ng/mL	urine	Ohio	1
41	Procaine	750 ng/mL	urine	Hong Kong	18
	Procaine	25 ng/mL	plasma	Canada	10
	Procaine	100 ng/mL	plasma	Jockey Club of Brasileiro	11
	Procaine	50 ng/mL	urine	Ohio	1
	Procaine	10 ng/mL	urine	California	2
	Procaine	25 ng/mL	urine	Washington	3
	Procaine	10 ng/mL	urine	New Mexico	4
42	Promazine	20 ng/mL	plasma	Jockey Club of Brasileiro	11
	Promazine	50 ng/mL	urine	Washington	3
	Promazine	25 ng/mL	urine	New Mexico	4
	Promazine	25 ng/mL	urine	Ohio	1
	Promazine	25 ng/mL	urine	California	2
43	Pyrilamine	5 ng/mL	plasma	Jockey Club of Brasileiro	11
	Pyrilamine	50 ng/mL	plasma	Ohio	1
44	Salicylates	750,000 ng/mL	urine	California	2
	Salicylates	750,000 ng/mL	urine	Washington	3
	Salicylates	750,000 ng/mL	urine	Ohio	1
	Salicylates	750,000 ng/mL	urine	New Mexico	4
45	Salicylic Acid	750,000 ng/mL	urine	Ohio	1
	Salicylic Acid	750,000 ng/mL	urine	International	6
	Salicylic Acid	750,000 ng/mL	urine	Texas	5
	Salicylic Acid	6,500 ng/mL	plasma	International	6
46	Terbutaline	10 ng/mL	urine	Ohio	1
47	Testosterone (epitestosterone)	20 ng/mL (geldings)	urine	International	6
	Testosterone	55 ng/mL (fillies & mares)	urine	International	6
48	Tetramisole	80 ng/mL	plasma	Jockey Club of Brasileiro	11
49	Theobromine	2,000 ng/mL	urine	Ohio	1
	Theobromine	2,000 ng/mL	urine	International	6
	Theobromine	2,000 ng/mL	urine	Texas	5

*BZE is the major urinary metabolite of cocaine.

For comparative purposes, the "thresholds" for human urine concentrations, as established by the Department of Health and Human Services' Substance Abuse and Mental Health Services Administration (SAMHSA), are listed below.

NB: The opiate testing cutoff concentrations were increased, effective December 1, 1998, from 300 ng/ml to 2,000 ng/ml.

Continued on next page

APPENDIX VII: *Continued*

Marijuana metabolite[1]	15 ng/ml	urine	SAMHSA	19
Cocaine metabolite[2]	150 ng/ml	urine	SAMHSA	19
Morphine	2,000 ng/ml	urine	SAMHSA	20
Codeine	2,000 ng/ml	urine	SAMHSA	20
6-Acetylmorphine[3]	10 ng/ml	urine	SAMHSA	20
Phencyclidine	25 ng/ml	urine	SAMHSA	19
Amphetamine	500 ng/ml	urine	SAMHSA	19
Methamphetamine[4]	500 ng/ml	urine	SAMHSA	19

1: Delta-9-tetrahydrocannabinol-9-carboxylic acid.

2: Benzoylecgonine.

3: Test for 6-AM when the morphine concentration exceeds 2,000 ng/ml.

4: Specimen must also contain amphetamine at a concentration > 200 ng/ml.

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APPENDIX IX: ACKNOWLEDGEMENTS

This National Policy was inspired by Mr Ted Bassett of the Keeneland Association, who suggested to Don Sturgill, General Counsel to the National HBPA, that the HBPA develop a national medication policy. This suggestion of Mr Bassett's resonated with that made by Kent Stirling of the Florida HBPA at the summer HBPA meeting in Boston in 2001. Don immediately alerted President John Roark and Executive Director Remi Bellocq of the National HBPA, and within days, Kent and Dr Thomas Tobin, with the assistance of Remi Bellocq and the National HBPA Medication Committee, began drafting this policy. This document, therefore, is a tribute to the leadership and foresight of Ted Bassett, Kent Stirling, Don Sturgill, John Roark, and Remi Bellocq, and all the members of the National HBPA Medication Committee.

The Medication Committee was well positioned to draft this document. Starting in 1994, under President Mel Bowman, the HBPA began supporting and encouraging research on therapeutic medication regulation. In August of that year, they supported an international workshop on Testing for Therapeutic Medications, Environmental and Dietary Substances in Racing Horses at the Maxwell H. Gluck Equine Research Center at the University of Kentucky. This workshop represented an intellectual turning point, in that it marked the formal academic acceptance of the concept of limited sensitivity testing for therapeutic medications in the United States.

The HBPA has also tackled the scientific problems facing medication control programs. In 1995, the Florida HBPA, under President Kent Stirling, initiated a chemical synthesis program for equine drug metabolite standards at the University of Kentucky (Appendix IV). Additionally, local HBPAs and the National HBPA under Presidents Bill Walmsley and Rick Hiles supported research on developing a scientific basis for regulatory thresholds for therapeutic medications (Appendix V).

This work on regulatory thresholds for therapeutic medications was accepted and published in the scientific literature, and it also attracted the attention of researchers. In 1998, the Journal of Veterinary Pharmacology and Therapeutics requested an overview of HBPA-supported research in this area. This review, which summarizes much of the work supported by the HBPA up to 1999, is attached to and made a part of this report.

This research on regulatory thresholds for therapeutic medications was also supported by the Kentucky Racing Commission, the Kentucky Equine Drug Council, and the dedicated efforts of members of the Equine Pharmacology, Experimental Therapeutics and Toxicology group at the University of Kentucky. Additionally, it is a pleasure to recognize the ongoing support of the faculty of the Gluck Equine Research Center and its director, Dr Peter Timoney. Finally, much of this document reflects the editorial contributions of Mrs Linda Keisel of Agricultural Communications Services in the College of Agriculture at the University of Kentucky and the ongoing support and contributions of Ms Amy Troppmann of the Gluck Equine Research Center.

APPENDIX X: SCIENTIFIC REVIEW

For a full scientific review of the thresholds material presented herein, please consult "Testing for therapeutic medications: analytical/pharmalogical relationships and 'limitations' on the sensitivity of testing for certain agents."J Vet Pharmacol Ther 1999;22:220-33 [KY AG Exp sta #98-14-134]

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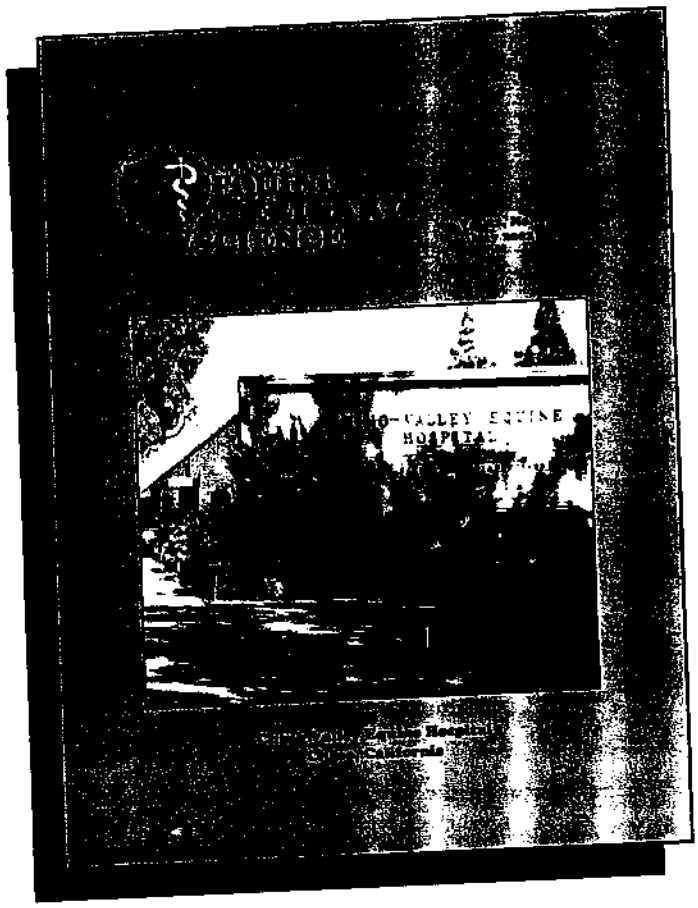

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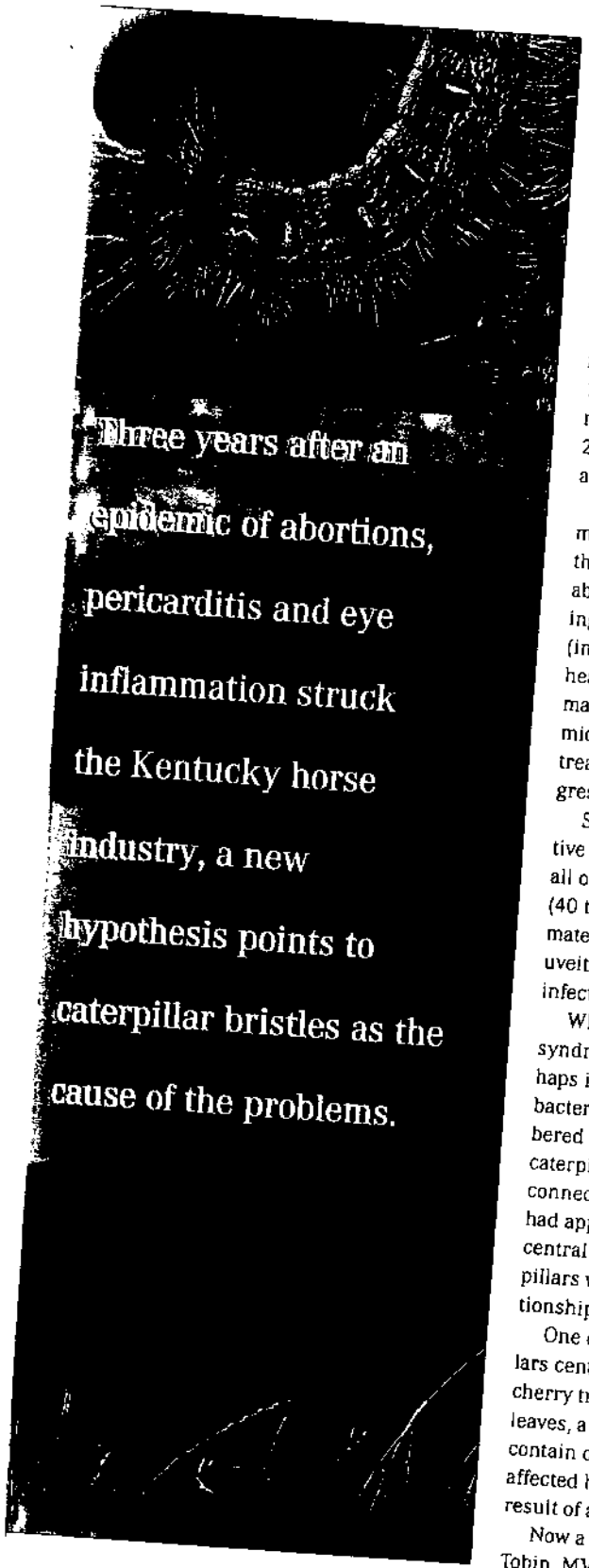
Appendix #5

TRACKING THE SOURCE OF MARE REPRODUCTIVE LOSS SYNDROME

***Equus*, Issue 327, pp. 44-49, January 2005.**

TRACKING THE SOURCE OF MARE REPRODUCTIVE LOSS SYNDROME

MIMI BRONKHORST; ED FITZGERALD (CATERPILLAR)



Three years after an epidemic of abortions, pericarditis and eye inflammation struck the Kentucky horse industry, a new hypothesis points to caterpillar bristles as the cause of the problems.

By Pat Adkins

Death visited the Bluegrass in 2001 during what should have been the season of life. On central Kentucky horse farms an epidemic of spontaneously aborted fetuses and stillborn foals cast a pall over the spring. Some foals born alive were weak and needed intensive nursing care. Breeders counted their losses and feared each day might bring more devastation. By season's end, approximately 17 percent of the pregnant Thoroughbred broodmares in the area had lost foals due in 2001 or 2002. Economic damage to the industry reached an estimated $336 million.

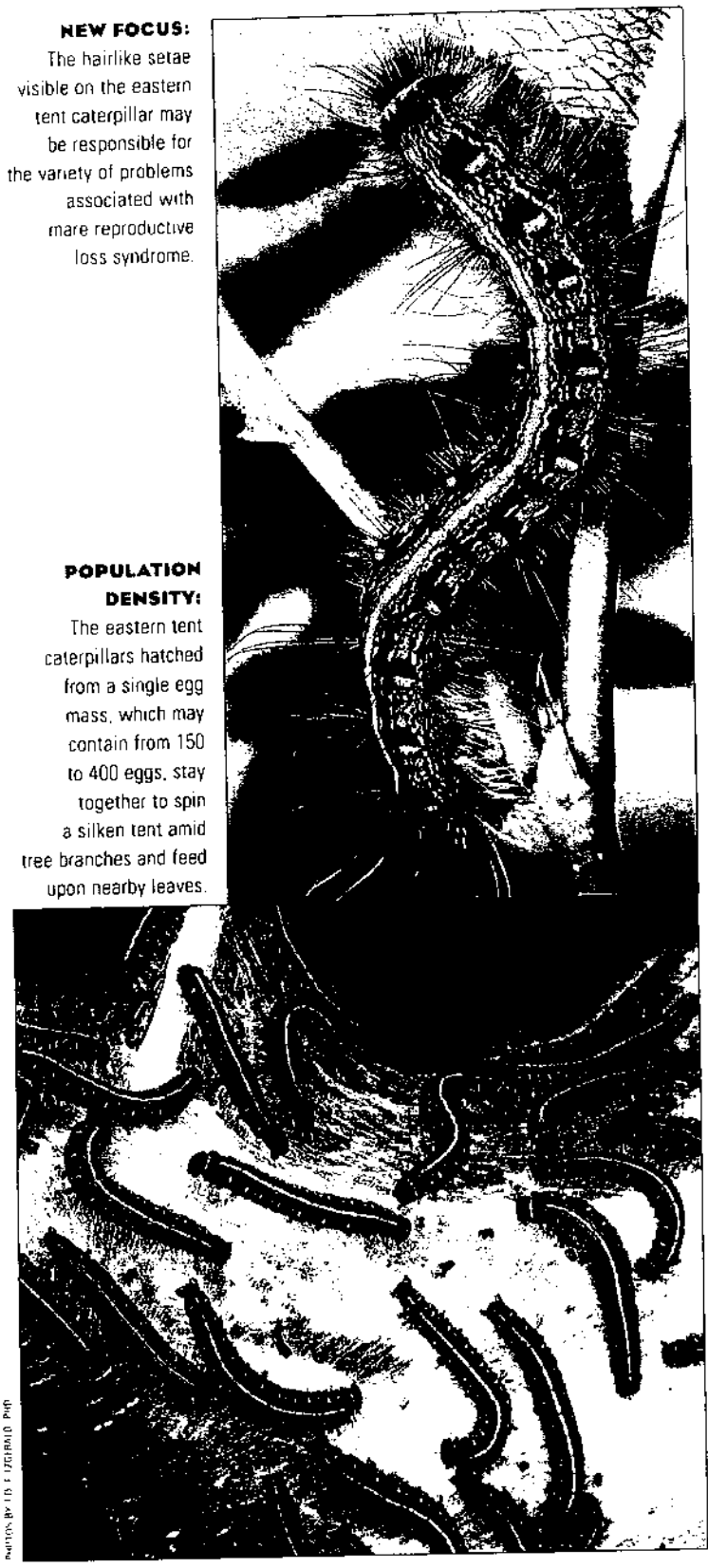
And the disaster wasn't limited to pregnant mares. Adult horses were affected in other ways, though the incidence of problems was considerably lower. Veterinarians found themselves treating an uncommon number of cases of pericarditis (inflammation of the fibrous sac enclosing the heart) and an unusual unilateral uveitis (inflammation of the pigmented structures within the middle portion of the eyeball). Despite intensive treatment, the inflammation invariably progressed to blindness in the single affected eye.

Soon the plague had a name: mare reproductive loss syndrome (MRLS), a designation used for all of its manifestations, including early fetal loss (40 to 120 days gestation), late fetal loss (approximately 220 to 250 days gestation), pericarditis, uveitis and *Actinobacillus* encephalitis, a bacterial infection of the brain.

What has remained elusive, however, is the syndrome's precise cause. Initially, a toxin, perhaps in the grass, was suspected. Some looked for bacteria. Others, particularly those who remembered the fetal losses that occurred following a caterpillar outbreak 20 years earlier, suspected a connection to the eastern tent caterpillar, which had appeared in overwhelming numbers across central Kentucky that spring. But even if the caterpillars were to blame, the exact nature of the relationship was the subject of much speculation.

One early toxin theory involving the caterpillars centered on their relationship with the black cherry trees prevalent in the area. The trees' leaves, a favorite food source for the caterpillars, contain cyanide, raising the possibility that the affected horses suffered cyanide poisoning as a result of accidentally ingesting the caterpillars.

Now a group of researchers, led by Thomas Tobin, MVB, PhD, DABT, at the Maxwell H. Gluck



NEW FOCUS: The hairlike setae visible on the eastern tent caterpillar may be responsible for the variety of problems associated with mare reproductive loss syndrome.

POPULATION DENSITY: The eastern tent caterpillars hatched from a single egg mass, which may contain from 150 to 400 eggs, stay together to spin a silken tent amid tree branches and feed upon nearby leaves.

PHOTOS BY LISA FITZGERALD, PhD

Equine Research Center at the University of Kentucky in Lexington, is proposing a new hypothesis to account for the unusual nature of MRLS. Formally known as the septic penetrating setal hypothesis of MRLS, it suggests that the barbed, hairlike structures known as setae, found on the outside of eastern tent caterpillars, are the source of the problem.

The hypothesis, which has yet to be proven with definitive scientific research, accounts for the possibility that the slender, rigid setae introduce bacterial pathogens into the horse's gut when he inadvertently ingests them. However, Tobin and his colleagues believe the likely explanation is more complex. They think the contaminated setae are capable of migrating from the gut and traveling rapidly via the bloodstream throughout the body to distant sites, including the heart, eye and especially the fetus of a pregnant mare.

Bacterial hitchhikers

According to the researchers' hypothesis, the process begins when a horse ingests the caterpillars. Fragments of the setae pick up bacteria from the horse's oral cavity, typically *Actinobacillus* and nonhemolytic *Streptococcus*. Both are consistently recovered from the umbilical tissue, lungs and placentas of horses diagnosed with MRLS. Then the septic setae begin a potentially devastating journey. Their fishhook-shaped barbs help them enter and migrate through various moving tissues. This is particularly true in the intestinal tract, where ongoing peristaltic movements propel the setae toward the thin-walled absorptive blood vessels. The setal fragments that enter the vessels travel rapidly through the bloodstream. Some remain in circulation, while others move through the vessel walls and into surrounding tissues.

When small amounts of septic material lodge in less vulnerable areas, such as muscles, the horse's immune system handles the potentially infective intrusion without clinically significant damage. However, some portions of the body are not as well protected by the immune system. Even a small amount of bacterial contamination in amniotic or other extracellular fluid—for instance, the liquid found in the eye or around the heart or brain—quickly leads to serious trouble. Bacteria in the amniotic fluid of mares who have been pregnant for 40 or more days grow rapidly and result in the death and expulsion of the fetus—within as few as 30 hours in experimental MRLS. Pregnancies of fewer than 40 days have not yet established amniotic fluid and, therefore, are less vulnerable

Using mathematical analysis in the lab

Tobin, whose specialty is toxicology, began working on MRLS by looking for a toxin such as cyanide but concluded that the evidence pointed to something else. He and his group then employed a sophisticated mathematical-analysis technique, known as the accelerated failure time survival model, which is designed to relate events to the time they occur. The technique had not been used previously in equine toxicology.

In the experiments that followed, ground-up eastern tent caterpillars and bacteria were delivered via nasogastric tube directly into the stomachs of pregnant mares, and they aborted without showing other clinical signs. The time that elapsed between the administration of the caterpillars and the abortions was mathematically predictable according to the size of the dose. Abortions occurred in as few as 30 hours if the dose was large enough. As the dose was reduced, the time between administration and abortion increased.

Another element the researchers considered during their trials was the type of bacteria that caused broodmares to abort. The bacteria introduced in the lab were different from the bacteria commonly found in horses who experience MRLS in the field. Yet the infective agents' action was the same: Somehow they traveled from the gut to the amniotic sac, where they caused the mare to abort her fetus. This finding led the researchers to hypothesize that the caterpillar setae are serving as a vehicle for a variety of bacterial hitchhikers.

From skepticism to interest to more research

In the research community, the new hypothesis was met with great skepticism at first, then growing interest. Some questioned whether setal fragments were being ingested until other researchers discovered them in the gut of necropsied horses, pigs and rats used in various studies. Those findings increased the plausibility of the hypothesis proposed by Tobin and his colleagues, although the exact role of the setae continues to be the subject of debate.

"Tom is insightful in coming up with this hypothesis and championing the idea of more research on it, but there has yet to be a definitive study published that establishes a cause-and-effect relationship," says Terry Fitzgerald, PhD, the entomologist who wrote the book *The Tent Caterpillars*, published by Cornell University in 1995. It was from Fitzgerald, a Distinguished Professor in the Department of Biological Sciences at the State University of New York–Cortland, that

FUNCTION

What are setae for?

Beyond whatever role they play in mare reproductive loss syndrome, the setae of eastern tent caterpillars serve an important purpose: The tiny, bristlelike hairs sense touch. Attached to nerve cells, they relay information about the environment to the caterpillar's brain.

Eastern tent caterpillars aren't the only creatures endowed with setae. The structures also help spiders to hear and to feel, and they are used to pull sticky silk from the spinneret. Earthworms, leeches and other annelids—invertebrate organisms that have a flat body divided into segments—use setae for locomotion and defense. And millions of setae on the bottom of a gecko's feet function as an adhesive, allowing the lizard to climb vertically and travel even when it is upside down.

COURTESY HENRY H. SOUTHGATE AND RICARDO BESSIN

TROUBLESOME BRISTLES: A microscopic view of a segment of a seta from an eastern tent caterpillar shows the sharp barbs that may help to propel the rigid structure from a horse's digestive tract to vulnerable sites throughout his body. The diameter of the seta (below) is comparable to that of an equine pulmonary capillary.

RESOURCES

Read more about MRLS online

Check out the Proceedings of the First Workshop on Mare Reproductive Loss Syndrome on the Web: www.ca.uky.edu/agc/pubs/sr/sr2003-1/sr2003-1.htm. A one-page summary of the hypothesis proposed by Thomas Tobin, MVB, PhD, DABT, and colleagues is available on page 75.

There also are many more details on how the research was performed, the mathematical model used, discussions of the mechanisms involved in moving setal fragments through the body and ideas for researchers interested in testing the hypothesis.

For more on MRLS—including caterpillar control, disease prevention, additional research and archives—go to www.uky.edu/ag/vetscience/mrls/index.htm.

Tobin learned about the nature of eastern tent caterpillars.

"Other researchers, such as Bill Bernard, DVM, Karen McDowell, PhD, and Bruce Webb, PhD, have performed critical studies that established that only the outside of the caterpillar, where the setae occur, causes problems," says Fitzgerald. "However, there is more on the outside of the caterpillar than setae, and these studies didn't establish that the setae were the components that caused the abortions. There seems to be some consensus forming around the idea that the setae affect the gut by introducing bacteria in some way. We need more scientific research."

Some of that research will be conducted at the Gluck Center. With funding from the Kentucky Thoroughbred Owners and Breeders Association, McDowell will study several aspects of the caterpillars' effects on pregnant mares. She plans to use four groups of pregnant mares in her experiments. One group will be given only caterpillar hairs; a second group will receive "polished" caterpillars, from which the hairs have been removed; a third group will receive the entire caterpillar; and a fourth group will be given nothing.

McDowell, whose early research found caterpillar setae in the intestine of pigs, also plans to study whether the mares' immune systems mount a response to the setae. The experiments are expected to yield new insights into the caterpillars and their role in transporting bacteria normally found in horses—but not in the caterpillars themselves—into vulnerable areas within the equine body.

Prevention and treatment

So what does the septic penetratin setal hypothesis mean to horse owne particularly those involved in breeding? Tobin, Fitzgerald, McDowell and others believe that it helps to reinfor

the notion, "no caterpillars, no problem," which was proposed to Tobin early on by Jimmy Henning, assistant extension director for agriculture and natural resources at the University of Kentucky. The hypothesis also indicates the advisability of taking certain precautions, especially during a caterpillar outbreak, when the creatures seem to be everywhere.

Caterpillar infestation can be prevented by removing and destroying the egg masses that encircle tree branches in winter and the tents that the insects spin in spring, and by applying insecticide. Keeping horses off the fields or muzzling them when the caterpillars become apparent can reduce the risk that they'll accidentally ingest any of the creatures' parts. And it's wise, as Fitzgerald notes, to pay attention to the horses' water source, too, because large numbers of caterpillars often collect in troughs.

If a horse, especially a pregnant mare, does come in contact with eastern tent caterpillars, rapid administration of antibiotics may be beneficial. "Our hypothesis offers a justification for the use of antibiotics," Tobin says. "But keeping horses away from the caterpillars is really the key."

Tobin points out that eastern tent caterpillars and similar species occur outside of Kentucky and may well be a little-recognized problem for horses in other areas of the country. He adds that it was the concentration of both very closely monitored pregnant mares and caterpillars in central Kentucky in 2001 that created a medical mystery and set the wheels of research turning.

"I am proud to say that it was all figured out very fast indeed," Tobin says. "The caterpillars were correctly pinpointed within three weeks and appropriate preventive measures put in place. Then, in 2002, within weeks of getting our hands on the next eastern tent caterpillar crop, we may well have pinpointed a unique pathogenic mechanism previously not described in biology or medicine. It was a major team effort, led by deans Scott Smith and Nancy Cox of the College of Agriculture, Dr. Peter Timoney, director of the Gluck Equine Research Center, and Dr. Lenn Harrison, director of the Livestock Diseases Diagnostic Center, with major and unstinting contributions from many equine practitioners and academic researchers. Significant financial support for MRLS research came from the U.S. Department of Agriculture Ag Research Service and Thoroughbred Charities of America."

The caterpillars are sure to return in high numbers some day, which is why all involved emphasize the need for more research. Armed with a newfound and hard-won understanding of the dangers, horse owners can be better prepared to prevent the kind of losses suffered in the spring of 2001. ●