Received: 26 November 2021 | Revised: 6 December 2021 | Accepted: 8 December 2021

DOI: 10.1002/dta.3212

CASE REPORT

WILEY

Case report: Synephrine, a plant substance yielding classic environmental clusters of hay related identifications in equine urine

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Funding information
National Horsemen's Benevolent and Protective Association; Commonwealth of Kentucky; National Institute of Food and Agriculture (NIFA); USDA Agriculture Research Service, Grant/Award Number: 58-6401-2-0025; Hatch Program, Grant/Award Number: 1010609; U.S. Department of Agriculture; National Institute of Food and Agriculture; United States Trotting Association, Columbus, Ohio; Equine Health and Welfare Alliance, Inc, Versailles, Kentucky

1 | INTRODUCTION

p-Synephrine, specifically the L-enantiomer, *R*-(−)-*p*-Synephrine, 4-[1-hydroxy-2-(methylamino)ethyl] phenol, $C_9H_{13}NO_2$, 167.21 g/mol is a phenylethylamine protoalkaloid/trace amine (Figure 1) found in plants and animals.[1–3] In August 2019, there was a reported urinary "presence" of Synephrine in a South African horse show event in Polokwane, fully consistent with local environmental conditions in South Africa (SA), which require a urinary screening limit of detection (SLOD) for Synephrine in horse racing.[4–6] Reviewing this matter, we identified 20 or so reported identifications of Synephrine, administratively "adverse analytical findings," in horse racing and sport horse events.[7,8] Furthermore, some of these identifications occurred as classic time and place "clusters," including a sequence of 8 identifications over a 22-day period in March 2019 in Mexico (Table 1).[9,10]

We now present our current understanding of the chemical, biological, geographic, and regulatory realities underlying these Synephrine identifications. Based on this analysis, we suggest the interim South African urinary SLOD of 50 ng/ml, as an interim SLOD for Synephrine and note the need for transparency when regulating substances such as Synephrine, present in both plants and animals across our regionally variable planet. We also note that Synephrine is related to two other plant substances, hordenine[12] and cathinone,[13] (Figure 1) for which SLODs have previously been presented.[12–14]

2 | THE SOUTH AFRICAN CASE AND RELATED IDENTIFICATIONS WORLDWIDE

This case report begins with a Synephrine identification reported in a show horse competing at Polokwane, SA, in August, 2019.[15] The person responsible (PR) was a distinguished international competitor, and to our knowledge, neither the horse nor the PR had previous medication violations. The PR became aware of a first Synephrine "positive" in SA when she and the horse returned to SA shortly before the Polokwane event. Her horse was fed standard South African Eragrostis hay from a reputable feedstuff merchant and on August 29, 2019, competed in the Grand Prix class, which event the horse won and was Federation Equestre Internationale (FEI) tested. On October 1, 2019, the PR received a test notice from the FEI indicating

Abbreviations: ARCI, Association of Racing Commissioners International; FEI, Federation Equestre Internationale; NHRL, National Horse Racing Laboratory; PR, person responsible; SA, South Africa; SLOD, screening limit of detection.

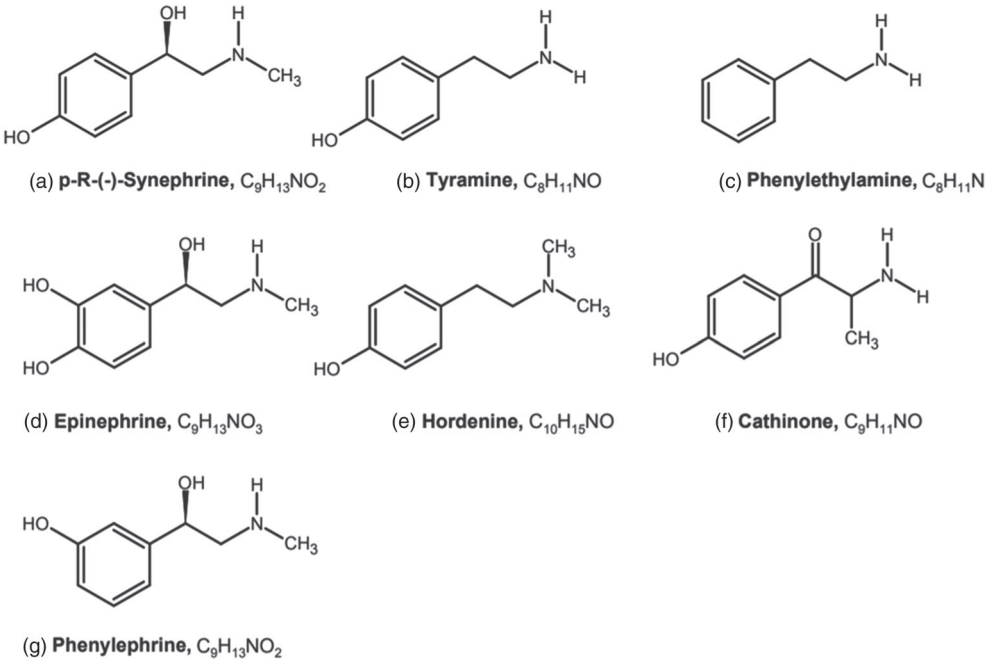

FIGURE 1 (a–g) Synephrine and related substances. (a) *R*-(−)-Synephrine, (b) tyramine, (c) phenylethylamine, (d) epinephrine, (e) hordenine, (f) cathinone, and (g) phenylephrine, a related synthetic pharmaceutical

TABLE 1 Worldwide Synephrine identifications, 2012 → -date

Date	Horse	Sport	Location	Source	Identification	Penalty	Reference
September 2019	Campbell	FEI show jumping	South Africa	Hay	Urine A sample only	2-month suspension	6
2019	8 horses	FEI show jumping	Mexico	Hay		Suspensions lifted	9, 10
February 7–10, 2019	Famorku	FEI show jumping	Spain	Hay	A and b samples	No fault	7
February 7–10, 2019	For Fun	FEI show jumping	Spain	Hay	A and b samples	No fault	8
May 3, 2018	Back in the game	Harness racing	New South Wales, Australia	Hay	A and b samples	Conviction but no penalty	11, 17
March 29, 2018	Future Stride	Harness racing	New South Wales, Australia	Hay	A and b samples	Conviction but no penalty	11, 17
March 2018	St Luke	TB racing	Australia	Paddock		Warning	18
February 27, 2018	Florist	Harness racing	New South Wales, Australia		A and b samples		19
February 28, 2016	My Hard Copy	Harness racing	New South Wales, Australia	Hay	Urine positive, blood negative	No penalty	20
June 21, 2012	Vancouver Gold	TB racing	Queensland, Australia		Positive swabs		21
January 11, 2012	Deep Cove	TB racing	Racing Victoria, Australia		Urine	Conviction but no penalty	22
January 15, 2012	Irish Golfer	TB racing	Racing Victoria, Australia				23
February 7, 2012	Zippy Zariz	TB racing	New South Wales, Australia		Urine		24
November 26, 2012	Willyclang	TB racing	New South Wales, Australia	Hay		No action, DQ	24

that the laboratory had "identified the presence of synephrine in the urine sample." Review of the Certificate of Analysis indicated that the corresponding blood sample was negative, so the "positive" call was based on urinary data only.

There was little question in the South African equine community as to the origins of this Synephrine identification. The PR had a broad sample of purchased/marketed South African feedstuffs analyzed (Table 2), and a preponderance of those identified as Eragrostis hay

TABLE 2 Synephrine content of Polokwane related South African hay samples

Sample	Matrix	Synephrine (μg/kg)
(1) August 2019 Eragrostis	Hay	141,500
(2) July 2019 Eragrostis	Hay	308,133
(3) June 2019 Lucerne Batch 1	Hay	1377.6
(4) July 2019 Lucerne Batch 2	Hay	<50.0
(5) June 2019 Eragrostis	Hay	5526.2
(6) July 2019 Lucerne Batch 3	Hay	<50.0

samples tested Synephrine "positive," one containing a not insignificant 300 mg/kg of Synephrine. South African Eragrostis hays are known to contain significant amounts of Synephrine, such that South African racing uses an SLOD to accommodate these higher than (world) average amounts of Synephrine in South African hays,[4] the SLOD being in the order of 50 ng/ml.

Review of available data shows the following concerning Synephrine identifications in horse urine. First, to our knowledge, Synephrine has never been reported detected in North American horseracing,[16] consistent with the fact that Synephrine is not listed in the Association of Racing Commissioners International (ARCI) Uniform Classification System for Foreign Substances. This absence of ARCI listing of Synephrine is likely related to the fact that Synephrine identifications in horseracing have been largely restricted to two Southern Hemisphere locations. An internet search for "Synephrine" (Table 1) brought up four reported Synephrine identifications in Australian Thoroughbred racing in 2012, in Victoria and New South Wales, with the last identification in November 2012 being traced to hay and no action taken against the trainer. More recently, a number of Australian Synephrine identifications have been reported in Harness racing in February, March, and May of 2018, with the last two identifications being attributed to environmental sources and no action taken against the trainers (Table 1).

Consistent with this Australian evaluation of the feedstuff origins of these Australian Synephrine identifications, a similar approach has been in place for some time in SA. Based on communications from Dr. Schalk de Kock, Director of the South African National Horse Racing Laboratory (NHRL), identifications of Synephrine in samples from South African racing are not unusual, including identifications at up to 50 ng/ml in urine and at considerably lower concentrations in plasma.[4] Based on these data, the South African NHRL apparently does not report Synephrine findings of <50 ng/ml or so in urine or their equivalent in plasma. In summary, Synephrine identifications in horse racing prior to 2019 have been largely restricted to two Southern Hemisphere geographic regions, SA and Australia, are recognized as being of feed-related origins, and are handled as such by the regulatory authorities.

We next reviewed a number of recent sport horse Synephrine identifications (Table 1), beginning with one of the more unusual grouping of defined clusters of adverse analytical findings in equine forensic science. These Synephrine cluster events occurred in Mexico between March 7 and 28, 2019, at three FEI regulated events. The first event was in San Miguel de Allende, Mexico, with three reported identifications of Synephrine in horses from this March 7–11 show. Next, March 11–14, two horses competing at an FEI event in Mexico City were reported "positive" for Synephrine, then, third, between March 28 and 30, five horses at an FEI monitored equine event in Balvanera, Mexico, were reported "positive" for Synephrine.[9,10] Overall, two classic three or more horse "clusters" of Synephrine identifications for a total of 10 horses test "positive" for the same naturally occurring substance within a 3-week period in a restricted geographic area.

The PRs were riders from Mexico, Uruguay, and Brazil. The provisional suspensions of the horses were all lifted as the FEI came to an agreement that the riders could not reasonably have known that the Teff Hay contained Synephrine. All horses had ingested Teff hay, both routinely and on the day of the drug tests, and the Teff hay had all been purchased from the same manufacturer. The producer of the Teff hay submitted the grass for analysis and showed the presence of Synephrine.[10]

We define a "cluster" as three or more horses trained by different individuals showing "positive" for unexplained trace amounts of the same substance within a restricted area and time, as we have outlined for Scopolamine.[11] The rationale is that it is unlikely that three or more trainers at the same location will independently and simultaneously decide to use trace amounts of the same substance on their horses. In this case, we have what appears to be eight or so horses in central Mexico in March 2019 whose trainers independently decided to use Synephrine in their horses, an unlikely explanation for these events.

To our knowledge, the samples taken from these horses were analyzed in the LGC Group Laboratory in England, and the A sample analytical reports presumably communicated to the FEI. This laboratory apparently reported no Synephrine identifications in 2018, compared with a total of 22 or so reported Synephrine identifications in 2019. The reason for the 2019 appearance of these Synephrine identifications is unclear but presumably relates to changes in the test sample origins and/or the testing procedures.

3 | DISCUSSION

Synephrine, specifically *R*-(−)-*p*-Synephrine (Figure 1a), is related to the naturally occurring monoamine alkaloids tyramine (Figure 1b) and phenylethylamine (Figure 1c), which, like Synephrine, function as neurotransmitters/neuromodulators in humans and as secondary metabolites in some plant species.[1,3] *p*-Synephrine is structurally related to the mammalian adrenergic agonist epinephrine (Figure 1d) and to the human pharmaceutical phenylephrine (Figure 1g). We also draw attention to the chemically related substance hordenine (Figure 1e),

identified as a naturally occurring plant substance reported in horse urine some 30 years ago,[12] and to cathinone (Figure 1f), another naturally occurring plant substance at times identified in horse urine.[13] To our knowledge, the human pharmaceutical most closely related to *p*-Synephrine is phenylephrine (Figure 1g), which came into medical use in 1938.

Synephrine exists as three structural isomers depending on the ortho, meta (phenylephrine, Figure 1g), or para positions of the phenolic hydroxyl group. Each of these three structural isomers exists as two enantiomers, for a total of six structural configurations of "Synephrine."[2,17] With regard to plant *p*-Synephrine, the most pharmacologically active enantiomer is the *R*-(−)-*p*-Synephrine or L-enantiomer and this is the predominant *p*-Synephrine enantiomer found in plants and also the major enantiomer of *p*-Synephrine recovered from human urine when the L-enantiomer is administered orally.[18] Evaluation of the chirality of the *p*-Synephrine found in an equine sample could therefore support the plant origins of an equine urinary *p*-Synephrine identification,[12] with a significant presence of the *S*-(+)-*p*-Synephrine or D-enantiomer being consistent with exposure to racemic, that is, pharmaceutical Synephrine, as Barker has shown with regard to aminorex.[19]

Synephrine occurs naturally in humans, being detected in both platelets and plasma, where it serves a neuromodulatory function,[3] an important consideration when evaluating the significance of Synephrine identifications in equine blood or urine samples. *p*-Synephrine, presumably chemically synthesized and racemic, is marketed in a number of European countries[14] but not in the United Kingdom and North America, and the pharmacokinetics of *p*-Synephrine suggest minimal potential for pharmacological responses following its oral administration to horses. In humans, the oral bioavailability of *p*-Synephrine has been described as "low," and the plasma half-life of *p*-Synephrine is brief, reported at about 2 h.[17,18] Based on these human data, the oral bioavailability and plasma half-life of *p*-Synephrine in horses are also likely to be "low," consistent with little likelihood of pharmacological responses following oral exposure of horses to dietary *p*-Synephrine, as is the case with orally administered hordenine[12]and isoxsuprine. [20]

Consistent with these interpretations, review of the scientific literature shows that the pharmacological responses of humans to orally administered Synephrine alone are minimal. In a study involving 75 healthy individuals taking 98 mg/day of Synephrine for 60 days, alone and in combination with hesperidin and naringin, no effect on heart rate was seen in the control group, a Synephrine dose equivalent to about 700 mg/day/horse.[21]

The above referenced analyses were performed on samples of commercial Eragrostis and Lucerne hay as supplied and fed to horses competing in the August 2019 Polokwane event, including the horse in question in this matter (Table 2). Samples are identified by month acquired and hay type. The analyses were performed by Food & Drug Assurance Laboratories (Pty) Ltd T/A FDA Laboratories, Pretoria, SA, an ISO-17025 accredited laboratory and reproduced with permission.

In plants, *R*-(−)-*p*-Synephrine is found at concentrations of up to 3 mg/kg in a citrus fruit and orange juice,[17,21] and Synephrine has long been used in Traditional Chinese Medicine. More importantly, recently acquired South African data on the concentrations of Synephrine in South African Teff hay show concentrations of up to 300 mg/kg or more, suggesting a potential for daily intake of Synephrine of several hundred milligrams per day of Synephrine for South African horses (Table 2). These levels of dietary exposure to Synephrine are consistent with the not infrequent identification of Synephrine in urine samples from South African racing and sport horses.[4]

A number of questions arise concerning the specific chemical identity of the substance giving rise these FEI reported Synephrine identifications. In the first place, the enantiomeric form of the Synephrine present in these samples has not been identified, even though identification of the *R*-(−)-*p*-Synephrine enantiomer would be fully consistent with plant origins of these identifications. Second, to our knowledge, no information has been made available concerning the concentrations of Synephrine identified in these urine samples, so it is not possible to relate these claimed Synephrine identifications to the currently in-place SLOD for Synephrine in South African racing.

An equally important consideration is the relationship, if any between, between the reported urinary "presence" and pharmacologically significant blood concentrations of Synephrine. This question arises because Synephrine is found in human urine as conjugates, consistent with the para position of the phenolic hydroxyl group on *p*-Synephrine, a well-known glucuronidation site[12] in equine drug metabolism. It is therefore likely that the bulk of the Synephrine present in these equine urine samples is actually glucuronidated Synephrine, which the testing laboratory released into the urine sample during a routine enzymatic hydrolysis step,[22–24] and following which hydrolysis step Synephrine itself was recovered from the enzymatically hydrolyzed sample. Simply put, the material(s) actually present ("presence") in the urine sample are most likely glucuronide conjugates of Synephrine,[18] as is the case with hordenine and isoxsuprine.[12,20,24] This is significant because conjugated substances are known to be excreted at readily detectable urinary concentrations as compared with the actual plasma concentrations of the parent substance in the animal in question. Plasma concentrations of Synephrine in these horses are most likely well below any pharmacologically significant concentration, because what is being reported "present" in the urine in these cases is most likely Synephrine enzymatically released from conjugated urinary metabolites of Synephrine.

A further consideration is the possible effect of urinary pH on urinary concentrations of Synephrine. Synephrine is a strongly basic substance and as such may readily concentrate in acidic urines. The classic contribution in this area is that of Gerken and colleagues, who showed that the basic medication lidocaine can concentrate up to 1000-fold in an acidic urine,[25] and there is no reason to believe that Synephrine will not equivalently trap in an acidic urine. The take home message is that urinary drug and drug metabolite concentrations have the potential to be highly variable, so concentrations of parent substance in serum/plasma are by far the most scientifically reliable indicators of potential pharmacologically effect. These factors are particularly relevant for a substance like Synephrine with the potential

to be present in urine as conjugated metabolites at readily detectable concentrations while being undetectable in plasma, as in the South African case in question.

These hay testing results and medication form concerns are fully consistent with and support the use of an SLOD in the order of 50 ng/ml in South African racing, based on the environmental substance approach implemented by the Ontario Racing Commission, namely, "to set limits high enough to cut-off the environmental noise and low enough to stop performance enhancement,"[26] which the in-place South African SLOD appears to have achieved.

Another important point of regulatory interest is the fact that no data are available concerning either the concentrations of Synephrine recovered from these equine urine samples or the limit of detection (LOD) of the presumably negative plasma testing data for the FEI cases. Such quantitative data are critically important because if sufficient data points become available, they make possible statistical evaluation of the environmental data. These data analyses then provide a scientific basis for any proposed SLODs, as shown by Machin et al.[27] in their outlier analysis and resultant proposed SLOD for naproxen in equine plasma samples.

With respect to the recently presented FEI Atypical Findings (ATFs) Policy communicated November 23, 2020,[28] we note that these Synephrine identifications meet all of the presented ATF policy criteria. These criteria include a requirement that there be identifications of the same prohibited substance arising from other samples taken at the relevant event(s), which criterion is met by the various Synephrine identifications reported in this communication. The second criterion is that there be ATFs arising from the same prohibited substance from other samples taken in previous events held at the same venue and/or in the same region, which criterion is also met. The third criterion is that samples taken from feed or bedding at the relevant event test positive for the substance in question, which criterion is also met. Finally, there is the matter of the concentration of the particular prohibited substance in the samples which, to the best of our knowledge of these reported synephrine events, are entirely characteristic of atypical findings.

Based on these criteria set forth by the FEI while this case report was being drafted, it is clear that the interim SLOD proposed in this communication is an appropriate interim SLOD for Synephrine, consistent with in-place regulatory practice in Southern Africa. We also respectfully draw attention to another important criterion not presented in the listed FEI or South African factors/criteria, namely, that the biologically expected *R*-(−)-*p*-Synephrine enantiomer found in plants should be/is the form identified in the urine samples particular if indeed the Synephrine identified is of Teff hay or related biological origin.

4 | CONCLUSIONS

In closing, review of the available data on this Polokwane Synephrine identification and the identifications listed in Table 1 makes clear that these identifications are most likely due to innocent and inadvertent exposure to local feedstuffs containing Synephrine. In SA and Australia, Synephrine is a recognized component of Teff hays and South African horse racing has an in-place SLOD to handle such identifications. It appears that the Southern Hemisphere regional nature of these Synephrine identifications has led to a lack of Northern Hemisphere awareness of Synephrine, both administratively and possibly also in analytical circles. Based on the data reviewed in this report, it is apparent that feedstuffs containing sufficient Synephrine to give rise to detectable concentrations of Synephrine, actually more likely conjugated Synephrine metabolites in equine urine, are present at times in Australian hays, apparently more frequently in South African hays, and also in hays presented to horses in Mexico and Spain. We also note that scientific evaluation of the specific enantiomeric form of Synephrine present in these samples would likely either support or deny plant origins for the Synephrine present in these identifications. Finally, based on the experience of our South African colleagues, we suggest that it is reasonable to use a 50 ng/ml of Synephrine recovered from equine urine as an interim urinary SLOD, pending development of a more scientifically rigorous and forensically satisfactory plasma/serum SLOD for Synephrine as *R*-(−)-*p*-Synephrine.

ACKNOWLEDGEMENTS

This research was made possible by research support from the Equine Health and Welfare Alliance, Inc, Versailles, Kentucky, and the United States Trotting Association, Columbus, Ohio. Further support came from the National Institute of Food and Agriculture, U.S. Department of Agriculture, Hatch Program under project KY014051 Accession Number 1010609, and also by grants from the USDA Agriculture Research Service Specific Cooperative Agreement #58-6401-2-0025 for Forage-Animal Production Research, the Kentucky Department of Agriculture, and the Kentucky Thoroughbred Association Foundation and by support for the Kentucky Agricultural Experiment Station as provided by the National Institute of Food and Agriculture (NIFA) and the Commonwealth of Kentucky. Other support includes research support from the National Horsemen's Benevolent and Protective Association and the Alabama, Arizona, Arkansas, Ontario, Canada; Charles Town, WV; Florida, Indiana, Iowa, Kentucky, Louisiana, Michigan, Minnesota, Nebraska, Ohio, Oklahoma, Oregon, Pennsylvania, Tampa Bay Downs, Florida, Texas, Washington State, and West Virginia Horsemen's Benevolent and Protective Associations. Published as paper #504 from T Tobin and the Equine Pharmacology, Therapeutics and Toxicology Program at the Maxwell H. Gluck Equine Research Center and Department of Veterinary Science, University of Kentucky.

The funding sources provided no role in the design of the study, nor in the collection, analysis, and interpretation of the data and writing of the manuscript.

CONFLICT OF INTEREST

GAM, CF, KB, and TT are veterinarians and equine forensic scientists who have testified in equine forensic science matters and related

areas. AMB, CF, and MNN are equine veterinarians who practice in the United States (CF) Europe (MNN) and South America and the Middle East (AMB) in equine sports-related areas.

AUTHOR CONTRIBUTIONS

JJM, KB, AMB, and MNN performed the primary regional regulatory and forensic literature searching and analysis and assembled the forensic data, with AMB and MNN focusing particularly on the European, Spanish, and South American veterinary and regulatory literature and experience. CF, Executive Director of the North American Association of Racetrack Veterinarians (NAARV), contributed to the writing and reviewed and approved the proposed interim SLOD for Synephrine and GAM, Director of the New York Drug Testing and Research Program, also contributed to the writing and reviewed and approved the proposed interim SLOD and its scientific basis from a regulatory scientist's point of view. TT coordinated, organized, and drafted the various drafts of this manuscript with ongoing contributions from all authors, and all authors reviewed and approved the final manuscript submitted for publication.

ETHICS STATEMENT

This research paper assembled, reviewed, and analyzed scientific, regulatory, and forensic data, and no animal experiments were performed.

DATA AVAILABILITY STATEMENT

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

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How to cite this article: Brewer K, Machin JJ, Maylin G, et al. Case report: Synephrine, a plant substance yielding classic environmental clusters of hay related identifications in equine urine. *Drug Test Anal*. 2022;1-7. doi:10.1002/dta.3212