"Trace" Benzoylecgonine Identifications in Post-Race Urines: Probable Sources and Regulatory Significance of Such Identifications

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Cocaine is an environmental contaminant in North America. Benzoylecgonine (BZE) is the major urinary metabolite of cocaine in horses and humans, and urinary detection of BZE is highly sensitive. Human workplace drug testing typically uses a confirmatory "cutoff" for BZE of 150 ng/ml in urine. A number of horse-racing jurisdictions have adopted similar urinary "cutoffs" for BZE; this communication presents the scientific basis for these cutoffs and lists jurisdictions with urinary cutoffs for BZE. Authors' addresses: Maxwell Gluck Equine Research Center, University of Kentucky, Lexington, KY 40546 (Camargo, Hughes, Tobin); UK Livestock Disease Diagnostic Center, Lexington, KY 40511 (Lehner); and Florida's Horsemen's Benevolent and Protective Association, Opa Locka, FL 33056 (Stirling); e-mail: fccama2@uky.edu (Camargo).

1. Introduction

Benzoylecgonine (BZE), the major urinary metabolite of cocaine in horses, is often identified and "called" at "trace"-level concentrations in post-race urines. This report sets forth the relationship between such trace-level identifications of BZE and the status of cocaine as a significant environmental contaminant.

In the United States, paper currency is commonly ("highly") contaminated with cocaine. In one study of 136 dollar bills, 79% yielded readily detectable cocaine, 50% carried microgram amounts, and 1.3 mg were recovered from one bill.

Because of the presence of cocaine in the environment, human workplace drug testing uses cutoffs or "limitations" designed to exclude BZE identifications resulting from innocent environmental contamination. These cutoffs are 300 ng/ml in screening tests and 150 ng/ml in confirmation tests; results below these cutoffs are not "reported out" forensically.

In horses, a 1-mg dose of cocaine may yield $\leq$100 ng/ml of urinary BZE. Because 1 mg of cocaine is unable to produce a pharmacological effect in a horse, there is no pharmacological or performance rationale for calling BZE concentrations of $\leq$100 ng/ml in horse urines.

Cocaine introduced directly into horse urine spontaneously hydrolyzes to BZE, whereas urine containing BZE that passed "through the horse" will contain other specific metabolites, including Ecgonine Methyl Ester (EME), para-hydroxy-BZE, and norcocaine. Because BZE alone in a urine sample can result from post-collection contamination, urinary identifications of BZE alone should be treated with caution.

NOTES

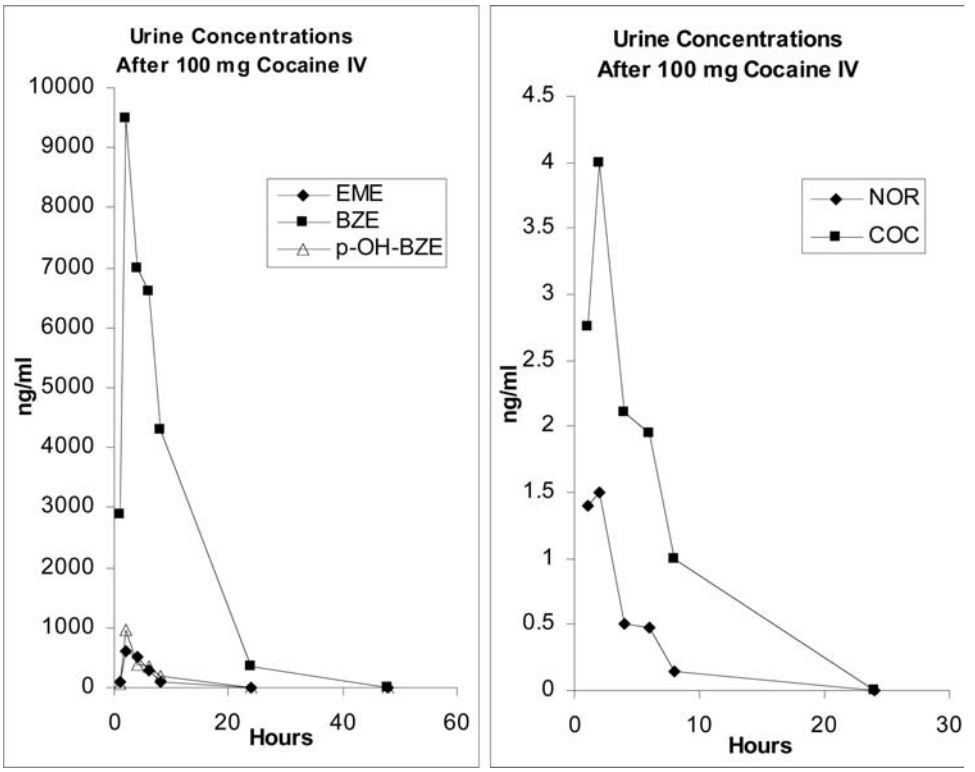

Fig. 1. Urinary concentrations of BZE, para-hydroxy-BZE, EME (left panel), cocaine, and norcocaine (right panel, note different y axis) after IV administration of 100 mg of cocaine to one horse. In horses, 200 mg/horse of cocaine intravenously has been reported to cause a performance-enhancing effect in treadmill studies, whereas a 50-mg dose produced no measurable effects.[11]

Many racing jurisdictions now have regulatory cutoffs for BZE in post-race urine of 50–150 ng/ml, supporting the above interpretations and consistency with practice in human forensics.

2. Background

The goal of this report is to review the relationship between trace-level identifications of BZE in equine urine and the status of cocaine as an environmental contaminant. BZE is the major urinary metabolite of cocaine in horses, and a frequent Association of Racing Commissioners International (ARCI) Class 1 identification in post-race urines. This report will outline what we know about BZE in horses so that we may better understand the regulatory significance of trace-level identifications of BZE in post-race urines.

A trace-level identification is identification at a concentration below those associated with pharmacological or performance effects. In this regard, a finding of BZE in a post-race urine creates the presumption that the horse was exposed to cocaine; what is not generally appreciated is how little cocaine is needed to produce such a BZE identification and how widely these small amounts of cocaine are distributed.

3. Horses, Cocaine, and Urinary BZE

BZE is the principal metabolite of cocaine found in horse urine along with other metabolites. Figure 1 shows the observed concentrations of BZE, EME, para-hydroxy-BZE, cocaine, and norcocaine after IV administration of 100 mg of cocaine to a horse. At 1 h post-administration, the urinary concentration of BZE was ~9200 ng/ml, and the concentrations of EME and para-hydroxy-BZE were ~600 ng/ml; the peak concentration of cocaine was only 4.0 ng/ml.[1] In round figures, the urinary concentration of the metabolite BZE was ~2300-fold greater than the urinary concentration of cocaine, a rather significant difference (Fig. 1).

4. BZE: A Highly Efficient Urinary Biomarker of Cocaine Exposure

BZE is a highly efficient urinary "biomarker" of cocaine exposure, because BZE is chemically unusual in that it carries two electrical charges, one positive and one negative, at normal pH values. In practical terms, this means that BZE is highly concentrated in horse urine at all urinary pH values. BZE is, therefore, an exceptionally sensitive urinary biomarker of exposure to cocaine, and exposure of animals or humans to very small amounts of cocaine give rise to relatively high urinary concentrations of BZE.

An equally important factor is that enzyme-linked immunosorbent assay (ELISA) tests for BZE are highly sensitive and are able to detect BZE concentrations of <0.5 ng/ml. The amount of cocaine required to produce a 20 ng/ml BZE concentration in

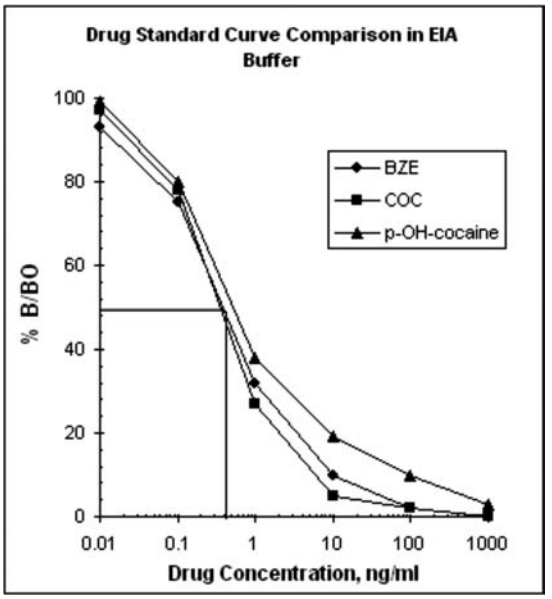

Fig. 2. ELISA test for cocaine and BZE. IC_{50}, or the concentration necessary to produce 50% of inhibition and thus the sensitivity of the test, for BZE is ~0.5 ng/ml. (Figure adapted courtesy of Neogen Corp.)

equine urine is ~0.2 mg. A human recreational dose of cocaine is ~50–100 mg; given the sensitivity of current testing, equine exposure to 0.2 mg (1/200 of a human dose) of cocaine has the potential to trigger a 20-ng/ml trace-level BZE identification in an equine urine sample (Fig. 2).[1]

Consistent with this scenario, when highly sensitive immunoassay screening was introduced into post-race testing in California, the immediate outcome was a number of low-level BZE identifications in six horses, some of which were conditioned by highly successful and respected trainers.[2] Since then, it has become clear that low-concentration BZE identifications are an expected outcome of highly sensitive immunoassay-based testing of post-race urine samples for cocaine/BZE in the United States.

Reviewing these matters, Kollias-Baker[3] noted that 2.5-mg amounts of cocaine, sufficient to yield detectable urinary concentrations of BZE for at least 24 hours post-exposure, "could easily be transferred from a cocaine abuser's hands to the mouth or muzzle of a horse" and yield concentrations similar to those "that are occasionally found in urine samples collected from show and race horses." Consistent with these interpretations, Waterman[4] of the Racing Medication and Testing Consortium notes that "the presence of cocaine in a horse's blood or urine is not a sure sign that somebody is trying to fix a race, because trace amounts of cocaine could be spread by casual contact with human users."

Spread of cocaine by casual contact is consistent with the fact that it is readily absorbed through human skin.[5] Dermal and mucosal exposures of horses may result in the presence of cocaine metabolites in urine. This is important, because, if any handler of a horse is exposed to cocaine, he/she may inadvertently expose the horse to the small amounts of cocaine that readily yield detectable BZE levels in the horse's urine.

5. Cocaine: An Environmental Contaminant

Cocaine is, for better or worse, a ubiquitous environmental contaminant in the United States. In 1995, an estimated 3.3 million U.S. citizens were cocaine users, consuming ~330 metric tons of cocaine a year.[6] This substantial quantity of cocaine is handled many times before being consumed, allowing contamination of numerous locations and items by both traffickers and users.

Paper currency, which moves from person to person, is the best studied example of cocaine contamination. In the United States, 79% of paper currency may be contaminated with cocaine. A study by the National Institutes of Health on 136 one-dollar bills collected in 14 American cities showed that 79% of these bills yielded at least 100 ng of cocaine per bill. More than one-half of these bills (54%) yielded 1000 ng/bill, and one bill, from Portsmouth, Ohio, yielded 1.3 mg of cocaine, a very significant amount of cocaine by analytical standards (Fig. 3).[7]

These results are well supported by other data. A related study suggests that 75% of circulating currency in the Los Angeles area is contaminated with residues of cocaine or other controlled substances. Similarly, 6 of 8 bills (75%) taken from civic dignitaries in Orlando, Florida, showed detectable amounts of cocaine. Other studies have shown that Canadian, American, and English paper money carries traces of cocaine, heroin, tetrahydrocannabinol (THC), and amphetamine-related compounds.[6]

Oyler et al.[7] interpreted their results in terms of "cross-contamination" from other currency or contaminated money-counting machines. They concluded that cocaine in amounts of $\leq$1.3 mg/bill did not signify that the currency was involved in a "drug transaction." These observations also suggest that every individual who handles U.S. paper currency has received, stored, and circulated small quantities of cocaine. These amounts, although generally minuscule, can at times amount to >1 mg/bill, an amount that is much more than sufficient to yield a urinary identification of BZE in a horse or a human.

In terms of cocaine as an environmental contaminant, these data show that exposure of a horse to the amount of cocaine not uncommonly found on a dollar bill in general circulation can trigger a BZE identification. In this regard, Figure 4 shows the positive cocaine identifications from the years 2000–2005 in jurisdictions reporting to the Association of Racing Commissioners International, showing a total of at least 52 reported cocaine/BZE identifications in nine different jurisdictions. Because the amount of cocaine on a single bill is sufficient to

Cocaine concentrations in United States Paper Currency ($1 denominations) from Selected Cities

City	Number positive (>0.1 μg/bill)	Number positive (>1.0 μg/bill)	Mean amount (μg/bill)	Range (μg/bill)
Baltimore, MD	9	9	75.7	0–597.0
Miami, FL	3*	2*	2.5	0–13.1
Chicago, IL	7	4	0.7	0–2.2
Honolulu, HI	10	5	3.0	0.2–9.9
Kansas City, KS	9	8	6.3	0–24.3
Las Vegas, NV	9	5	3.9	0–13.9
Los Angeles, CA	9	6	3.9	0–11.4
Minneapolis, MN	8	6	63.8	0–559.8
Spanish Fort, AL	9	7	9.0	0–70.3
Ft. Wayne, IN	9	6	3.8	0–16.6
Pittsburgh, PA	4	1	0.4	0–2.6
Yellowstone, WY	5	2	1.9	0–14.5
Whitefish, MT	7	4	0.9	0–3.0
Portsmouth, OH	10	9	136.9	0.5–1327.0

* $N = 10$ for all collection points except Miami, FL, where $N = 6$

Fig. 3. Amount of cocaine in micrograms extracted from 136 randomly selected one-dollar bills from 14 American cities. Note that 79% of the bills contain traceable amounts of cocaine, and one particular bill from Ohio contained 1.3 mg of cocaine.[7]

yield a post-race urinary concentration of BZE in a horse of 50–100 ng/ml, the question then becomes what, if anything, is the performance and therefore, forensic significance of these trace-level urinary identifications of BZE.

For most of these reported identifications, the material identified was BZE; however, its concentration was not identified. For Indiana, most of a number of identifications in the fall of 2005 involved BZE urinary concentrations of ~30 ng/ml. Even low concentrations are a problem for horsemen, because they may face significant fines and/or suspensions for identifications over which they have little or no control.

Lack of appreciation for the ease with which low urinary concentrations of BZE can be achieved is not uncommon. With reference to the above identifications in Indiana, an equine veterinarian at Purdue University stated that she "doubted that cocaine would show up in a horse's blood or urine unless it had been given the drug" and offered as her opinion that "somebody would have to have their hands pretty much coated with it for you to see it come off at detectable levels."[4] These comments are in sharp contrast with those of informed industry professionals.

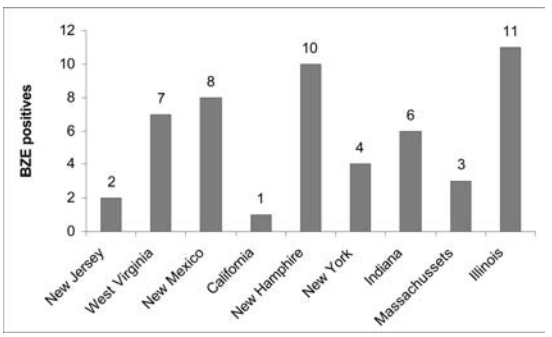

Fig. 4. The vertical bars show the number of cocaine/BZE positives for each of the listed jurisdictions for the years 2000–2005.

6. Cocaine as an Environmental Contaminant: Practical Consequences

The fact that cocaine is an environmental contaminant has a number of practical consequences in human forensics. In the first place, the question for law-enforcement personnel is how much cocaine needs to be found on paper money to "link" the money to drug trafficking. In this regard, the U.S. courts have ruled that to be admissible as evidence, the traces of cocaine must be markedly different in amount (presumably greater) than those that might be expected on bank notes in the relevant geographic area.

A second consequence of the fact that cocaine is an environmental contaminant is the existence of a federally defined cutoff or limit on the lowest amount of cocaine or metabolite of cocaine that is considered "actionable" in human forensics. This limit or cutoff is 300 ng/ml for screening, and 150 ng/ml for confirmatory tests, which considerably higher concentrations than the ones that have been the subject of recent positives in equine urine in some jurisdictions.[8]

Given that cocaine is a ubiquitous environmental contaminant in North America, the next question to address is how much cocaine a horse must be exposed to or administered to yield an identifiable

urinary concentration of BZE, the major urinary metabolite of cocaine, and how these concentrations may relate to performance effects in horses.

7. Pharmacological and/or Performance Effects of Cocaine

There are two ways in which cocaine can influence racing performance. The first is by means of its well-known central nervous system stimulant effects, and the second mechanism is through its local anesthetic properties.

We have studied the local anesthetic properties of cocaine in some detail. Cocaine is the least potent local anesthetic that we investigated, requiring a dose of >5 mg per site for a significant local anesthetic effect.[9] Such a dose of cocaine can give rise to urinary concentrations of BZE of ~500 ng/ml, a concentration far exceeding many of the reported concentrations of BZE on which regulatory action has been taken in some jurisdictions.

In earlier work, we studied the relationship between the dose of cocaine and central nervous system stimulation.[10] This work used a variable interval responding apparatus, which allowed us to sensitively measure the central nervous system stimulation produced by cocaine. The minimum dose to produce transient central nervous system stimulation was 4 mg in one particularly sensitive horse. If we reduce this dose four-fold and assume that 1 mg is the highest no effect dose (HNED) for a central stimulant effect, we arrive at a very conservative urinary limit or cutoff of ~100 ng/ml of BZE.

Our colleagues at the Ohio State University[11] have studied the effects of IV cocaine administration in horses running on a treadmill, and they have come up with more conservative estimates of the dose of cocaine required to produce a performance effect. In their hands, a dose of 50 mg/horse of cocaine was considered a no-effect dose in terms of possible performance-enhancing effects. A dose of 50 mg/horse, a dose that did not produce an effect, would yield a urinary concentration of cocaine metabolites in micrograms or thousands of nanograms (these concentrations are far higher than the proposed 150 ng/ml cutoff limit). Reviewing these results, Sams[12] has suggested that "cocaine doses >100 mg administered intravenously 5 min before exercise are necessary to produce an effect on exercise" and he noted that after such doses "urine concentrations of BZE . . . would be expected to be several micrograms (several thousands nanograms) per milliliter."

A recent study by Queiroz-Neto et al.[13] suggests that the HNED for cocaine in a behavior chamber model is 0.02 mg/kg or ~10 mg/horse and that this dose elicited a peak urinary concentration of BZE of 550 ng/ml at 2 h post-administration. This dose, 10 mg/horse, is considerably less than the one reported by McKeever et al.[11] (200 mg/horse to produce an effect in the horse). This is presumably because Queiroz-Neto et al.[13] investigated behavioral changes, whereas McKeever et al.[11] looked at performance effects.

These findings make clear that urine identifications of 100–150 ng/ml or less of BZE are, for forensic purposes, irrelevant trace-level identifications.

8. BZE Alone in Equine Urine

In closing, a finding of BZE alone in a urine sample should be treated with regulatory caution. This is because cocaine added directly to horse urine spontaneously hydrolyzes to yield BZE, but few, if any, of the other cocaine metabolites are found through the horse sample.[1] Our colleagues at Horseracing Forensic Laboratory (HFL)[14] point to the likelihood of some of the original cocaine remaining identifiable in the sample, and they also noted that the pH and storage conditions of the urine sample influence the spontaneous hydrolysis of cocaine into BZE. However, the point remains: a finding of BZE alone, in the absence of other identifiable phase 1 cocaine metabolites, may be caused by post-collection contamination, and a BZE identification by itself cannot rigorously exclude a post-collection source.

9. Currently in Place Limits, Cutoffs, or Thresholds for Cocaine/BZE

A Major North American Jurisdiction

These pharmacologically based cutoffs are in good agreement with regulatory cutoffs currently in place in a number of North American jurisdictions. At one time, a major North American jurisdiction included in their cocaine ELISA screens a 50 ng/ml BZE "calibrator." In this jurisdiction, a sample would need to yield an ELISA reading greater than that produced by 50 ng/ml of BZE to attract further regulatory attention, and this jurisdiction is not represented in the data of Figure 4.

Ohio

In 1999, the Ohio authorities introduced a cutoff for BZE of 150 ng/ml, the same as that in place in human drug testing. Figure 4 shows that no cocaine identifications were reported in Ohio from 2000 to 2005.

Louisiana

The State of Louisiana recognizes BZE as an environmental contaminant, and no disciplinary action is taken if the concentrations found are consistent with environmental contamination and could not have influenced the performance of the horse. In this regard, recent communications show that this threshold is set at 150 ng/ml of BZE, and Figure 4 shows that no cocaine identifications were reported in Louisiana from 2000 to 2005.

Illinois

In early 2005, the State of Illinois introduced a screening cutoff for BZE in post-race urine of 300 ng/ml and a confirmation cutoff of 150 ng/ml. Above

these concentrations, the finding is treated as a full cocaine/BZE identification; below these concentrations, the horsemen is notified, and a considerably less severe action taken. Figure 4 shows that there were 11 BZE positives in Illinois between 2000 and 2005, after which time the State of Illinois introduced its cutoff for BZE.

Florida

Several years ago, the Florida Horsemen's Benevolent and Protective Association and Florida Division of Pari-Mutuel Wagering agreed on an "in-house" unpublished threshold of 100 ng/ml of BZE in urine threshold. Identifications below this threshold result in a fine for the trainer, but no further consequences are incurred; Florida is not represented in the data of Figure 4.

Washington State

Since 2004, the Washington State rules of racing treat cocaine as an environmental contaminant, and ≤50 ng/ml of urinary BZE does not activate regulatory sanctions. Washington State is not represented in the data of Figure 4.

Oklahoma

At the time that this manuscript was submitted for publication, we were informed that Oklahoma was preparing to formally recognize BZE as a "trace common substance," an environmental contaminant at concentrations of ≤150 ng/ml. We also note that Oklahoma is not represented in the data of Figure 4.

10. The National Horsemen's Benevolent and Protective Association Proposed Policy on Drug Testing and Therapeutic Medication

As set forth above, low-concentration urinary identifications of BZE have occurred around the world; a number of jurisdictions now have formal published thresholds, regulatory limits, or in-house regulatory limits for cocaine. The National Horsemen's Benevolent and Protective Association "Proposed Policy for Drug Testing and Therapeutic Medication" was published in the January 2003 issue of the *Journal of Equine Veterinary Science,* and it proposed a cutoff of 150 ng/ml of BZE in urine.

11. Conclusion

A number of horse-racing jurisdictions have adopted urinary cutoffs for BZE of 50–150 ng/ml, broadly similar to those in place for human workplace drug testing. This communication presents the scientific basis for these cutoffs in equine forensic science and lists no less than seven North American jurisdictions with proposed or in place urinary cutoffs for BZE.

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References

1. Lehner AF, Hughes CG, Woods WE, et al. A liquid chromatographic-electrospray tandem MS/MS method for quantitation of equine cocaine Metabolites, in *Proceedings.* 13th International Conference of Racing Analysts and Veterinarians 2000;413–419.
2. *Sports Illustrated*, v70, n9, 2-27-89. Edited by C. Neff.
3. Kollias-Baker C. A review of possible environmental sources of drug positives, in *Proceedings.* 48th Annu Conv Am Assoc Equine Pract 2002;186–189.
4. Charles Wilson, The Associated Press, 12-8-2005. Cocaine in horse drug test raises questions. http://web.lexis-nexis.com/universe. Accessed on July 21, 2006.
5. Baselt RC, Chang JY, Yoshikawa DM. On the dermal absorption of cocaine. *Journal of Analytical Toxicology*. 1990; 14(6):383–384.
6. Sleeman R, Burton F, Carter J, et al. Drugs on money: in the war on illegal drugs it is important to distinguish "drug" money from "innocent" money. *Anal Chem* 2000;72: 397A–403A.
7. Oyler J, Darwin W, Cone E. Cocaine contamination of United States paper currency. *J Anal Toxicol* 1996;20:213–216.
8. Wingert W. Lowering cutoffs for initial and confirmation testing for cocaine and marijuana: large-scale study of effects on the rates of drug-positive results. *Clin Chem* 1997; 43:100–103.
9. Harkins JD, Mundy GD, Stanley S, et al. Determination of highest no effect dose (HNED) for local anaesthetic responses to procaine, cocaine, bupivacaine and benzocaine. *Equine Vet J* 1996;28:30–37.
10. Shults T, Combie J, Dougherty J, et al. Variable-interval responding in the horse: a sensitive method of quantitating effects of centrally acting drugs. *Am J Vet Res* 1982;43: 1143–1146.
11. McKeever KH, Hinchcliff KW, Gerken DF, et al. Effects of cocaine on incremental treadmill exercise in horses. *J Appl Physiol* 1993;75:2727–2733.
12. Sams RA. Review of possible sources of exposure of horses to natural products and environmental contaminants resulting in regulatory action, in *Proceedings.* 43rd Annu Conv Am Assoc Equine Pract 1997;220–223.
13. Queiroz-Neto A, Zamur G, Lacerda-Neto JC, et al. Determination of the highest no-effect dose (HNED) and of the elimination pattern for cocaine in horses. *J Appl Toxicol* 2002; 22:117–121.
14. Dumasia MC, Teale P, Williams RB, et al. Effect of pH, temperature and length of storage on cocaine degradation and benzoylecgonine formation in the horse, in *Proceedings.* 15th International Conference of Racing Analysts and Veterinarians 2004;87–92.