Detection of Morphine in Blood and Urine Samples from Horses Administered Poppy Seeds and Morphine Sulfate Orally

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Abstract

The objective of this study was to determine if the administration of poppy seeds to horses would result in detectable concentrations of morphine in urine and blood samples, as has been shown to occur in humans. In this study blood and urine samples were collected following administration of poppy seeds and morphine sulfate orally to four horses. Urine samples were subjected to enzyme-linked immunosorbent assay (ELISA) for the presence of morphine. All urine samples testing positive by ELISA, as well as plasma samples collected after administration of 10-g doses of poppy seeds, were analyzed by gas chromatography-mass spectrometry for the presence of morphine. Morphine was detectable in the plasma samples for at least 4 h after administration of 10 g of poppy seeds. Morphine was detectable in urine samples for up to 24 h after administration of 10 g, 5 g, and 1 g of poppy seeds and 426.7 pg of morphine as morphine sulfate. The results of this study indicate that horses that consume or are administered poppy seeds may have detectable concentrations of morphine in their urine and plasma for hours after administration.

Introduction

Opiates, such as morphine, are commonly used as analgesics and pre-anesthetic drugs in many species including humans. In horses, however, opiates can cause central nervous system stimulation and, therefore, are only occasionally used, and then generally in combination with sedatives or tranquilizers (1). Morphine, however, has been occasionally detected in urine samples collected from horses after racing. Typically, the concentrations of morphine found in these post-race urine samples are < 50 ng/mL, and in most cases morphine is not present in detectable concentrations in the corresponding plasma samples. The presence of any opiate at any concentration in post-race urine samples, however, is of concern to racing authorities because in the past these drugs have been administered to horses in illicit attempts to enhance their racing performance. Therefore, the presence of morphine in a post-race urine sample is a violation of the rules of racing, although evidence for environmental contamination may be considered as a mitigating factor.

The opiate morphine is derived from the opium poppy Papaver somniferum, a plant native to the Far and Middle East, but widely cultivated throughout the world. Although it is illegal to knowingly cultivate P. somniferum in the United States, it is not uncommon to find the plants in gardens throughout the U.S. In addition, seeds of the plant are commonly imported into North America for use in baking and production of poppy seed oil. The poppy plant and seeds contain variable amounts of naturally occurring opiates such as morphine, codeine, and thebaine. It has been well documented that the consumption of poppy seeds by human subjects, generally in the form of baked goods, can result in the excretion of detectable concentrations of morphine in urine (2-4). Therefore, the objective of this study was to determine whether the administration of poppy seeds to horses would result in detectable concentrations of morphine in the urine and plasma.

Materials and Methods

Reagents and solvents

β-Glucuronidase type L-II containing sulfatase activity from Patella vulgata (cat. no. G-8132) was purchased from Sigma Chemical (St. Louis, MO). Methanol (cat. no. 230-4) and dichloromethane (cat. no. AH300-4) were purchased from Burdick & Jackson (Muskegon, MI). N,O-bis-(Trimethylsilyl)trifluoroacetamide (BSTFA) (cat. no. 38828) was purchased from Pierce Chemical (Rockford, IL). All other reagents and chemi-
animal metabolites were reagent grade or better and used as obtained from commercial sources.

**Standard solutions**

Morphine sulfate standard solution containing 1.00 mg of morphine per milliliter (cat. no. M-9524) was purchased from Sigma Chemical and morphine-d₃ standard solution containing 100 mg of morphine-d₃ per milliliter (cat. no. M-003) was purchased from Cerilliant Corp. (Austin, TX). Working solutions containing morphine at a concentration of 10.0 ng/mL and morphine-d₃ at a concentration of 10.0 ng/mL were prepared by diluting the standard solutions with methanol.

Morphine calibrators for ELISA analysis were prepared at concentrations of 1, 5, 10, 100, and 1000 ng/mL from drug-free horse urine and the working solution of morphine. A positive control sample for ELISA analysis was prepared at a concentration of 10 ng/mL from drug-free horse urine and the working solution of morphine. A positive control sample for gas chromatographic-mass spectrometric (GC–MS) analysis were prepared at concentrations of 2.0, 5.0, 10.0, 20.0, 50.0, 150.0, and 300.0 ng/mL in drug-free horse urine and 1.0, 2.0, 5.0, 10.0, 15.0, and 20.0 ng/mL from drug-free horse plasma and the working solution of morphine. A certified reference material containing morphine-3-ß-glucuronide at a concentration of 437 ng/mL was obtained from the College of American Pathologists (Northfield, IL) and was diluted 1:10 (v/v) with water for use as a positive control sample for GC–MS analyses.

**Animal studies**

Four healthy Thoroughbred (n = 3) or Quarter Horse (n = 1) mares (aged 10 to 14 years and weighing 530 to 622 kg) were used in this study, which consisted of the administration of six different treatments as shown in Table I. A different treatment was administered each week to all of the horses for six weeks. In general, except where indicated, the horses were held off feed overnight and then fed the poppy seed dose in a small amount of grain. For treatment 6, the horses were given their normal hay ration 2 h before administration of the poppy seeds. All horses were kept in box stalls during the study, fed hay, and allowed free access to water.

Blood samples were collected from the jugular vein using vacuum containers and needles, and urine samples were collected via a Foley catheter placed in the bladder using a sterile technique. Blood and urine samples were collected before and at 1, 2, 4, 8, 12, and 24 h after each treatment. Plasma, separated from other blood components by centrifugation, and urine samples were frozen at -20°C until analyzed. The experimental protocol was approved by and followed the guidelines of The University of California Institutional Animal Care and Use Committee.

**Morphine content of poppy seeds**

Four batches of poppy seeds were obtained from commercial sources. One-gram aliquots of each batch of poppy seeds were mixed with 5 mL of 10% potassium hydroxide in methanol (containing morphine-d₃ at a concentration of 250 ng/mL) and heated at 65°C for 2 h. A 25–100-μL aliquot of each of the hydrolysates was then diluted to 5 mL with water, adjusted to pH 9.0–9.5, and extracted with dichloromethane/isopropanol (3:1, v/v). Calibrators containing 25, 50, 100, 200, 400, and 800 ng of morphine per milliliter were prepared in 10% potassium hydroxide in methanol (containing morphine-d₃ at a concentration of 250 ng/mL) and were processed concurrently with the poppy seeds. The organic extracts were then isolated from the aqueous phases and evaporated to dryness under N₂ at 65 ± 5°C. The residues were mixed with 20 mL of BSTFA and heated at 65 ± 5°C. The morphine content of the poppy seeds was determined by GC–MS in the electron-impact mode of ionization under selected ion monitoring conditions. Ions at m/z 429 and 432 were monitored throughout the run. The ion areas at the retention times of the morphine and morphine-d₃ derivatives were measured and the ion area ratios (A₄29/A₄32) were calculated. Area ratios were plotted against the corresponding morphine concentrations of the calibrators. The morphine content was then calculated from the slope and intercept of the non-weighted linear regression equation and the corresponding ion area ratios of the poppy seed extracts. Batches 1 through 4 contained 7.3, 69.3, 29.5, and 73.2 μg/g of morphine, respectively. Poppy seeds from batch 4 were administrated to the horses at the doses indicated (Table I).

**Animal specimens**

Horse urine samples were screened for opiates using the immunoassay procedure (Opiate ELISA, Elisa Technologies Corp., Inc., division of Neogen Corp., Lansing, MI) described. Urine samples determined to be positive by immunoassay, as well as plasma samples collected after administration of the 10-g doses of poppy seeds, were extracted and subjected to GC–MS analysis for identification and determination of total morphine concentration as will be described here.

**Immunoassay**

The Opiate ELISA tests were performed as described by the kit manufacturer. All assay steps were performed at room tem-

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Table I. Treatment Groups for Administration of Poppy Seeds and Morphine Sulfate

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Dose Administered</th>
<th>Fed versus Not-fed</th>
<th>Method of Administration</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>10 g of poppy seeds</td>
<td>Not-fed for 12 h</td>
<td>Via nasogastric tube</td>
</tr>
<tr>
<td>2</td>
<td>5 g of poppy seeds</td>
<td>Not-fed for 12 h</td>
<td>Via nasogastric tube</td>
</tr>
<tr>
<td>3</td>
<td>1 g of poppy seeds</td>
<td>Not-fed for 12 h</td>
<td>Via nasogastric tube</td>
</tr>
<tr>
<td>4</td>
<td>500 mg of morphine sulfate</td>
<td>Not-fed for 12 h</td>
<td>Via nasogastric tube</td>
</tr>
<tr>
<td>5</td>
<td>5 g of poppy seeds</td>
<td>Not-fed for 12 h</td>
<td>In approximately 100 g of grain</td>
</tr>
<tr>
<td>6</td>
<td>5 g of poppy seeds</td>
<td>Fed</td>
<td>Via nasogastric tube</td>
</tr>
</tbody>
</table>
per temperature. The assay was started by adding 20 μL of a calibrator, test, or control sample to a sample well, along with 100 μL of the morphine-HPA solution. After 60 min of incubation, the solutions were removed, and the wells were washed three times with wash solutions. Substrate solution (TMB Microwell Peroxidase Substrate, Kirkegaard and Perry, Gaithersburg, MD) was then added to all wells, and after 60 min of incubation the absorbances were read at 650 nm in a microtiter plate reader (EL310 Microplate Autoreader, Bio-Tek Inc, Winooski, VT).

Analytical procedure for determination of morphine and total morphine in plasma

Preparation of samples. Duplicate 1-mL aliquots of test samples collected after the 10-g poppy seed treatment, calibrators, and control samples were pipetted into 13 x 100-mm test tubes containing 50 ng of morphine-d₃ as internal standard. One complete set of test samples, calibrators, and control samples was treated with β-glucuronidase (as described) for determination of total morphine concentration. The other set was not treated with β-glucuronidase and was used to determine the free morphine concentration.

Hydrolysis of conjugates. A 1-mL aliquot of β-glucuronidase reagent (2500 units/mL in sodium acetate buffer) was pipetted into each tube of one of the sets of test samples, calibrators, and control samples. The contents of each tube were vortex mixed for 5–10 s, and the tubes were placed in an incubator at 70°C for 2 h. The tubes were then removed from the incubator and allowed to cool to room temperature.

Extraction. Two milliliters of 1M carbonate buffer (pH 9) was added to each tube in both sets of test samples, calibrators, and control samples, and the contents of each were vortex mixed for 5–20 s. Four milliliters of n-butyl chloride/dichloromethane (3:1; v/v) was added to each tube and the tubes were placed on an end-over-end mixer at 17 revolutions per min for 10 min. The tubes were then centrifuged and the aqueous phases were frozen by immersing the tubes in a dry ice/acetone bath. The organic phases were decanted into conical tubes and evaporated under nitrogen in a water bath at 40 ± 5°C.

Derivatization. The residue in each tube was derivatized with 50 μL of PFPA and 50 μL of acetonitrile at 70°C for 60 min. The excess reagent was evaporated under nitrogen in a water bath at 40 ± 5°C, and the residues were dissolved in 50 μL of toluene.

GC–MS analysis. A Hewlett-Packard model 5890A GC 5970B mass selective detector was used throughout. A DB-5MS capillary GC column (15 m x 0.25-mm i.d., 0.25-μm film thickness, J&W Scientific, Folsom, CA) was used. The injector temperature was 250°C and was operated in the splitless mode. Over 60 min of incubation, the solutions were removed, and the wells were washed three times with wash solutions. Substrate solution (TMB Microwell Peroxidase Substrate, Kirkegaard and Perry, Gaithersburg, MD) was then added to all wells, and after 60 min of incubation the absorbances were read at 650 nm in a microtiter plate reader (EL310 Microplate Autoreader, Bio-Tek Inc, Winooski, VT).

Quantitative determination of morphine in plasma. The peak-area ratios for morphine from each test sample, calibrator, and positive control sample were calculated by dividing the area of the ion at m/z 414 at the retention time of the morphine derivative by the area of the ion at m/z 417 at the retention time of the morphine-d₃ derivative. The resulting peak-area ratios versus concentrations of the corresponding calibrators were plotted, and the calibration line was determined by linear non-weighted regression. A correlation coefficient of at least 0.999 was deemed acceptable. Concentrations of morphine in test samples and control samples were determined from the slope and intercept of the corresponding regression equation. The limits of quantitation (LOQ) of the method for morphine and total morphine in plasma were 2 and 5 ng/mL, respectively.

Analytical procedure for determination of total morphine in urine

Preparation of samples. Five-milliliter aliquots of test samples, calibrators, and control samples were pipetted into 16 x 125-mm test tubes containing 250 ng of morphine-d₃ as internal standard. The samples were vortex-mixed to assure dissolution of the internal standard and adjusted to pH 5 (± 0.5) with 6N hydrochloric acid or dilute ammonium hydroxide solution.

Hydrolysis of conjugates. Two milliliters of β-glucuronidase reagent (2500 units/mL in sodium acetate buffer) was pipetted into each tube. The contents of each tube were vortex mixed for 5–10 s, and the tubes were placed in an incubator at 65°C for 3 h. The tubes were then removed from the incubator and allowed to cool to room temperature. Two milliliters of 0.1M potassium phosphate buffer (pH 6.0) was added to each tube and the contents of each were vortex-mixed for 5–20 s. The contents of each tube were adjusted to pH 6 (± 0.5), if necessary, with 1N hydrochloric acid or dilute ammonium hydroxide solution. Each tube was then centrifuged at 1500–2000 rpm for 5 min.

Solid-phase extraction. Solid-phase columns (Bond Elut Certify, 10-mL LRC, cat. no. 1211-3052, Varian, Inc. Sample Preparation Products, Harbor City, CA) were conditioned by successively eluting to waste 2 mL of methanol and 2 mL of water. The flow was stopped as soon as the solvent reached the top of the sorbent bed. The test samples, calibrators, and control samples were decanted into the corresponding column reservoir, and the flows were adjusted so that the solutions flowed through the columns in not less than 2 min. Each column was then rinsed successively with 2 mL of water, 1 mL of 0.1M acetate buffer (pH 4.0), and 2 mL of methanol. The sorbent beds were then dried under full vacuum for 30 s. The analytes were eluted with 2 mL of dichloromethane/isopropanol/ammonium hydroxide (78:20:2, v/v/v) and then evaporated under nitrogen in a water bath at 65 ± 5°C.

Derivatization. The residue in each tube was derivatized with 50 mL of BSTFA at 60°C for 15 min.

GC–MS analysis. A Hewlett-Packard model 5890A GC 5970B mass selective detector was used throughout. A DB-5MS capillary GC column (15 m x 0.25-mm i.d., 0.25-μm film thickness) was used. The injector temperature was 250°C and was...
operated in the splitless mode. The oven temperature was set at 150°C (held for 1.0 min after injection) and then increased at 10°C/min to 280°C (held for 1 min and returned to 150°C). The carrier gas was ultra high-purity helium (99.999%), and the initial flow rate was 1 mL/min. The sample injection volume was 1 μL.

The mass selective detector was operated under electron ionization (EI) conditions and mass spectra were acquired in the full-scan mode (50 to 550 amu at 1.3 scans/s) and in the selected ion monitoring (SIM) mode of operation. Selected ion monitoring data were used to determine total morphine concentration. The monitored ions were m/z 234, 236, and 429 for morphine and m/z 239 and 432 amu for morphine-d3. The dwell time for each ion was 20 ms.

Quantitative determination of total morphine in urine. The peak-area ratios for morphine from each test sample, calibrator, and positive control urine samples were calculated by dividing the area of the ion at m/z 429 at the retention time of the morphine derivative by the area of the ion at m/z 432 at the retention time of the morphine-d3 derivative. The resulting peak-area ratios versus the concentrations of the corresponding calibrators were plotted, and the calibration line was determined by linear non-weighted regression. A correlation coefficient of at least 0.999 was deemed acceptable. Concentrations of morphine in test samples and control samples were determined from the slope and intercept of the corresponding regression equation. The LOQ of the method for total morphine in urine was 1.0 ng/mL.

Identification criteria. The retention times of the ions at m/z 234 and 236 (qualifying ions) for the morphine derivative in the test sample were within 0.05 min of the retention times of the qualifying ions from the morphine derivative from the standard solution. The relative ion area ratios of the qualifying ions for morphine in the extract of the test samples were within ± 20% of the values of the same ions from the morphine standard. The chromatographic peak shape was approximately Gaussian with a narrow base, baseline separation from neighboring peaks, and little evidence of tailing.

Statistical analysis
Data are expressed as mean plus or minus standard error of the mean (SE). Repeated measures analysis of variance (ANOVA) was used to test for differences in urine morphine concentrations following administration of 5-g doses of poppy seeds. The 5-g poppy seed dose was administered via a nasogastric tube or orally, and the horses were either held off feed for 12 h before the administration or allowed free access to their normal hay ration. Differences were considered significant if the p value was less than 0.05.

Results

The results of GC–MS analysis indicated that a gram of the poppy seeds fed to the horses in this study contained 73.2 μg of morphine. Therefore, the total amounts of morphine administered to the horses in the form of poppy seeds were 732 μg/10 g, 366 μg/5 g, and 73.2 μg/1 g. The 500-μg dose of morphine sulfate administered to the horses contained 426.7 μg of morphine.

No behavioral changes were observed following administration of any of the treatments. The horses remained quiet throughout the study period, and no excessive locomotor activity was observed.

After administration of the 10-g dose of
poppy seeds, free morphine was not present in detectable concentrations in any of the plasma samples. However, after treatment of the plasma samples with β-glucuronidase to hydrolyze conjugates of morphine, morphine was detectable in samples collected through 4 h. Although the concentrations of morphine in these plasma samples were above the limit of detection (LOD = 1 ng/mL), they were less than the lower LOQ.

In contrast to the plasma samples, morphine was detectable by ELISA and identified by GC–MS analysis of urine samples collected from horses administered poppy seeds or morphine sulfate (Figure 1). Peak urine concentrations of total morphine following administration of 10-, 5-, and 1-g doses of poppy seeds ranged from 44.7 to 213 ng/mL, 40.3 to 119 ng/mL, and 2.7 to 27.6 ng/mL, respectively. When 426.7 μg of morphine was administered orally as morphine sulfate, peak total morphine concentrations ranged from 6.7 to 66.7 ng/mL. Peak concentrations of morphine in the urine occurred within 4 h of administration of all treatments.

When 10- or 5-g doses of poppy seeds or a 426.7-μg dose of morphine as morphine sulfate was administered, morphine was detectable by ELISA in all of the urine samples collected from the horses for 24 h. The urine concentrations of morphine 24 h after administration ranged from 4 to 15 ng/mL for the 10-g dose and from 4.4 to 14.0 ng/mL for the 5-g dose of poppy seeds. When the 426.7-μg dose of morphine was administered, urine concentrations ranged from above the LOD but below the LOQ, to 7.1 ng/mL 24 h after administration.

When a 1-g dose of poppy seeds was administered, morphine was detectable by ELISA (data not shown) and GC–MS (Figure 1) in the urine samples collected from 1 horse for 8 h and from 3 horses for 24 h after administration. Following administration of the 1-g dose of poppy seeds the concentrations of morphine in the urine samples 8 h after administration ranged from above the LOD but below the LOQ, to 8.6 ng/mL.

The concentrations of morphine in urine samples collected after 5-g doses of poppy seeds were administered did not differ significantly (p > 0.05) whether the horses were held off feed or were fed before administration or whether the poppy seeds were administered via a nasogastric tube or fed in a small amount of grain (Figure 2). Peak urine concentrations of morphine occurred within 4 h of administration, and morphine was detectable in urine samples collected 24 h after administration of all of the 5-g poppy seed treatments (Figure 2).

**Discussion**

The results of this study demonstrate that the administration of poppy seeds to horses can produce detectable plasma and urinary concentrations of morphine. For example, morphine was detectable in plasma samples collected from horses administered 10 g of poppy seeds after the plasma was subjected to enzyme hydrolysis, which allows for the detection of conjugated metabolites. Furthermore, morphine was detectable by the Opiate ELISA and GC–MS methods in urine samples collected for at least 24 h following the administration of either a 10- or 5-g dose of poppy seeds. Even when only a 1-g dose of poppy seeds was administered, morphine was detectable in urine samples from all of the horses for at least 8 h after administration. These results are particularly significant because many racing chemistry laboratories use ELISA methods to screen post-race urine samples for the presence of morphine and confirm any positive findings using GC–MS methodology similar to that employed in this study.

The morphine content of the different batches of poppy seeds analyzed for this study varied from 7.3 to 73.2 μg/g. The batch with the highest concentration of morphine was administered to the horses in this study in order to increase the probability that at least some of the samples would contain detectable concentrations of morphine. Obviously, if a horse consumed poppy seeds containing smaller amounts of morphine, the detection period and the peak concentration of morphine in the samples could be less than those determined in this study.

In the present study, morphine pharmacokinetic parameters were not determined because plasma concentrations of morphine were less than the LOQ. In previous studies in horses, however, the disposition and pharmacokinetics of serum morphine concentrations were described (7–9). For example, following administration of a 0.1-mg/kg intravenous (IV) dose of morphine, plasma morphine concentration-time data were best described by a three-compartment open model with a plasma t₁/₂ of 87.9 min (9). In that study, which used a GC–electron capture detector method with a LOD for morphine of 0.2 ng/mL, morphine could be detected in the plasma for 48 h after administration (8).

Intra-articular administration of morphine to ponies also resulted in detectable concentrations of morphine in the plasma, but these concentrations were much higher than those determined in the present study, presumably because the morphine doses were much higher (10). In addition, both free morphine and two metabolites, morphine-3-glucuronide and morphine-6-glucuronide, were detected in the plasma following intra-articular administration of morphine. For example, when 15 mg of morphine sulfate was administered into the tarsocrural joints of eight ponies, the peak plasma concentrations of free morphine occurred 30 min after injection, and the mean peak concentration was 7.1 ng/mL (10). The peak plasma concentrations of morphine-3-glucuronide, which was the major metabolite present in the plasma, occurred 2 h after administration and were greater than 10 ng/mL in all of the ponies.

Urine concentrations following intravenous administration of morphine have also been reported. For example, using a method with a limit of detection of 0.2 ng/mL, morphine was detectable in the urine for up to 144 h after administration of a 0.1-mg/kg dose of morphine intravenously (8). The peak urinary concentration of total morphine in that study occurred 2 h after administration, and the mean concentration was 21,894 ng/mL. By contrast, in the present study the peak urine concentration of morphine occurred 1 h after administration of a 10-g dose of poppy seeds that contained 732 μg of morphine, and the mean (+ SE) peak concentration of morphine was 108.1 ± 42 ng/mL. When a 1-g dose of poppy seeds, containing 73 μg of morphine, was administered, peak concentrations of morphine in the urine never exceeded 30 ng/mL. Thus, the peak concentrations of total morphine in the urine associated
with administration of poppy seeds were substantially less than those detected after IV administration of 0.1 mg/kg morphine. Nevertheless, after administration of a 1-g dose of poppy seeds, morphine was present in detectable concentrations in the urine from all of the horses for 8 h and in 3 out of 4 of the horses for 24 h.

The pharmacodynamics of morphine in the horse have also been reported (7,9–11). Morphine, like most opiates, produces CNS stimulation in horses that is manifested by increased locomotor activity (7). In one study, the excitatory response of morphine in horses was not observed at IV doses below 0.6 mg/kg (7). In a different study, however, morphine, given at an IV dose of 0.12 mg/kg, was associated with significant increases in heart rate, respiratory rate, cardiac output, and systemic blood pressure for up to 30 min after administration (11). In addition, in the latter study, subjective changes in behavior, described as the development of dysphoria followed by euphoria, were also noted for up to 15 min in some horses after IV administration of the 0.12 mg/kg dose.

In the present study, physiological parameters were not monitored after administration of the poppy seeds or the morphine sulfate. No behavioral changes, however, were noted in any of the horses after administration of any dose of poppy seeds or morphine sulfate. This is not surprising when one considers the doses of morphine administered and the route of administration. For example, the highest dose of poppy seeds administered contained 732 μg of morphine, which would correspond to a mean dose of approximately 0.0013 mg/kg in these horses. This dose was approximately 1% of the dose reported to produce changes in cardiovascular parameters for 30 min and subjective behavioral effects in horses for 15 min after administration (11). Furthermore, oral administration of morphine is probably associated with extensive first-pass metabolism as it is in other species.

The results of this study demonstrate that morphine was detected for up to 24 h in urine samples from horses administered poppy seeds, even though the total dose of morphine administered in each treatment was less than 1 mg. Therefore, these findings have important practical and regulatory implications. In various racing jurisdictions, the finding of any concentration of morphine in a urine sample collected from a horse in an athletic competition could result in severe penalties for the owner, trainer, and in some cases, rider of the horse. These findings are generally considered prima facie evidence of deliberate morphine administration to the horse. The results of this study provide evidence that contamination of the horse’s feed with poppy seeds could result in detectable concentrations of morphine in official test samples collected before or after competition. In addition, because P. somniferum is occasionally found in gardens, these plants are also a hypothetical, though unlikely, source of environmental morphine contamination to the horse. We are currently unaware of any validated methods to discriminate between morphine administered to horses as a pharmaceutical formulation and morphine ingested in the form of poppy seeds and plants. However, Casella et al. (12) have demonstrated that the presence of thebaine in urine can be used in humans to differentiate between morphine, heroin, and poppy seed consumption. Acetylmorphine and total codeine to total morphine ratios have also been proposed as indicators of heroin use in humans (12,13). In this study, however, the total concentrations of morphine in urine were low, making detection of minor metabolites and other potential biomarkers highly unlikely. In addition, the horse so rapidly and extensively metabolizes codeine by O-demethylation that little or no codeine can be detected after administration.

Although pharmacodynamic activity due to the morphine content of the poppy seeds administered in this study was not evident, the study was not designed to evaluate those responses. In addition, the scientific literature does not provide sufficient evidence to indicate whether the concentrations of morphine reported in this study are likely associated with pharmacodynamic responses in horses, although the doses administered were approximately 1% of the lowest dose reported to produce a detectable effect in the horse (11). Thus, regulatory authorities should be aware that environmental sources of morphine do exist in the U.S.; therefore, the finding of morphine in official test samples should not be taken as proof of morphine administration.

References


Manuscript received June 11, 2001; revision received August 10, 2001.