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Petition for Rulemaking regarding No-effect Thresholds

Introduction

Pursuant to 5 U.S.C. § 553(e) and 16 C.F.R. § 1.9, the National Horsemen's Benevolent and Protective Association ("National HBPA") submits this Petition to request that the Federal Trade Commission ("FTC" or the "Commission") adopt a rule to create no-effect thresholds for certain substances found in racehorses under the anti-doping and medication control ("ADMC") program of the Horseracing Integrity and Safety Act of 2020 ("HISA" or the "Act").[1] A no-effect threshold, also known as a no-effect screening limit or no-effect cutoff, is a number below which no trainer or owner will be punished for innocent and pharmacologically irrelevant concentrations of foreign substances that have no effect on a horse.

[1] Codified at 15 U.S.C. §§ 3051–3060.

The purpose of the ADMC program is to protect racehorses from "the influence of medications, other foreign substances, and methods that affect their performance" and that "mask or deaden pain in order to allow injured or unsound horses to train or race."[2] But infinitesimally minimal levels of medications and other foreign substances that are detectable by modern laboratory testing do not enhance performance or mask pain. Therefore, they should not trigger a penalty in racehorses. The National HBPA petitions the FTC to adopt no-effect thresholds within the ADMC program to protect trainers and owners of horses with detectible but minimal and pharmacologically irrelevant concentrations of foreign substances from being wrongfully punished for "adverse" analytical findings over which they had no control and which did not affect the performance of the horse.[3]

In addition, because eighty-four percent of the substances on the HISA Authority's list of permitted substances do not contain allowable limits, which are required by law,[4] the FTC must issue allowable limits itself to bring the HISA Authority's list into compliance with the Act.

No Prejudice to Litigation

At the outset, please note that the National HBPA believes that HISA is an unconstitutional delegation of legislative and executive authority to a private non-

[2] 15 U.S.C. § 3055(b)(1-2).
[3] For a general discussion of the concept, see P.L. Toutain and V. Lassourd, "Pharmacokinetic/pharmacodynamic approach to assess irrelevant plasma or urine drug concentrations in postcompetition samples for drug control in the horse," *Equine Vet. J.* (2002) 34 (3) 242-249, attached as Exhibit 1.
[4] 15 U.S.C. § 3055(c)(1)(B).

governmental organization: the HISA Authority. For that reason, among others, the National HBPA and several state affiliates brought suit in federal court against the Commission and the HISA Authority seeking to declare HISA unconstitutional and enjoin its enforcement. On November 18, 2022, the United States Court of Appeals for the Fifth Circuit ruled in favor of the National HBPA and declared HISA to be unconstitutional.[5] This litigation is ongoing. The case is back before the Fifth Circuit, awaiting a decision after oral argument was heard on October 4, 2023.[6] The case returned to the court because shortly after the first Fifth Circuit ruling, Congress passed a one-sentence amendment to 15 U.S.C. § 3053(e). But the National HBPA believes the constitutionally significant flaws in HISA remain post-amendment. Specifically, among other reasons, § 3053(a) still requires that all rulemaking must begin with a proposal from the Authority—not the FTC. And § 3053(c) still requires that the FTC *must* approve all rules proposed by the Authority as long as they are consistent with HISA and the procedural rule promulgated by the FTC. Finally, under § 3053(c) the FTC cannot modify the rules during the promulgation process but can only "make recommendations to the Authority to modify the proposed rule or modification." The National HBPA acknowledges that Congress claims to have added new language in § 3053(e) allowing the FTC to modify Authority rules, but those rules can be modified only *after* promulgation: "The Commission ... may abrogate, add to, and modify the rules of the Authority

[5] See *National HBPA v. Black*, 53 F.4th 869 (5th Cir. 2022), available at https://www.ca5.uscourts.gov/opinions/pub/22/22-10387-CV0.pdf.
[6] Fifth Circuit case number 23-10520; audio available at https://www.ca5.uscourts.gov/OralArgRecordings/23/23-10520_10-4-2023.mp3

promulgated in accordance with this chapter ....” The ADMC regulation that the National HBPA is petitioning the FTC to modify is one such regulation that can be modified after the fact because it has already been promulgated.[7] Therefore, the National HBPA submits this Petition for Rulemaking without prejudice to its position in its litigation against the HISA Authority and the Commission.

Interest of the Petitioners

The National HBPA is the world’s largest Thoroughbred horsemen’s organization, representing approximately 30,000 horse owners and trainers throughout the United States and Canada through its 28 affiliates. A not-for-profit association founded in 1940 and headquartered in Lexington, Kentucky, the National HBPA has greatly expanded its responsibilities as the racing industry has become more complex. In addition to its original benevolent mission, the National HBPA is the leading force for horsemen on the national stage, advocating for the advancement of the sport through safety and integrity initiatives, promoting racing, and assisting in the development of aftercare programs for retired horses.

The National HBPA is “an interested person,” able to file a petition under 5 U.S.C. § 553(e) because it is both interested and a person. As an association, it meets the definition of a “person” found in 5 U.S.C. § 551(2). And it is interested

[7] HISA Anti-Doping and Medication Control Rule, 88 F.R. 5070-5201, Jan. 26, 2023, available at https://www.govinfo.gov/content/pkg/FR-2023-01-26/pdf/2023-00957.pdf, approved by the FTC in “Order Approving the Anti-Doping and Medication Control Rule Proposed by the Horseracing Integrity and Safety Authority,” March 27, 2023, available at https://www.ftc.gov/system/files/ftc_gov/pdf/P222100CommissionOrderAntiDopingMedication.pdf.

because its Thoroughbred horse owners and trainers are subject to the ADMC program regulations at issue[8] and requested to be modified by this Petition.

The Problem #1: Blameless Horsemen are Wrongfully Punished

The HISA Authority and its enforcement arm, the Horseracing Integrity & Welfare Unit ("HIWU") have caused a crisis in the horseracing industry. Innocent owners and trainers are being wrongfully maligned and punished as cheaters after HIWU finds a pharmacologically irrelevant, miniscule amount of a foreign substance in a horse that has no effect whatsoever on its performance or health. Numerous owners and trainers are losing their reputations for integrity, hundreds of thousands of dollars in lost earnings and legal fees, and in some instances their careers—all because the HISA Authority does not recognize no-effect thresholds for its laboratory testing. The FTC must intervene to stop this injustice.

Modern laboratory testing has reached a level of detection that is both highly impressive and also incomprehensible to the untrained mind. Some tests now report results in picograms per milliliter. A picogram is one trillionth of a gram. As with all scientific endeavors, the ethical scientist and regulator must ask not only, "Can we do this?" but also, "Should we do this?" If the result is to punish racehorse owners and trainers for picogram-level detections of substances without accounting for no-effect thresholds, the answer to the second question is "No."

A classic example of miniscule levels of prohibited substances having no effect on everyday life is the amount of cocaine found on dollar bills. A 1998 study

[8] *Id.*

cited by the National Institutes of Health found that cocaine was present on almost 80% of dollar bills tested from several different cities.[9] The study hypothesized that when a bill used to snort cocaine is run through a bank's money-counting machine, it cross-contaminates all the other bills in the machine because cocaine powder is extremely fine and is thrown into the air by these machines. But to prosecute everyone with a trace amount of cocaine in his wallet for cocaine possession would be a grave injustice. Therefore, the federal government, in instances like this, establishes no-effect thresholds.

No-effect thresholds are common in the human world of drug testing. For example, the Department of Transportation, in its drug testing of commercial airline pilots, allows a no-effect threshold of 150 nanograms (one billionth of a gram) of cocaine metabolite per milliliter of urine.[10] It allows 500 nanograms per milliliter of methamphetamine and 100 nanograms per milliliter of oxycodone. By contrast, HIWU publishes a zero-tolerance oxycodone policy. It places the drug on its banned list and does not publish a no-effect threshold for racehorses.[11]

Another federal department with a 100 nanogram per milliliter no-effect threshold for oxycodone is the Department of Health and Human Services. It

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[9] Oyler J, Darwin WD, Cone EJ. Cocaine contamination of United States paper currency. J Anal Toxicol. 1996 Jul-Aug;20(4):213-6. DOI: 10.1093/jat/20.4.213. Erratum in: J Anal Toxicol. 1998 Jul-Aug;22(4):15. PMID: 8835657; available at https://pubmed.ncbi.nlm.nih.gov/8835657/.

[10] 49 C.F.R. § 40.85.

[11] HISA Authority Banned Substances, last updated Mar. 1, 2023, available at https://hisadev.wpengine.com/wp-content/uploads/2023/03/HISA_BannedProhibitedList_Report_030223a.pdf (retrieved June 17, 2024), attached as Exhibit 2.

creates the Mandatory Guidelines for Federal Workplace Drug Testing Programs that govern 275,000 urine specimens tested each year by federal agencies.[12] Its guidelines mandate that laboratories engaged in drug testing for federal agencies must utilize its published list of no-effect thresholds, which it calls "cutoffs."[13]

The Environmental Protection Agency ("EPA") is another federal agency that recognizes no-effect thresholds. The EPA is well aware that many of the pharmaceuticals that HIWU is testing for are found in trace amounts in American drinking water. One count identified 24 major metropolitan areas that had detectable levels of pharmaceuticals.[14] According to the investigation, the drugs are ingested by humans, and some passes through them and is flushed down the toilet. Other drugs are dumped directly down the sink or toilet.[15] Then, the wastewater is treated before being sent to rivers and treated again before being sent as drinking water, but the process cannot fully remove all the pharmaceuticals.[16] Because the

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[12] 88 Fed. Reg. 70768.

[13] The list can be found at 88 Fed. Reg. 70781, available at https://www.govinfo.gov/content/pkg/FR-2023-10-12/pdf/2023-21734.pdf (retrieved June 29, 2024).

[14] Jeff Donn, Martha Mendoza, and Justin Pritchard, Associated Press, "Drugs found in drinking water," Sept. 12, 2008, available at http://www.h2oengineeringllc.com/doc_library/Drugs%20found%20in%20drinking%20water%20-%20USATODAY.pdf.

[15] Harvard Health Publishing, Harvard Medical School, "Drugs in the water," June 1, 2011, available at https://www.health.harvard.edu/newsletter_article/drugs-in-the-water#:~:text=In%20contrast%20to%20the%20uncertainty,female%2Dto%2Dmale%20ratios.

[16] For example, water treatment fails to completely remove phenytoin, atenolol, hydrochlorothiazide, sotalol, and carbamazepine epoxide. See Maria Huerta-Fontela, Maria Teresa Galceran, and Francesc Ventura, "Occurrence and removal of pharmaceuticals and hormones through drinking water treatment," *Water Research*, Volume 45, Issue 3, January 2011, Pages 1432-1442, DOI

EPA knows that a zero tolerance level is unattainable and because it knows that scant pharmaceuticals are harmless, it sets no-effect thresholds for the substances it measures.[17]

Notably, the EPA measures foreign substances in drinking water in micrograms per milliliter, or one millionth of a gram per milliliter.[18] That is *one million times larger* than the picogram per milliliter level for which some HIWU laboratories are testing. The EPA recognizes that reporting picogram levels of foreign substances in American drinking water would needlessly alarm the public of the presence of foreign substances that the EPA knows have no effect. Therefore, the EPA sets a no-effect threshold much higher than that tested by HIWU.

The federal government also does not test individuals for the presence of pharmaceuticals below the no-effect threshold at which they are found in drinking water for the obvious reason that such substances may be present as a result of environmental transfer from drinking tap water.

Environmental transfers account for a major reason why federal government agencies establish no-effect thresholds. Environmental transfers are inadvertent transactions that spread a foreign substance from one being or object to another. In the example of cocaine on dollar bills, the bank counting machine is responsible for

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10.1016/j.watres.2010.10.036, available at https://www.sciencedirect.com/science/article/abs/pii/S0043135410007451 (retrieved June 26, 2024).

[17] Environmental Protection Agency, "National Primary Drinking Water Regulations," available at https://www.epa.gov/ground-water-and-drinking-water/national-primary-drinking-water-regulations#two (retrieved June 24, 2024).

[18] That is the milliliter equivalent of the milligrams per liter that it uses.

the environmental transfer from one bill to another. Because environmental transfers often occur in minimal amounts and without the knowledge of the recipient, it would be unfair to hold the recipient responsible for the presence of the foreign substance. For example, illicit drugs are also found in trace amounts in drinking water, and we do not prosecute humans for ingesting such tiny amounts.[19] And in horses, the problem of responsibility is compounded by the fact that the person being held responsible for the presence of the substance is not the one doing the ingesting. At times, a horse is exposed to a substance against its will—as for example when a groom accidentally touches a horse's mouth without having washed his hands properly. Or more commonly, it is the horse who is doing the ingesting all on its own—with no control from its owner or trainer. For example, when a groom ingests methamphetamine and urinates in a racing stall, trace elements of the drug are present. When the next horse shows up for the race and licks the residue, it may be exposing its owner and trainer to ruin. Unbelievably, because of HIWU, this is happening.

For example, John Pimental had a clean record for nearly a half-century as a respected trainer before HIWU upended his life, tarnishing his reputation and ruining his finances for alleged infractions that are not performance enhancing. One of his horses tested positive for 193 trillionths of a gram per milliliter of

[19] Muñiz-Bustamante L., Caballero-Casero N., Rubio S., "Drugs of abuse in tap water from eight European countries: Determination by use of supramolecular solvents and tentative evaluation of risks to human health." Environ Int. 2022 Jun;164:107281. DOI: 10.1016/j.envint.2022.107281. Epub 2022 May 6. PMID: 35561596, available at https://pubmed.ncbi.nlm.nih.gov/35561596/.

methamphetamine, a street drug which unfortunately is not unknown among some in the backstretch population, who may come in contact with racehorses, which can then inadvertently test positive for the "adverse" analytical finding. According to an in-depth investigation by Thoroughbred Daily News,[20] ex-FBI HIWU agents descended upon Pimental's stable, searching everywhere and pouncing on an old container of thyroid medication for which he produced a prescription for his pony. Treating him as a common criminal, the heavy-handed agents threatened him with exorbitant penalties and then said they would cut him a break if he became a whistleblower. The charges have cost him untold thousands of dollars to defend. Ultimately, he was suspended 15 months and fined $10,000 for not knowing that he should have thrown away his pony's thyroid medication under HIWU's new rules, and his methamphetamine case is ongoing.[21] But the harm to his reputation will never be undone.

In a similar case, a 72-year-old trainer with five decades of licensure, Mike Lauer, spent almost $50,000 and five months trying to clear his name and reputation from HIWU allegations that cost him even more in lost clients.[22]

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[20] T.D. Thornton, "HISU Descends Upon a Shedrow, Upending Life for a Mom-and-Pop Stable," *Thoroughbred Daily News*, Oct. 29, 2023, available at: https://www.thoroughbreddailynews.com/hiwu-descends-upon-a-shedrow-upending-life-for-a-mom-and-pop-stable/.

[21] "John Pimental Gets 15-Month Suspension, $10,000 Fine," *Thoroughbred Daily News*, Feb. 6, 2024, available at https://www.thoroughbreddailynews.com/john-pimental-gets-15-month-suspension-10000-fine/.

[22] T.D. Thornton, "A Diabetes Drug's Outsized, Contested Role in Horse Racing's Anti-Doping Crusade," *Thoroughbred Daily News*, Jan. 30, 2024, available at https://www.thoroughbreddailynews.com/a-diabetes-drugs-outsized-contested-role-in-horse-racings-anti-doping-crusade/.

Ultimately, HIWU concluded that a groom who had ingested his prescription diabetes medication Metformin at lunch then unintentionally contaminated Lauer's horse by touching its mouth while fitting the gelding with a bit and bridle. Yet HIWU still suspended Lauer for 75 days and fined him $2,600.

These horror stories are pushing other trainers, like Rusty Arnold, to simply admit to violations they did not commit just to get HIWU off their back. Arnold recently accepted a 7-day suspension, a $1,000 fine, and a lost $40,000 purse for his horse's owner for a scant finding of 3 parts per billion per milliliter urinary detection of a metabolite of Tramadol, which has no effect in horses at a level below 50 parts per billion.[23] Arnold believes his horse was contaminated outside any area that he or his employees controlled. In a recent statement proclaiming his innocence, he asserted, "HIWU undoubtedly knows that many of these positives are due to environmental contamination."[24] He explained, "Horses are grazing animals. They eat dirt. They love to lick smelly wet spots in stalls. They eat manure. They lick the walls of ship-in stalls. It is unreasonable to think we can control this." Yet the HISA Authority is wrongfully holding owners and trainers responsible: "the system in place is unfairly hurting livelihoods and reputations while doing nothing to make our horses and industry safer." Arnold laid the blame on overzealous

[23] "Arnold Receives Seven-Day Suspension for Tramadol Positive; Raises Contamination Questions," *Thoroughbred Daily News*, Mar. 7, 2024, Available at https://www.thoroughbreddailynews.com/arnold-receives-seven-day-suspension-for-tramadol-positive-raises-contamination-questions/.

[24] "Arnold: Horsemen must speak up — loudly — about testing policies that defy realities of our environment," National HBPA website, Mar. 7, 2024, available at https://nationalhbpa.com/arnold-horsemen-must-speak-up-loudly-about-testing-policies-that-defy-realities-of-our-environment/, attached as Exhibit 3.

enforcement of scant laboratory findings: "HIWU's system of gotcha chemistry is not helping but instead harming racing." Arnold concluded, "HISA has become a dictatorship. They are the judge, jury and executioner. Whether with open eyes or out of ignorance, they have let their enforcement arm throw common sense and fairness out the window." As a solution, Arnold recommended the adoption of a "no-effect threshold level … that has no impact on performance."

Arnold's punishment and statement has caused a furor in the industry. Arnold is universally renowned for being a talented trainer who follows the rules. In response to his rallying cry to "speak up together to regulators – loudly," horsemen everywhere are beginning to tell their horror stories of run-ins with HIWU and to demand no-effect thresholds. Over 750 horsemen, and counting, have signed a National HBPA initiated petition to the four congressional sponsors of HISA, deriding HIWU's gotcha chemistry, bemoaning Arnold's unfair punishment, and demanding no-effect thresholds.[25]

The outcry against punishment for microscopic levels of Metformin has even led to a change in policy by HIWU. But the change is not enough. In addition to Mike Lauer (above), George Weaver,[26] Jonathan Wong,[27] and four other trainers

[25] "Horsemen for common-sense testing; racing must end 'gotcha' chemistry," Petition to Senator Mitch McConnell, Representative Andy Barr, Representative Paul Tonko, Senator Kirsten Gillibrand, available at https://mstr.app/b6ec128d-9d3a-458f-8864-e19b4966cb19, attached as Exhibit 4.

[26] Bill Finley, "Weaver Trained Horse Test positive for Metformin," *Thoroughbred Daily News*, Apr. 11, 2024, available at https://www.thoroughbreddailynews.com/weaver-trained-horse-tests-positive-for-metformin/.

have had horses test positive for Metformin. Metformin is particularly susceptible to environmental transfers because it is the third most prescribed medication to humans in America.[28] As Weaver's attorney, Drew Mollica said, "The groom who cares for the horse is on the medication. This is a clear case of contamination. The consequences that HIWU seeks to impose are career killers with no basis in reality." In response, the HISA Authority issued its more than 20th rule change by press release,[29] declaring that all active provisional suspensions for Metformin positive tests will be deferred until completion of a review of all scientific information regarding Metformin detections.[30] The National HBPA wishes that a thorough review of the scientific literature on Metformin had been conducted prior to setting an unrealistic, seemingly limit-of-detection threshold for the drug—either by the HISA Authority or by the FTC, which is supposed to be overseeing the HISA Authority. The problem with the belated rule suspension is that earlier trainers' reputations have already been tarnished. Rather than wait for the HISA Authority to issue a no-effect threshold for Metformin that it should have issued last year, the

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[27] Sue Finley and Stefanie Grimm, "Wong Suspended for Metformin; 'Our Game Has Been Hijacked' Says Attorney," *Thoroughbred Daily News*, July 2, 2023, available at https://www.thoroughbreddailynews.com/wong-suspended-for-metformin-our-game-has-been-hijacked-says-attorney/.
[28] "The Top 50 Drugs Prescribed in the United States," *Healthgrades*, last medical review Sept. 22, 2022, available at https://www.healthgrades.com/right-care/patient-advocate/the-top-50-drugs-prescribed-in-the-united-states (retrieved June 17, 2024).
[29] "NHBPA Motion to Supplement the Record," June 6, 2024, Case No. 23-10520, Doc. 191 at p. 5, attached as Exhibit 5.
[30] "HISA Announcement Regarding Metformin," *HISA*, June 4, 2024, at p. 1, attached as Exhibit 6, also available at https://hisaus.org/news/hisa-announcement-regarding-metformin.

FTC should review the scientific research below and issue the no-effect threshold now. And it should do the same for other foreign substances.

While the HISA Authority has proposed other rule changes to its ADMC program as recently as May, the modifications do not go far enough for at least three reasons. First, the proposed changes leave too much discretion to HIWU, which will lead to subjective decision-making and unequal treatment of similarly situated individuals. For example, the proposed changes acknowledge that the presence in a racehorse of cocaine, methamphetamine, and oxycodone may be the result of "unintentional transfer" from a human abusing those drugs, but the decision of whether this occurred is left to the "discretion" of HIWU after "any investigation it deems fit."[31] Second, the no-effect thresholds that the HISA Authority proposes are significantly lower than those utilized by other scientists, and it is not clear on what scientific studies, if any, they are based. For example, the HISA Authority proposed a no-effect threshold for Metformin that is ten times lower than one proposed by published scientific experts.[32] Third, the proposed rule changes still do not set no-effect thresholds for well over 90% of the foreign substances tested by HIWU.[33] The HISA Authority's failure to establish no-effect thresholds for the vast majority of the foreign substances for which it tests leaves

[31] "Proposed Modifications to the ADMC Rules (Updated May 14, 2024)," *HISA*, at p. 50, available at https://bphisaweb.wpengine.com/wp-content/uploads/2024/05/Proposed-Modifications-to-the-ADMC-Rules-Updated-May-14-2024.pdf.
[32] Compare 0.5 nanogram per milliliter, *id*. at p. 200, with 5 nanograms per milliliter, *infra* at n. 38.
[33] *Id*. at Appendix 1 to Rule Series 4000, pp. 106-262.

its proposed list of banned and controlled substances deficient and doomed to perpetuate unjust outcomes.

The Problem #2: The HISA Authority is violating the Act.

HISA requires the HISA Authority, in developing its ADMC program rules, to set "allowable limits of permitted medications, substances, and methods."[34] But the HISA Authority has not done so. Its published list of permitted medications, substances, and methods contains allowable limits for only 45 of the 274 substances listed.[35] The list is woefully insufficient. Eighty-four percent of the listed substances contain no allowable limits whatsoever. Therefore, the HISA Authority's list of permitted substances is in violation of the law. The FTC must modify the HISA Authority's list to bring it into compliance with the Act.

The Solution: No-effect Thresholds

A. The solution to the problem plaguing the horseracing industry of false positive tests for foreign substances, false accusations of wrongdoing, and false punishments doled out to innocent horsemen is the adoption of no-effect thresholds. A no-effect threshold is a laboratory testing detection level below which a medication or substance has no effect on the performance or health of the racehorse.

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[34] 15 U.S.C. § 3055(c)(1)(B).

[35] HISA Authority Controlled Medications, last updated Mar. 1, 2023, available at https://hisadev.wpengine.com/wp-content/uploads/2023/03/HISA_ControlledProhibitedList_Report_3.02.23.pdf (retrieved June 17, 2024), attached as Exhibit 7.

The National HBPA petitions the FTC to adopt no-effect thresholds for *all substances* tested by HIWU.

B. In particular, the National HBPA petitions the FTC to issue a rule *as soon as possible* to create the following no-effect thresholds, which are all supported by peer-reviewed, scientific research.

1) 20-Hydroxecdysone

No-effect threshold: 2 nanograms / milliliter in urine

Supporting scientific study: Kimberly Brewer, Clara Fenger, Abelardo Morales-Briceño, Andreas F. Lehner, George A. Maylin, and Thomas Tobin, "20-Hydroxyecdysone identification in performance horses – case reports and review," *Pferdeheilkunde – Equine Medicine* 40 (2024) 1 (January February) 10-17, DOI 10.21836/PEM20240102.[36]

2) Fentanyl

No-effect threshold: 50 picograms / milliliter in plasma/serum

Supporting scientific study: Kimberly Brewer, Clara Fenger, Abelardo Morales-Briceño, Andreas Lehner, and Thomas Tobin, "Trace level identifications of fentanyl and eutylone in equine plasma, pharmacological significance and probable origins – a case report and analysis," *Pferdeheilkunde – Equine Medicine* 39 (2023) 6 (November/December) 550-556, DOI 10.21836/PEM20230606.[37]

3) Metformin

[36] Attached as Exhibit 8.
[37] Attached as Exhibit 9.

No-effect threshold: 5 nanograms / milliliter in plasma/serum

Supporting scientific study: Kimberly Brewer, Clara Fenger, Abelardo Morales-Briceño, Andreas F. Lehner, George A. Maylin, Robert Holland, and Thomas Tobin, "Metformin as an environmental substance transferring to horses – a case report and analysis," *Pferdeheilkunde – Equine Medicine* 40 (2024) 2 (March/April) 1-7, DOI 10.21836/PEMBrewer_85.[38]

4) Methamphetamine

No-effect threshold: 850 picograms / milliliter in plasma/serum

Supporting scientific study: Kimberly Brewer, Abelardo Morales-Briceño, Robert Holland, George Maylin, Clara Fenger, Andreas F. Lehner, and Thomas Tobin, "Trace-level detections of methamphetamine in racing horses: a review and forensic analysis," *Pferdeheilkunde – Equine Medicine* 40 (2024) 5 (September/October) 428-439, DOI 10.21836/PEM20240501.[39]

5) Xylazine

No-effect threshold: 200 picograms / milliliter in plasma

Supporting scientific study: L.E. Macomber, Glenys Noble, Kimberly Brewer, Abelardo Morales-Briceño, Mary A. O'Connell, Rodney Eisenberg, Clara Fenger, Andreas F. Lehner, and Thomas Tobin, "Regulatory thresholds for xylazine – review and analysis based on recent pharmacokinetic data,"

[38] Attached as Exhibit 10.
[39] Attached as Exhibit 11.

Pferdeheilkunde – Equine Medicine 40 (2024) 2 (March/April) 1-6, DOI 10.21836/PEMTobin.[40]

C. In addition, the National HBPA also petitions the FTC to issue a rule to create *scientifically based*, no-effect thresholds *as soon as possible* for the following substances, which are causing imminent harm to the horseracing industry.

1) Ethyl Glucuronide

In an unpublished, confidential letter dated May 2, 2024, HIWU General Counsel Michelle Pujals asserted, "Based upon the results of the administration study and other available information, HISA's ADMC Committee has determined that there will be a Threshold of 10 ng/mL for Ethyl Glucuronide under the ADMC Program. As of May 1, 2024, findings over this Threshold will be reported as Atypical Findings because Ethyl Glucuronide is an endogenous substance."[41] The FTC should demand a copy of this "administration study," evaluate it, and conduct its own study to determine the correct no-effect threshold for Ethyl Glucuronide. An endogenous substance is one that is naturally produced in a horse's body. Therefore, to set a scientifically sound, no-effect threshold, the FTC should

[40] Attached as Exhibit 12.

[41] Under the HIWU Atypical Findings Policy, an owner or trainer is guilty until proven innocent by presenting "any possible explanation for the presence of the substance in the Covered Horse's system. If the Atypical concerns an endogenous Prohibited Substance, HIWU will request that the trainer provides [sic] relevant veterinary information for the Covered Horse." Available at https://assets.ctfassets.net/6mwruzwftvzd/5GhiPGzAM0gOrHeXJYrYRE/164b8669c718d546cd9ca5f88ce732e9/HIWU_atypical_findings_R1.pdf (retrieved June 24, 2024).

test a number of Thoroughbred racehorses for endogenous Ethyl Glucuronide and perform a statistical analysis of the results, taking into account seasonal variations, regional variations, and individual equine behavior like dunking feed into water before consumption. Also, the FTC should rely on peer-reviewed, scientific studies like that in the following footnote, which found the lowest limits of detection for Ethyl Glucuronide to be 100 nanograms / milliliter and the lowest limits of quantitation and confirmation to be 500 nanograms / milliliter.[42]

2) Pemoline

Pemoline is a central nervous system stimulant that was originally thought to occur in horses as a simple oxidation product of aminorex, which is an equine metabolite of levamisole,[43] which is an antiparasitic drug approved for use in veterinary medicine in the United States.[44] Now, Pemoline is also considered to be of natural and presumably botanical origins[45] because it is

[42] Youwen You, Cornelius E. Uboh, Lawrence R. Soma, Fuyu Guan, Xiaoqing Li, Jeffrey A. Rudy, and Jinwen Chen, "Biomarkers of alcohol abuse in racehorses by liquid chromatography/tandem mass spectrometry," *Rapid Commun. Mass Spectrom.* 2007; 21: 3785-3794 at 3790, DOI: 10.1002/rcm.3282, attached as Exhibit 13.

[43] J. Gutierrez, R.L. Eisenberg, N.J. Koval, E.R. Armstong, J. Tharappel, C.G. Hughes, & T. Tobin, "Pemoline and tetramisole 'positives' in English racehorses following levamisole administration," Irish Veterinary Journal 63, 498 (2010), attached as Exhibit 14.

[44] "Levamisole," Drug Enforcement Administration, February 2024, available at https://www.deadiversion.usdoj.gov/drug_chem_info/levamisole.pdf.

[45] By comparison, aminorex has been shown to appear in racehorses when they have naturally fed on plants from the Brassicaceae family. See George Maylin, Clara Fenger, Jacob Machin, Sucheta Kudrimoti, Rodney Eisenberg, Jonathan Green, and Thomas Tobin, "Aminorex identified in horse urine following consumption of

routinely detected without the administration of levamisole at low concentrations in urine samples in France, Germany, and South Africa.[46] Dr. Yves Bonnaire, former Director of the French Laboratoire des Courses Hippiques, reported that it naturally occurred in concentrations up to 5 nanograms / milliliter in urine samples, and Dr. Terence Wong of the Hong Kong Jockey Club Laboratory reported a finding from a French horse at an estimated concentration of 2.5 nanograms / milliliter in urine.[47] Therefore, the draft study on Pemoline recommends a conservative no-effect threshold of 2 nanograms / milliliter in blood plasma serum.[48]

3) Tramadol

Tramadol is a pain relief medication and schedule IV controlled substance.[49] Morphine is a much more potent and pharmacologically active opiate than Tramadol, so the no-effect threshold for Tramadol should be at least as high as that of morphine, which runs between 50 and 120 nanograms / milliliter in urine.[50]

Barbarea vulgaris; a preliminary report," Irish Veterinary Journal 72:15 (2019), attached as Exhibit 15.

[46] Kimberly Brewer, Abelardo Morales-Briceño, Robert Holland, George Maylin, Clara Fenger, Andreas F. Lehner, and Thomas Tobin, "Pemoline, A Central Nervous System Stimulant Occurring Naturally in Equine Samples in Europe and Elsewhere: A Review and Analysis," at p. 5, draft attached as Exhibit 16.

[47] *Id.*

[48] *Id.* at p. 11.

[49] Manraj Dhesi, Kenia A. Maldonado, Preeti Patel, Christopher V. Maani, "Tramadol," available at https://pubmed.ncbi.nlm.nih.gov/30725745/ (retrieved June 25, 2024).

[50] Fernanda Camargo, A.F. Lehner, W. Karpiesiuk, Kent Stirling, Pierce V. Kavanagh, Noel Brennan, Mark Dowling, and Thomas Tobin, "Review of

Proposed Rule Text

Petitioner proposes that the FTC issue a rule substantially similar to the following:

Rule 4212 shall be amended by adding the following as a newly designated subsection:

(f) Allowable limits, or no-effect thresholds, shall be established for all medications, substances, and methods listed in Appendix 1 to Rule Series 4000 in the column titled, "Screening limit" or, as proposed, "Screening Limit / Threshold / Minimum Reporting Level (MRL)." A no-effect threshold, also known as a no-effect screening limit or no-effect cutoff, is a laboratory testing detection level below which no owner or trainer will be punished for innocent and pharmacologically irrelevant concentrations of foreign substances that have no effect on a horse. The no-effect thresholds shall be based on peer-reviewed, scientific research.

(1) The no-effect threshold for 20-Hydroxecdysone shall be 2 nanograms / milliliter in urine.

(2) The no-effect threshold for Fentanyl shall be 50 picograms / milliliter in plasma/serum.

(3) The no-effect threshold for Metformin shall be 5 nanograms / milliliter in plasma/serum.

(4) The no-effect threshold for Methamphetamine shall be 850 picograms / milliliter in plasma/serum.

Environmental Morphine Identifications: Worldwide Occurrences and Responses of Authorities," (2005) Maxwell H. Gluck Equine Research Center Faculty Publications, 90, attached as Exhibit 17.

(5) The no-effect threshold for Xylazine shall be 200 picograms / milliliter in plasma.

(6) The no-effect threshold for Ethyl Glucuronide shall be based on a statistical analysis of the endogenous Ethyl Glucuronide present in a given population of Thoroughbred racehorses examined, taking into account seasonal variations, regional variations, and individual equine behavior like dunking feed into water before consumption. The threshold shall not be based on an administration study.

(7) The no-effect threshold for Pemoline shall be 2 nanograms / milliliter in blood plasma serum.

(8) The no-effect threshold for Tramadol shall be 50 nanograms / milliliter in urine.

Conclusion

For the foregoing reasons, Petitioner National HBPA respectfully requests that the Commission initiate rulemaking to establish no-effect thresholds for 20-Hydroxecdysone, Fentanyl, Metformin, Methamphetamine, Xylazine, Ethyl Glucuronide, Pemoline, Tramadol, and all other medications or substances being tested by HIWU. Setting no-effect thresholds will protect honest horsemen from false allegations of cheating, and it will fulfill a congressional mandate.

Exhibit

1

EQUINE VETERINARY JOURNAL
Equine vet. J. (2002) **34** (3) 242-249

Pharmacokinetic/pharmacodynamic approach to assess irrelevant plasma or urine drug concentrations in postcompetition samples for drug control in the horse

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Keywords: horse; drug; doping; pharmacokinetics; pharmacodynamic; control; irrelevant concentration

Summary

The current performance of analytical techniques used for drug control in horses lead the Regulatory Authorities to decide whether trace levels of drugs legitimately used for therapeutic medication should or should not be reported. Here, we propose a well-ordered and nonexperimental pharmacokinetic/pharmacodynamic approach for the determination of irrelevant drug plasma (IPC) and urine concentrations (IUC). The published plasma clearance is used to transform an effective (marketed) dose into an effective concentration (EPC). EPC is transformed into an IPC by applying a safety factor (SF). This method is based on several assumptions (e.g. drug effects reversibly driven by plasma concentration, linearity of drug disposition). The suitability of the computed IPC and IUC can be checked by calculating the residual amount of drug at IPC and computing a minimal drug withdrawal time. It is concluded that controlling the drug effect (using drug or any analyte concentration as a marker) rather than the drug exposure will be more demanding and also makes urine a less than ideal matrix.

Introduction

Difficulties with the findings of analytical techniques, currently used in drug control in horses, have been encountered when very low levels of drugs employed in the treatment of racehorses have been detected on the day of racing (Houghton 1994; Tobin 1995). In such situations, the regulatory authorities have to decide whether trace levels should be reported (i.e. considered as a potential threat to the integrity of competition) or ignored (i.e. considered as biologically irrelevant and of no concern).

Two approaches can be adopted to take a decision. The first consists of reporting any detected drug, which is operationally equivalent to fixing the reporting level at the level of detection (LOD) of the selected analytical technique. Here the aim is to control any drug exposure; this is mandatory for prohibited substances or masking agents for which the most sensitive analytical technique should be used.

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A second approach consists of reporting only concentrations that are above a selected cut-off value, in order to control competition fairness without preventing the possibility of providing proper veterinary care. It has to be based not only on appropriate pharmacokinetic and pharmacodynamic data (i.e. biological data) but also on the selection (implicit or explicit) of safety factors which should reflect what is considered as acceptable or unacceptable by the regulatory authorities.

The objective of the present report is to outline a general and nonexperimental approach for the determination of irrelevant drug plasma concentrations (IPC) and irrelevant drug urine concentrations (IUC) from data in the literature (meta-analysis) in terms of pharmacological and/or clinical effect.

Definitions

For the purpose of this article, the irrelevant plasma drug concentration (IPC, i.e. drug or metabolite) and irrelevant urine drug concentration (IUC, i.e. drug or metabolite) are defined as plasma or urine concentrations which guarantee the absence of any relevant drug effect and for which there will be no regulatory action. Relevant or irrelevant drug effects are qualified later (see section on safety factor).

Assumptions in computing IPC and IUC

The proposed estimation of IPC relies on 3 basic assumptions:

(i) The drug effects are driven reversibly by plasma concentrations and a relationship exists between the overall drug exposure and drug effect. The total area under the plasma concentration vs. time curve (AUC) is used as an index of drug exposure.

(ii) In horses, the currently used (approved) dosage regimens for the drugs under investigation are appropriate, i.e. they correspond to an efficacious dose which does not greatly exceed the ED_{50} (i.e. dose giving half-maximal effect).

(iii) The drug disposition is linear (neither dose- nor time-dependent) within the range of concentrations under consideration and plasma clearance is a parameter (not a variable) relating the recommended dose to plasma concentrations (for further explanations on linearity, see Gibaldi and Perrier 1982).

Computational steps to estimate IPC and IUC

Step 1: computation of an effective plasma concentration (EPC)

For any drug, calculation of the average EPC over the dosing interval corresponding to the standard dosage regimen is given by equation 1:

$$EPC = \frac{\text{standard dose (per dosing interval) x F}}{\text{plasma clearance (per dosing interval)}} \quad (1)$$

In equation 1, standard dose is the generally recommended dose per dosing interval, plasma clearance is the pharmacokinetic parameter which expresses the body's capacity to eliminate the drug, F is the bioavailability factor lying between 0 and 1. The EPC will be calculated using i.v. data only so that F = 1, as the EPC is the same whatever the route of drug administration. Therefore, equation 1 can be reduced to equation 2:

$$EPC = \frac{\text{standard dose (per dosing interval)}}{\text{plasma clearance (per dosing interval)}} \quad (2)$$

Step 2: computation of irrelevant plasma concentration (IPC)

An irrelevant plasma concentration (IPC) can be deduced from EPC by applying a safety factor (SF) to EPC (equation 3):

$$IPC = EPC/SF \quad (3)$$

The selection of an SF is mainly a regulatory decision (see Discussion).

Step 3: determination of irrelevant urine concentration (IUC)

An irrelevant urine concentration (IUC) can be derived from IPC using equation 4:

$$IUC = IPC \times Rss \quad (4)$$

In equation 4, Rss is the steady-state urine to plasma concentration ratio.

Step 4: checking the appropriateness of IPC and IUC

After computing IPC and IUC, the suitability of the proposed figures can be checked by computing the amount of drug remaining in the body when the plasma concentration is equal to IPC and by calculating the shortest possible withdrawal time (WT) of the drug, i.e. the time after i.v. administration during which the drug can be detected at concentrations higher to IPC or IUC.

The residual amount (RA) of drug in the body when the plasma concentration is equal to IPC is given by equation 5:

$$RA = IPC \times Varea \quad (5)$$

Where Varea is the volume of distribution calculated by the area method. This RA can then be compared to the recommended dosage regimen and should be lower than a given percentage of the recommended dose (e.g. 1%).

Estimation of the drug WT after i.v. administration can be obtained with the following equation (6):

$$WT = 1.44 \times \text{selected half-life} \times \text{Log}\left(\frac{\text{intercept of selected half-life}}{IPC}\right) \quad (6)$$

where the selected half-life is the half-life which encompasses the calculated IPC (generally the terminal half-life) and the intercept of the selected half-life is the plasma concentration at time zero when the (terminal) phase of interest begins to decay.

Supplementary considerations for computing and checking IPC and IUC

The computation of EPC (equation 1) requires the selection of a dose. For many drugs, there is only a standard dose but for others (e.g. tranquilisers) different dose levels can be used, depending on the desired effect. For those drugs, the lowest recommended dose should be selected when computing an EPC. Alternatively, the dose corresponding to the effect most relevant to doping control and for which the drug has the greatest potency should be selected.

When very different values of plasma clearance are reported in the literature, the highest clearance should be selected to ensure a conservative approach. When similar clearances are reported, mean or median can be determined. For treatments involving multiple drug administrations, clearance (generally expressed as l/kg/h or ml/kg/min) should be expressed for the recommended dosing interval (e.g. l/kg/24 h). For treatments involving a single dose administration, the period should be selected case by case taking into account a conservative estimate of the possible duration of drug action.

The computation of IPC (equation 3) requires the selection of a safety factor (SF). The selection of safety factors (SF) is a conjoined policy and a scientific decision (see Discussion). A default SF of 500 (i.e. 10 x 50) can be proposed, with 50 to transform an effective plasma concentration close to EC_{50} into an ineffective one and 10 to take interindividual variability into account (Calabresse 1983) (Fig 1).

In Figure 1, Emax, ED_{50} and h are parameters. For drug A, h = 1 and the equation follows the classical Emax model. For drugs B and C, h = 0.5 or 2, respectively. Visual inspection of the curves shows that the effect corresponding to a dose equal to $ED_{50}/50$, i.e. a dose of 0.2, is highly dependent on the h value. For h = 1, the effect is equal to 2% Emax, for h = 0.5 and h = 2, the effects are 12.4 and 0.04% Emax, respectively (see Fig 1 inset). The figure suggests that for drugs with a shape factor below 1, a SF higher than 500 should be selected. Conversely, a lower SF can be used for drugs in which the shape factor is higher than 1.

The determination of IUC requires knowing the urine to plasma concentration ratio, i.e. Rss. Rss is seldom reported in scientific literature and has to be approximated from published raw data (or from published figures).

For calculating an amount of drug in the body, Varea is the appropriate volume term when the computation is performed for a single dose administration and Vss (steady state volume of distribution) is the appropriate volume term when the computation is performed for steady state conditions, but these numerical values are close enough for either to be usable for assessing the suitability of a computed IPC.

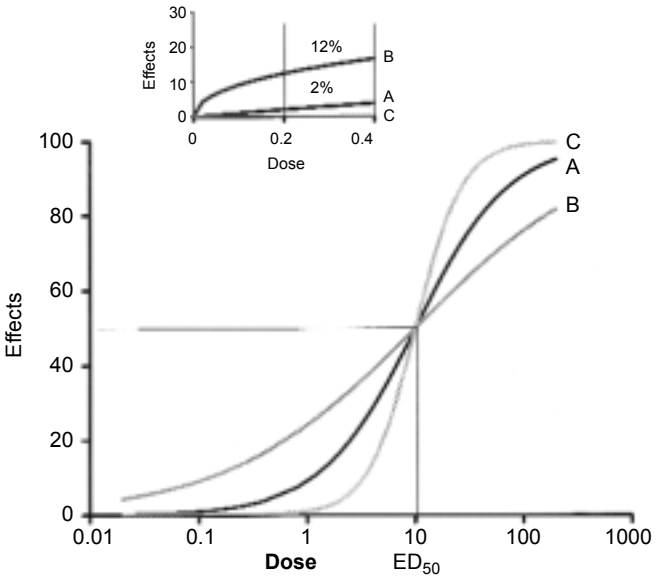

Fig 1 : Selection of a safety factor (SF) and shape factor of the dose-effect relationship model. This figure shows the shape of the dose-effect relationship for 3 hypothetical drugs (A,B,C) simulated with a Hill model of the form:

$$Effect = \frac{Emax \times Dose^h}{ED^h_{50} + Dose^h}$$

Emax = maximal possible effect (fixed at 100); ED_{50} (fixed at 10) = dose producing half Emax; h = shape factor; Dose = simulated dose. For full explanation, see text.

It is also desirable, when checking the appropriateness of IPC and IUC, to have a rough estimate of the withdrawal time (WT) required to reach the IPC after administering a standard dose. This should be obtained from i.v. data, because these provide information on the actual drug WT (irrespective of the pharmaceutical formulation) and the WT obtained in this way should be the shortest possible. In contrast, the data obtained by extravascular route is influenced by the bioavailability factors, some being due to the pharmaceutical formulation and not to the drug (e.g. a slow process of absorption leading to a prolonged terminal half-life).

In equation 6, the selected half-life should be the half-life that encompasses the IPC (Fig 2) and, for many drugs, this should be the 'terminal half-life'. However, for some drugs the IPC will be lower than the level of quantification (LOQ) of the current analytical method and the computation of withdrawal time using the last observed plasma half-life could be misleading, because there is no guarantee that this is the terminal half-life encompassing the IPC. If the reported urine terminal half-life is longer than the terminal plasma half-life (often the case) despite the fact that the terminal half-times are theoretically the same for plasma and urine, and if IPC is lower than the plasma LOQ, WT should also be calculated using IUC and urine data. The phenylbutazone (PBZ) case provides a good illustration of the proposed method (see next section).

An example: computational steps for the determination of an IPC and IUC for phenylbutazone

Step 1 - selection of the recommended dosage: The regular dosage regimen for phenylbutazone (PBZ) is 4.4 mg/kg/24 h (maintenance dose), but an 8.8 mg/kg/24 h dose is also recommended as an i.v. loading dose; the 4.4 mg/kg/24 h dose has been selected as the standard dose.

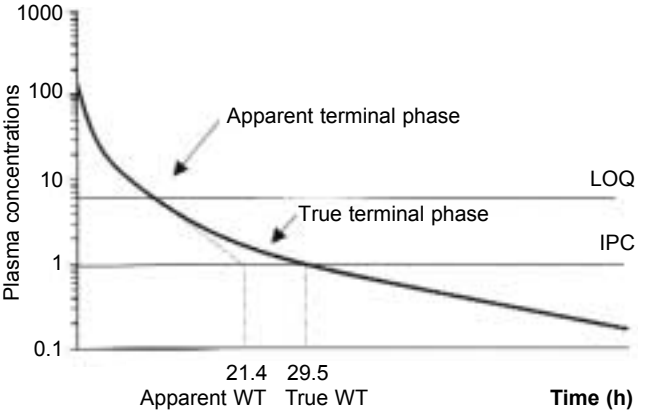

Fig 2: Selection of a terminal half-life for the computation of a minimal withdrawal time (WT) for a hypothetical drug with an IPC equal to 1. The curve was simulated with the equation:

$$C(\mu g/ml) = 100\ exp(-1t) + 30\ exp(-0.2t) + 3\ exp(-0.04t)$$

The LOQ is 5 µg/ml and only the 2 first phases are quantitatively measurable. If only the data above LOQ are used, a bi-exponential equation has to be selected to fit the data which leads to an estimated WT of 21.4 h, whereas it is actually 29.5 h.

Step 2 - computation of EPC: PBZ plasma clearance has been measured by many authors; mean reported values range from 16.3 to 41.3 ml/kg/h (Soma *et al.* 1983; Lees *et al.* 1987; Mills *et al.* 1996). The most conservative value is obtained by taking the highest clearance value, i.e. 41.3 ml/kg/h (Mills *et al.* 1996) or 991 ml/kg/24 h. Using equation 7:

$$EPC = \frac{4400\ \mu g/kg/24\ h}{991\ ml/kg/24\ h} = 4.44\ \mu g/ml \qquad (7)$$

This EPC is consistent with the EC_{50}, which was directly determined by Toutain *et al.* (1994) using a PK/PD approach in horses subjected to experimental arthritis (EC_{50} from 1.5 to 4.3 µg/ml depending on the pharmacodynamic endpoint). Lees *et al.* (1987) reported that the inhibitory action of PBZ on thromboxane B_2 formation was still 50% 24 h after an i.v. PBZ administration (4.4 mg/kg), giving an EC_{50} of about 2 µg/ml. In addition, in this experiment, it was necessary to wait 48 h to reach a not effective plasma PBZ concentration (i.e. about 0.1 µg/ml). Drevemo *et al.* (1994) evaluated the effect of PBZ at a low plasma concentration by studying locomotion in horses with low grade distal forelimb lameness by means of a quantitative kinetic method. They concluded that PBZ may engender a significant change in the locomotor pattern of horses with low grade lameness and that the effect may last longer than 48 h after PBZ administration (2.5 mg/kg b.i.d.). The authors concluded that the PBZ effect lasted for plasma concentrations lower than 2 µg/ml (their LOQ) and it was concluded from this clinical trial that a plasma concentration of about 0.1 µg/ml still has some therapeutic meaning. The IPC should, therefore, be lower than 100 ng/ml. Finally, these results taken together suggest that the EPC of PBZ is around 1 µg/ml and that the IPC should be lower than 100 ng/ml.

For several drugs, not only the drug but also its primary metabolite(s) can participate in the overall drug effect. This is the case for oxyphenbutazone (OPBZ), a primary metabolite of PBZ in horses. Therefore, the question is whether this active metabolite should be taken into account when computing an EPC for PBZ.

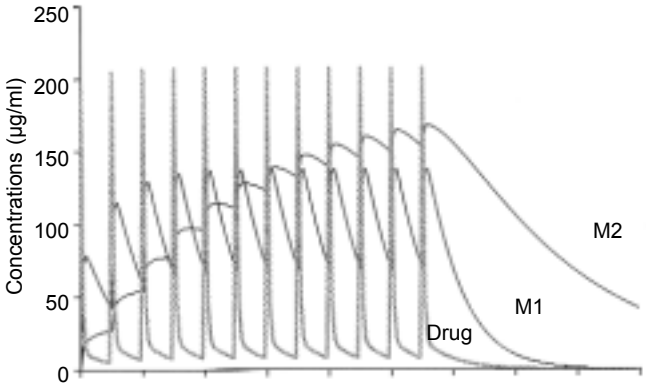

Fig 3: Time development of plasma concentration for a hypothetical drug and its 2 primary metabolites (M1 and M2) during and after a multiple drug administration (half-life of 8.36 h). M1 and M2 obey a monocompartmental model and have intrinsic terminal half-lives of 4.17 and 34.6 h, respectively.

The answer is negative, as the calculated EPC is solely the observed plasma PBZ concentration for which effects are expected (i.e. taking into account the action of all the active metabolites), and not the actual PBZ concentration at the receptor level required to trigger a given effect. In other words, with the proposed approach, PBZ is the 'marker analyte' of the PBZ response and the proposed EPC value has no mechanistic value.

Step 3 - computation of an IPC for PBZ: Using equation 8 and the default value for SF (500) for illustration:

$$IPC = \frac{4440 \text{ ng/ml}}{500} = 8.88 \text{ ng/ml} \quad (8)$$

rounded to give an IPC = 10 ng/ml, which is much lower than the 5 µg/ml threshold fixed in North America (ARCI) (Tobin *et al.* 1999).

Step 4 - determination of IUC for PBZ: Soma *et al.* (1983) published plasma and urine PBZ concentrations after i.v. and oral PBZ administration. They demonstrated an excellent correlation between the decrease in urinary and plasma concentrations after oral (r = 0.95) and i.v. (r = 0.99) administration. In addition, after oral administration (8.8 mg/kg every 24 h for 8 consecutive days) the urinary concentrations at 24, 48, 72, 96 and 120 h, i.e. 24 h after each administration, were about 4–6 µg/ml, i.e. of the same order of magnitude as the plasma concentration and suggesting a urine to plasma ratio (Rss) of about 1.

Similar results are reported in the monograph compiled by Canada's Agriculture and Agri-food Equine drug evaluation programme (Anon 1994), and Gerring *et al.* (1981) reported a urine to plasma concentration ratio of 0.2 to 1.

Using equation 9 and fixing Rss at 1:

$$IUC = 10 \text{ ng/ml} \times 1 = 10 \text{ ng/ml} \quad (9)$$

It is noteworthy that the computed IUC is lower than the LOD (about 50 ng/ml) reported in the literature; and that the urinary concentration of PBZ can be modified dramatically by

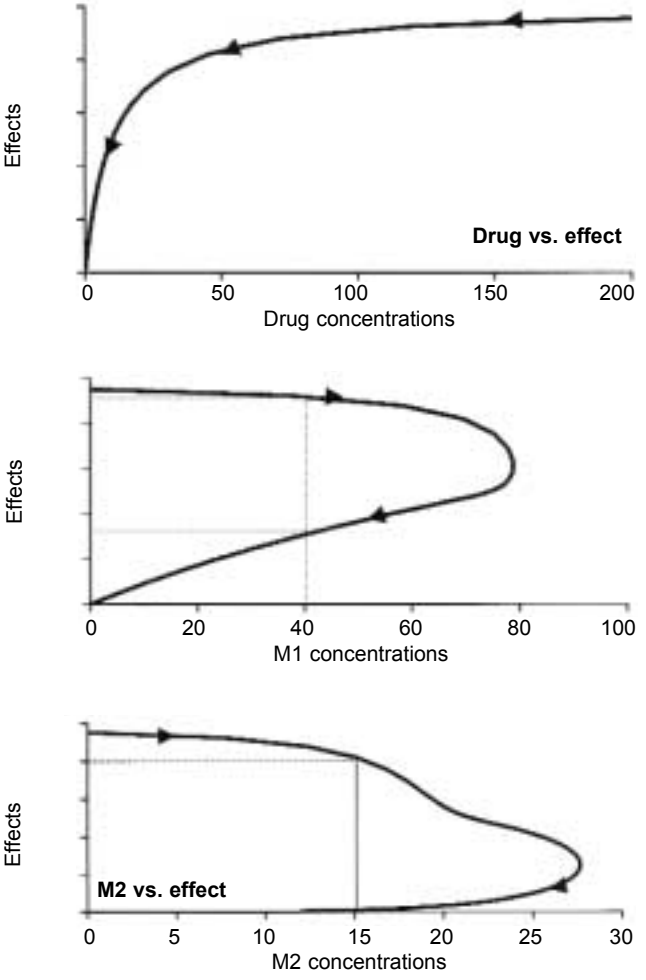

Fig 4: Relationship between plasma analyte concentrations (drug, M1 or M2 as for Fig 3) and effect for a single i.v. drug administration (for full explanation, see text).

the urinary pH. It was shown in postrace urine from horses racing in Kentucky that urinary PBZ concentrations can by multiplied by 200 when the pH increases from 4 to 8.5 (Houston *et al.* 1985). As urinary pH does not influence plasma clearance, it is probable that a PBZ IUC of 10 ng/ml is much more conservative for a horse with a basic urine pH than for one with an acid urine pH (see Discussion).

Step 5 - computation of residual PBZ amount for IPC: The residual amount (RA) of PBZ can be calculated from IPC and Varea (see equation 5). Several authors have published Varea for PBZ with figures ranging from 0.152 l/kg (Soma *et al.* 1983) to 0.37 l/kg (Toutain *et al.* 1994). Using equation 10:

$$RA = 10 \text{ µg/l} \times 0.37 \text{ (or } 0.15) \text{ l/kg} = 3.7 \text{ (or } 1.5) \text{ µg/kg} \quad (10)$$

This residual amount is lower than 1% of the recommended dose (4400 µg/kg).

The minimal PBZ withdrawal time (WT) can be computed using the mean parameters obtained by Mills *et al.* (1996) (their Table 1) for a dose of 8.8 mg/kg by i.v. route:

$$C \text{ (µg/ml)} = 80\, e^{-18.55t(h)} + 33\, e^{-0.14t(h)}$$

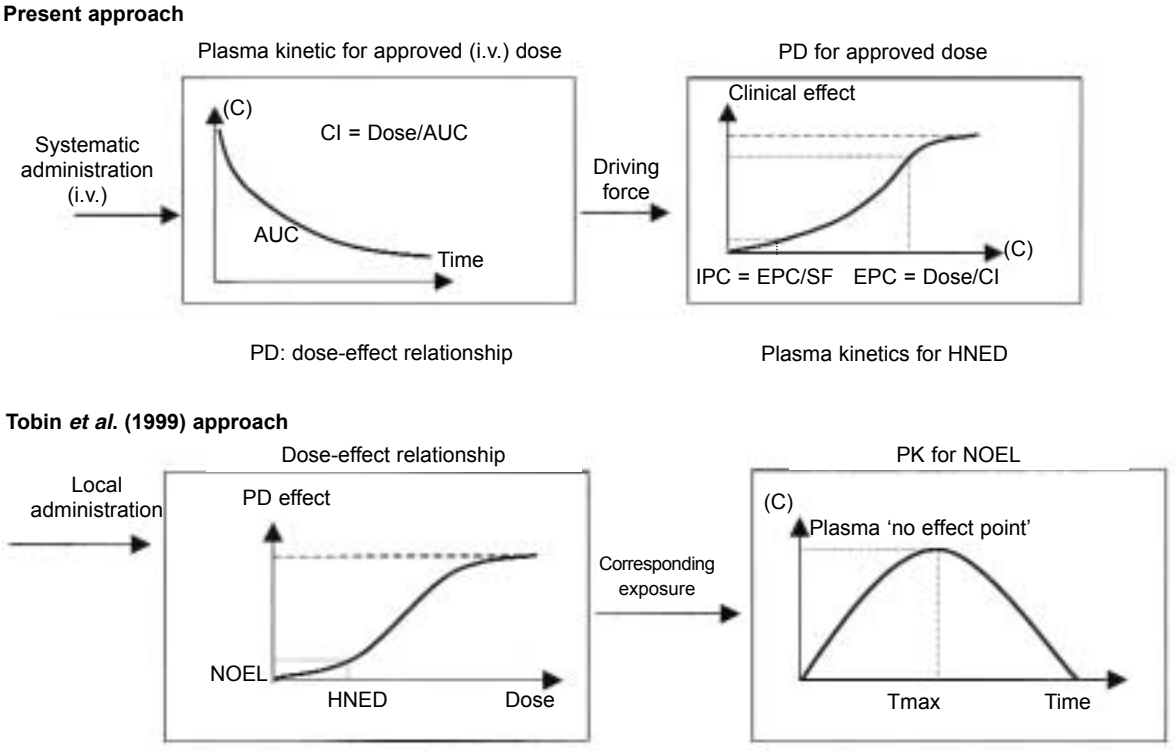

Fig 5: Present vs. approach of Tobin et al. *(1999) to determine an irrelevant or 'no effect' point plasma concentration (for full explanation, see Discussion).*

By solving this equation for IPC, different WT can be estimated for different single doses, namely 58 h for an i.v. dose of 8.8 mg/kg and 53 h for a 4.4 mg/kg dose. Similar results are obtained with the simplified equation 6, in which the intercept Yz is equal to 33 µg/ml and the terminal half-life equals 4.95 h.

Using the equation proposed by Lees *et al.* (1987) for a dose of 4.4 mg/kg:

$$C\ (\mu g/ml) = 34.5\ e^{-5.9t(h)} + 29.76\ e^{-0.115t(h)}$$

The estimated WT required to reach the IPC is 75.5 h for an 8.8 mg/kg dose and 69.5 h for a 4.4 mg/kg dose.

These figures assumed that the reported terminal half-life (4.95 h from Mills *et al.* [1996] and 6 h from Lees *et al.* [1987]) would be the true terminal half-life of PBZ, i.e. the plasma half-life which would be measured if the plasma concentrations had been measured over 3 days. There is some evidence that this is not the case and that there is a supplementary phase that cannot be detected accurately from the plasma disposition curve as the LOQ is too high. A terminal half-life of about 8 h can be calculated graphically from the urine data published by the Canadian Agriculture and Agrifood Equine drug evaluation programme (Anon 1994). If we accept that the terminal half-life in urine is theoretically equal to the terminal half-life in plasma (Gibaldi and Perrier 1982), then the true terminal plasma half-life for PBZ is 8 h, rather than 4 or 6 h. Therefore, a minimal WT slightly longer than 70 h should be expected after i.v. administration of PBZ at the recommended dosage regimen. This conclusion fits the experimental data, a delay of about 80 h being obtained graphically from figures published (Anon 1994) for i.v. PBZ administration of 2.0 g.

The issue of active and inactive metabolites in IPC and IUC calculation

The analyte(s) selected to calculate the IPC or IUC can be the drug itself or its active moiety (e.g. dipyrone, which is transformed within a few minutes into the active 4-methylaminoantipyrine or 4-AMM), and this is the most desirable situation. However, the drug or its active moiety may not be eliminated in the urine in sufficient amounts, thereby making appropriate monitoring impossible, and an inactive metabolite has to be selected. A relevant question in the context of this present approach is whether an inactive metabolite can be used to calculate IPC.

As we are concerned with both IPC and IUC control, it must be ensured that this inactive (or weakly active) metabolite is an authentic tracer of the overall drug activity. For this, the ratio of the plasma or urinary marker (metabolite) to the plasma active principle (drug, active moiety) must be known, and be more or less constant over the period of the IPC or IUC control.

For an analyte to be a marker (tracer) of the active principle (hereafter named 'tracee'), the slopes of the terminal phase of the tracer and of the tracee should be the same as this guarantees a constant ratio between them. The conditions for this are 2-fold: (i) the intrinsic half-life of the marker (i.e. the half-life which will be obtained after administering the tracer by i.v. route) should be shorter than or equal to the half-life of the tracee; (ii) the system is in pseudo-distribution equilibrium (after a single dose administration) or in steady state conditions (after a multiple dose-administration). This explains the fact that inactive metabolites cannot always be used if the purpose of drug control is to ensure a lack of relevant effect (Figs 3, 4).

For example, in Figure 3, plasma concentrations for the drug

and its 2 metabolites, M1 and M2, were simulated for a multiple dose administration (every 12 h for 6 days). Visual inspection of the figures shows that the drug and M1 rapidly reach the steady-state conditions, i.e. that the same plasma concentration profile is repeated after each administration. In contrast, the steady-state conditions are not totally achieved after 6 days for M2. This is due to the fact that the time to reach steady-state conditions is governed by the terminal half-life of each analyte, which is much higher for M2 than M1. This simulation shows that the M2/drug ratio can be different at the beginning of the drug administration up to the attainment of steady state conditions and that a high M2 concentration can be measured whereas drug concentration (and effect) are very low. In this kind of situation, M2 is not an appropriate analyte for a drug control aimed at the control of a noneffect level. Instead, using M2 will be operationally equivalent to controlling drug exposure.

In Figure 4, the effect was simulated using a classical Emax equation with drug plasma concentrations as the driving concentration. This figure shows that there is a perfect relationship between plasma drug concentrations and effect, i.e. when plasma drug concentrations decrease (after i.v. administration) the drug effect decreases. The relationship between M1 plasma concentrations and effect is different and exhibits a hysteresis phenomenon, i.e. after drug administration, M1 concentrations increased to reach a maximum concentration (80) and then began to decrease to 0. It can be seen that for a given M1 concentration (e.g. 40) there are two possible effects, one nearly total and the other of about 30. For M2, a plasma concentration of about 15 can correspond to either a nearly full effect or to a lack of effect. This figure shows that a snapshot inactive metabolite concentration cannot predict univocally a drug effect. Similarly, a hysteresis phenomenon can also exist between the plasma drug concentration itself and the effect.

Discussion

A drug control can be based on one of 2 lines of reasoning: control of drug exposure or control of the absence of a (relevant) drug effect. Any analyte (drug, active or inactive metabolite) can be used for monitoring drug exposure and sensitivity will depend on analytical performance. This approach is sound for prohibited substances which have no place in horse racing. On the other hand, the control of drug exposure for therapeutic medications raises the problem of ‘trace’ concentrations and, due to the permanent improvement of analytical techniques, some drugs used in horses can still be detected long after their effect has totally vanished. Under these conditions, the drug control programme can become a limiting factor in the use of drugs for proper veterinary care of racehorses.

Here we present a standardised approach for the treatment of data that has already been published. It is by definition a nonexperimental approach, which differs from the one proposed by Tobin *et al.* (1999) in which a dose-effect relationship is established experimentally, then a dose without effect and finally the corresponding plasma concentration is measured. The advantage of the present approach is that it is inexpensive, because it consists of performing a meta-analysis on already existing data. Its main limit is that it can only be applied to drugs for which the effects are driven by plasma concentration, i.e. drugs that act systemically. It cannot be applied to drugs with presystemic effect (i.e. which act before reaching the general circulation). This is the case for local anaesthetics or any drug which can be administered by local route (e.g. glucocorticoids). The approach outlined by Tobin *et al.* (1999) is recommended for these classes of drug. The 2 approaches are compared in Figure 5, together with their domains of application.

In the present (nonexperimental) approach, either a plasma drug or active metabolite concentration drives the effect at the biophase level, i.e. is the independent variable with respect to the concentration-effect relationship. The approved (marketed) dose is considered as the pivotal dose giving a relevant overall clinical response which, divided by plasma clearance, gives an effective plasma concentration (EPC). A safety factor (SF) is used to transform EPC into an irrelevant plasma concentration (IPC). The advantages of this method are its cheapness, the fact that it is based on clinical response rather than on pharmacodynamics (surrogate effect) and its independence from route of administration (providing its action is systemic) and from formulation. The limits of the approach are apparent with drugs which can be administered locally (biophase exposure preceding plasma exposure), drugs for which the effect lasts much longer than their actual presence in the body (anabolic), the need to obtain a quantitative measurement of plasma or urine analytes and the depreciation of urine as a matrix for doping control, and of any inactive metabolite if the aim is to control the presence or absence of relevant effect. In the approach of Tobin *et al.* (1999) (experimental), the plasma drug (metabolite) concentration does not drive the effect but is conceptualised as a marker of a presystemic biophase drug exposure to the highest no effect dose (HNED). The HNED has to be determined experimentally using a critical pharmacological effect (rather than an overall clinical response) by performing a dose-effect relationship. An analyte marker (drug, metabolite) is then used to establish a ‘no effect point’ or no effect level (NOEL), i.e. a cut-off plasma or urine concentration corresponding to the HNED. As quoted by Tobin *et al.* (1999), the limit of this approach is that ‘if the drug is administered by different routes of administration, or repeated doses, or a different formulation or with another therapeutic rational, then the analytical pharmacological data base reported (here) may not necessarily be applicable to the specific regulatory circumstances’.

The present approach relies on several assumptions, the central one being that drug effects are reversibly driven by the plasma concentration profile and the present method cannot be used for drugs (e.g. steroids) whose effects persist long after the drug has disappeared.

The action of some drugs is related more to the achievement of a minimal plasma concentration than to overall exposure (e.g. anti-arrhythmics, anaesthetics), leading to an all-or-nothing type of response rather than a graded dose-response. The present approach is not recommended for such drugs, because an AUC fraction (more or less important and which is dose-dependent), does not participate in their action.

The present approach requires selecting a pivotal dose to compute an EPC and we suggest choosing the approved dosage regimen (e.g. as indicated in the marketing authorisation). Therefore, the clinical response expected by clinicians rather than selected pharmacodynamic effects is used as efficacy endpoint, which guarantees the relevance of EPC. One difficulty may arise for drugs which have obtained a marketing authorisation with too high a dosage thanks to a good safety margin. This is probably the case for acepromazine in horses, which is marketed in France at 500 μg/kg and at 130–260 μg/kg in the UK but has proved

effective at a much lower dose (Freestone *et al.* 1991; Wood *et al.* 1992). To deal with this situation, the lowest effective dose can be selected to compute the EPC or, alternatively, a more conservative SF can be selected.

The plasma clearance needs to be known for the computation of EPC and is known, in horses, for many drugs. If the drug disposition is linear, clearance is a genuine parameter allowing transformation of an efficacious dose into an overall average efficacious plasma concentration. It should be noted that an efficacious plasma concentration is a drug (i.e. molecule) property that is independent of the route of administration or formulation. EPC can also be obtained tentatively from an extravascular route of administration by dividing the measured AUC by the recommended dosing interval. The advantage of computing an EPC using clearance is that clearance is a species parameter reflecting only the drug (molecule) property, whereas an AUC obtained by extravascular administration is also influenced by the route of administration, formulation etc, AUC is therefore less attractive when international harmonisation is required.

The computation of EPC also requires determination of a time interval corresponding to the duration of a relevant effect. For drugs with a marketing authorisation for an approved dosing interval, this dosing interval (often 24 h) will be the best to consider. In contrast, for drugs used in a single dose administration (e.g. tranquiliser), it is necessary to evaluate the duration of the action in order to express clearance for a relevant duration of time.

To transform an EPC into an IPC an *a priori* safety factor has to be selected. The selection of an SF is a policy and a scientific decision. It is the responsibility of the regulatory authorities to determine what is acceptable (or unacceptable) in terms of drug effect. The magnitude of the SF also involves, for example, the reliability of the data, the nature of the effect, the possibility of large interindividual variability between the population subgroups, the existence of different effects for which a drug has different potencies and the shape of the dose-effect relationship. A default value of 500 would take into account the interindividual variability and transform an efficacious plasma concentration into an irrelevant one. The interindividual factor (10) is relatively large and can be reduced if the interindividual plasma clearance has been documented from population kinetics. The 50 factor to convert EPC to IPC holds if the selected dose is close to the ED_{50} and if the dose-effect relationship obeys the Emax model. If these 2 conditions are fulfilled, the residual effect is about 2% Emax. If the selected dose is much higher than the ED_{50}, it will be necessary to increase this factor. Similarly, the SF should be increased if the dose-effect relationship obeys a Hill model with a slope factor lower than 1 (Fig 1). The slope factor is known for some drugs in the horse, e.g. for NSAIDs the slope factor for PBZ was higher than 1 (Toutain *et al.* 1994) whereas, in human medicine, the slope factor is considered to be lower than 1 for betablockers (Campbell 1990).

It is interesting to note that fixing SF at infinity is operationally equivalent to controlling drug exposure, as any detected drug will be reported; and fixing SF at 1 is operationally equivalent to accepting drugs at therapeutic levels, showing that the present approach is very general. For PBZ, the threshold has been fixed at 5 µg/ml in North America (ARCI) (Tobin *et al.* 1999) which is precisely the EPC reported here. In this case, an SF equal to 1 has been implicitly applied.

The drug control is generally undertaken using urine as a matrix rather than plasma. However, it should be kept in mind that only plasma concentration is governing the effect and, in the framework of the present approach, urine becomes a surrogate matrix for plasma.

As plasma concentration is also the driving concentration for urine excretion rate, a relationship exists between plasma and urine drug concentrations, but the urine to plasma ratio is not a robust factor. After a single dose administration, it is not a parameter but a variable, with values ranging between 0 (just after drug administration) and a theoretically constant value (Rss) in pseudoequilibrium conditions. For a multiple drug administration, the urine to plasma ratio is permanently changing until steady state conditions are reached, when Rss can be constant over a more or less large fraction of the dosing interval. In addition, Rss can be influenced by water diuresis (urine volume) and some physiological factors, such as urine pH (Houston *et al.* 1985). For all these reasons, urine is a less than ideal matrix for controlling a no effect drug level and changing the drug control policy could make urine much less attractive (appropriate) than for controlling drug exposure.

Another consequence of the proposed approach could be the necessity to abandon many metabolites currently used for drug control. If the purpose of drug control is to control exposure (e.g. cocaine, masking agents), any analytes (drug, active or inactive moiety) can be used for monitoring. In contrast, if the purpose is to control an IPC or IUC for a drug recognised as a therapeutic agent in horse, the recourse to an inactive metabolite can become debatable, because measuring a snapshot inactive metabolite concentration can be very misleading, as explained in Figures 3 and 4.

The minimal WT of a drug should be checked to make sure that IPC and IUC are acceptable. It should be remembered that the WT is not a pivotal issue for regulatory authorities. Given the IPC, it will be the responsibility of the drug company to estimate the WT of their commercial preparations for their selected dosage regimen and route of administration. In this paper, WT is computed (without any statistical protection) in order to assess the acceptability of the proposed IPC and IUC.

It should be understood that the concept of detection time (Tobin *et al.* 1999) (or WT) is fundamentally different from that of IPC (or threshold). A threshold is a drug parameter, whereas detection time is a variable influenced by many factors unrelated to the drug properties, such as dose, route of administration, pharmaceutical formulation or LOD.

In conclusion, this paper proposes a well-ordered and nonexperimental approach on the basis of pharmacokinetics and clinical data for the determination of what are the appropriate analytical performances, for drug control, on the day of racing. Its purpose is to discriminate between concentrations of drugs which could threaten the integrity of racing, and irrelevant concentrations which can be detected using operational analytical tools. The present approach holds only for drugs acting systemically. It is also emphasised that such a change to drug control policy could make urine a much less appropriate matrix than plasma for the control of drug exposure and that a snapshot inactive metabolite measurement (urine or plasma) cannot be used unequivocally to control a drug effect. Finally, controlling drug effect will require not only an LOD to check presence or absence of an analyte, but also quantification of the analyte in plasma and urine.

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Exhibit

2

HORSERACING INTEGRITY AND SAFETY AUTHORITY

HISA Prohibited Substances List

Banned Substances

Last updated on March 1st, 2023

BANNED

HORSERACING INTEGRITY AND SAFETY AUTHORITY

HISA STATUS / SS[1]	SUBSTANCE	ACTION	COMMERCIAL / DEVELOPMENTAL NAME(S) WHERE AVAILABLE
S1	Δ-1-androstene-3, 17diol	Anabolic	Lacks FDA approval. DEA Schedule III.
S1	Δ-1-androstene-3, 17dione	Anabolic	Lacks FDA approval. DEA Schedule III.
S1	Δ-1-dihydrotestosterone	Anabolic	Lacks FDA approval. DEA Schedule III.
S1	19-Norandrostenediol	Anabolic	Lacks FDA approval. DEA Schedule III.
S1	19-Norandrostenedione	Anabolic	Lacks FDA approval. DEA Schedule III.
S1	19-Noretiocholanolone	Anabolic	Lacks FDA approval.
S1	1-androstenediol (5α androst-1-ene-3β, 17βdiol)	Anabolic	Lacks FDA approval. DEA Schedule III.
S1	1-androstenedione (5αandrost-1-ene-3, 17dione)	Anabolic	Lacks FDA approval. DEA Schedule III.
S1	1-testosterone (17βhydroxy-5α- androst-1en-3-one)	Anabolic	Lacks FDA approval. DEA Schedule III.
S0	2-Aminoheptane (Tuaminoheptane)	Sympathomimetic / Vasoconstrictor	Lacks FDA approval
S4	2-androstenol(5α-androstane-2-en-17-ol)	Pheromone / Reproductive Hormone	Lacks FDA approval
S4	2-androstenone (5α-androst-2-en-17-one)	Pheromone / Reproductive Hormone	Lacks FDA approval
S0	3,4-methylenedioxypyrovalerone (MDVP)	Stimulant	Lacks FDA approval
S4	3-androstenol (5α-androst-3-en-17-ol)	Pheromone / Reproductive Hormone	Lacks FDA approval
S4	3-androstenone (5α-androst-3-en-17-one)	Pheromone / Reproductive Hormone	Lacks FDA approval

1 – SPECIFIED SUBCLASSIFICATION
Specified substances designated with an X

BANNED

HORSERACING INTEGRITY AND SAFETY AUTHORITY

HISA STATUS / SS[1]	SUBSTANCE	ACTION	COMMERCIAL / DEVELOPMENTAL NAME(S) WHERE AVAILABLE
S4	4-androstenediol (androst-4-ene- 3β,17βdiol)	Anabolic	Lacks FDA approval. DEA Schedule III.
S4	4-chloromethatandienone	Anabolic	Lacks FDA approval. DEA Schedule III.
S1	4-Hydroxytestosterone	Anabolic	Lacks FDA approval. DEA Schedule III.
S4	5-androstenedione (androst-5- ene- 3,17dione)	Anabolic	Lacks FDA approval. DEA Schedule III.
S1	5α-Andros-2-ene-17one	Anabolic	Lacks FDA approval. DEA Schedule III.
S1	5α-Androstane-3α, 17 α-diol	Anabolic	Lacks FDA approval. DEA Schedule III.
S1	5α-Androstane-3α, 17 β-diol	Anabolic	Lacks FDA approval. DEA Schedule III.
S1	5α-Androstane-3β, 17α -diol	Anabolic	Lacks FDA approval. DEA Schedule III.
S1	5α-Androstane-3β, 17β -diol	Anabolic	Lacks FDA approval. DEA Schedule III.
S1	5β-androstane-3 α, 17βdiol, androst-4-ene3α,17α-diol	Anabolic	Lacks FDA approval. DEA Schedule III.
S1	7-keto-dhea	Anabolic	
S1	7α-hydroxy-dhea	Anabolic	
S1	7β-hydroxy-dhea	Anabolic	
S5	Acebutolol	Antihypertensive	Generic
S0	Acecarbromal	Sedative / Hypnotic	Lacks FDA approval

1 – SPECIFIED SUBCLASSIFICATION
Specified substances designated with an X

BANNED

HISA STATUS / SS[1]	SUBSTANCE	ACTION	COMMERCIAL / DEVELOPMENTAL NAME(S) WHERE AVAILABLE
S0	Acefylline	Bronchodilator	Lacks FDA approval
S0	Acemetacin	NSAID	Lacks FDA approval
S0	Acenocoumarol	Anticoagulant	Lacks FDA approval
S0	Acetanilide	NSAID	Lacks FDA approval
S5	Acetazolamide	Carbonic Anhydrase Inhibitor	Generic
S0	Acetohexamide	Insulin secretion	Discontinued, no FDA-approved product commercially available
S0	Acetophenazine	Antipsychotic	Discontinued, no FDA-approved product commercially available
S0	Acetophenetidin (Phenacetin)	NSAID	Lacks FDA approval
S0	Acetylmorphine	Opioid analgesic	Lacks FDA approval
S0	Aclidinium bromide	Bronchodilator	Tudorza Pressair; Dualkir Pressair (with formoterol)
S0	Adinazolam	Sedative / Anxiolytic	Lacks FDA approval
S0	Adiphenine	Antispasmodic	Lacks FDA approval
S0	Adrafinil	Stimulant	Lacks FDA approval
S4	5-Aminoimidazole-4-carboxamide ribonucleotide (AICAR)	Metabolic modulator	Lacks FDA approval

1 – SPECIFIED SUBCLASSIFICATION
Specified substances designated with an X

HISA STATUS / SS[1]	SUBSTANCE	ACTION	COMMERCIAL / DEVELOPMENTAL NAME(S) WHERE AVAILABLE
S0	Alclofenac	NSAID	Lacks FDA approval
S0	Alcuronium	Muscle relaxant	Lacks FDA approval
S5	Aldosterone	Diuretic	Lacks FDA approval
S6	Alendronate	Bisphosphonate	Fosamax, Binosto
S2	Alexamorelin	Growth Hormone	Lacks FDA approval
S0	Almotriptan	Selective Serotonin Receptor Agonist	Generic
S0	Alpha-pyrrolidinovalerophenone (Alpha PVP and “Bath Salts”)	Stimulant /Hallucinogen	Lacks FDA approval. DEA Schedule I.
S2	Alpha-casozepine	Sedative	Lacks FDA approval
S0	Alphadolone (Alfadolone) acetate	Anesthetic	Lacks FDA approval
S0	Alphaprodine	Opioid analgesic	Lacks FDA approval. DEA Schedule II.
S0	Alphenal	Barbiturate / Anticonvulsant	Lacks FDA approval
S0	Alpidem	Anxiolytic	Lacks FDA approval
S0	Alprenolol	Antihypertensive	Generic
S0	Althesin	Anesthetic	Lacks FDA approval
S5	Althiazide	Diuretic	Lacks FDA approval

1 – SPECIFIED SUBCLASSIFICATION
Specified substances designated with an X

BANNED

HISA STATUS / SS[1]	SUBSTANCE	ACTION	COMMERCIAL / DEVELOPMENTAL NAME(S) WHERE AVAILABLE
S6	Altrenogest Intact males, geldings, spayed females	Progestogen / Estrus Suppression	Regumate
S0	Alverine	Antispasmodic	Lacks FDA approval
S0	Amantadine	Dopamine agonist	Gocovri, Osmolex ER
S0	Ambenonium	Cholinesterase Inhibitor	Discontinued, no FDA-approved product commercially available
S0	Ambroxol	Mucolytic	Lacks FDA approval
S0	Ambucetamide	Antispasmodic	Lacks FDA approval
S0	Amfepramone	Antidepressant	Lacks FDA approval
S0	Amfetaminil	Stimulant	Lacks FDA approval
S0	Amidephrine	Stimulant	Lacks FDA approval
S5	Amiloride	Diuretic	Midamor
S0	Amineptine	Antidepressant	Lacks FDA approval
S4	Aminoglutethimide	Aromatase inhibitor	Discontinued, no FDA-approved product commercially available
S0	Aminomethylbenzoic acid	Anti-fibrinolytic	Lacks FDA approval
S0	Aminometradine	Diuretic	Lacks FDA approval
S0	Aminopterin	Anti-neoplastic / Immunosuppressive	Lacks FDA approval
S0	Aminopyrine (Pyramidon)	Analgesic	Lacks FDA approval
S0(x)	Aminorex	Stimulant	Lacks FDA approval. DEA Schedule I.

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BANNED

HISA STATUS / SS[1]	SUBSTANCE	ACTION	COMMERCIAL / DEVELOPMENTAL NAME(S) WHERE AVAILABLE
S0	Amiphenazole	Respiratory Stimulant	Lacks FDA approval
S5	Amisometradine	Diuretic	Lacks FDA approval
S0	Amisulpride	Antipsychotic	Barhemsys
S5	Amlodipine	Antihypertensive	Norvasc
S0	Ammonium Chloride	Chemical neurectomy	Generic
S0	Ammonium Sulphate	Chemical neurectomy	Lacks FDA approval
S0	Ammonium Sulphide	Chemical neurectomy	Lacks FDA approval
S0	Amobarbital	Bartiburate / Anticonvulsant	Lacks FDA approval. DEA Schedule II.
S0	Amoxapine	Antidepressant	Generic
S0	Amperozide	Antipsychotic	Lacks FDA approval
S0	Amphetamine	Stimulant	Adzenys XR-ODT, Dyanavel XR, Evekeo. DEA Schedule II.
S0	Amphetaminil	Stimulant	Lacks FDA approval
S0	Ampyrone	NSAID	Lacks FDA approval
S0	Amyl nitrite	Antihypertensive/ CNS Depressant	Lacks FDA approval
S0	Amylocaine	Local anesthetic	Lacks FDA approval
S2	Anamorelin	Growth Hormone	New Drug Application submitted; currently lacks FDA approval
S4	Anastrozole	Anti-estrogen	Arimidex

1 – SPECIFIED SUBCLASSIFICATION
Specified substances designated with an X

HISA STATUS / SS[1]	SUBSTANCE	ACTION	COMMERCIAL / DEVELOPMENTAL NAME(S) WHERE AVAILABLE
S1	Andarine	Selective Androgen Receptor Modulator (SARM)	Lacks FDA approval
S1	Androst-4-ene-3α,17β diol	Anabolic	DEA Schedule III.
S1	Androst-4-ene-3β,17α diol	Anabolic	DEA Schedule III.
S1	Androst-5-ene-3α,17α diol	Anabolic	DEA Schedule III.
S1	Androst-5-ene-3α,17β diol	Anabolic	DEA Schedule III.
S1	Androst-5-ene-3β,17α diol	Anabolic	DEA Schedule III.
S4	Androstatrienedione (Androsta-1,4,6-triene-3,17-ddione)	Anabolic	DEA Schedule III.
S4	Androstenediol (androst-5-ene-3β, 17βdiol)	Anabolic	DEA Schedule III.
S4	Androstenedione (androst-4-ene-3, 17dione)	Anabolic	DEA Schedule III.
S4	Androsterone (3 βhydroxy-5 α – androstan-17-one)	Anabolic	DEA Schedule III.
S0	Anileridine	Opioid analgesic	Discontinued, no FDA-approved product commercially available. DEA Schedule II.
S0	Anilopam	Opioid analgesic	Lacks FDA approval
S0	Anisindione	Anticoagulant	Discontinued, no FDA-approved product commercially available
S0	Anisotropine (Octatropine methylbromide)	Anticholinergic	Discontinued, no FDA-approved product commercially available

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BANNED

HISA STATUS / SS[1]	SUBSTANCE	ACTION	COMMERCIAL / DEVELOPMENTAL NAME(S) WHERE AVAILABLE
S0	Antipyrine	NSAID	Lacks FDA approval
S0	Apazone (Azapropazone)	NSAID	Lacks FDA approval
S0	Apocodeine	Dopamine agonist	Lacks FDA approval
S0	Apomorphine	Opioid analgesic	Kynmobi, Apokyn
S0	Aprindine	Antiarrhythmic	Lacks FDA approval
S0	Aprobarbital	Barbiturate	Lacks FDA approval. DEA Schedule III.
S0	Apronalide	Sedative / Hypnotic	Lacks FDA approval
S2	ARA-290	Erythropoiesis	FDA Orphan Drug status
S0	Arecoline	Stimulant	Lacks FDA approval
S3	Arformoterol	Beta-2 agonist-bronchodilator	Brovana
S2	Argon	Hypoxia Inducible Factor activating	
S4	Arimistane (Anrosta-3,5-diene-7,17-dione)	Anabolic	Lacks FDA approval
S2	Asialo EPO	Erythropoiesis	
S0	Atenolol	Antihypertensive	Tenormin

1 – SPECIFIED SUBCLASSIFICATION
Specified substances designated with an X

HISA STATUS / SS[1]	SUBSTANCE	ACTION	COMMERCIAL / DEVELOPMENTAL NAME(S) WHERE AVAILABLE
S0	Atomoxetine	Stimulant	Strattera
S0	Azacylonol (γ-pipradrol)	CNS depressant	Lacks FDA approval
S0	Azaperone	Sedative	Stresnil
S0	Azapetine	Vasodilator	Lacks FDA approval
S0	Azapropazone	NSAID	Lacks FDA approval
S0	Azathioprine	Immunosuppressor	Imuran
S0	Azatidine (Azatadine)	Antihistamine	Discontinued, no FDA-approved product commercially available
S5	Azosemide	Diuretic	Lacks FDA approval
S0	Bambuterol	Beta-2 agonist-bronchodilator	Lacks FDA approval
S0	Bamifylline	Bronchodilator	Lacks FDA approval
S0	Barbital (Barbitone)	Sedative / Hypnotic	Lacks FDA approval. DEA Schedule IV.
S4	Bazedoxifene	Selective Estrogen Receptor Modulator (SERM)	FDA-approved in combination with Premarin as Duavee
S0	Beclamide	Anticonvulsant	Lacks FDA approval
S0	Bemegride	Stimulant	Lacks FDA approval

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BANNED

HISA STATUS / SS[1]	SUBSTANCE	ACTION	COMMERCIAL / DEVELOPMENTAL NAME(S) WHERE AVAILABLE
S0	Benactyzine	Anticholinergic	Lacks FDA approval
S0	Benapryzine	Anticholinergic	Lacks FDA approval
S0	Benazepril	Antihypertensive	Lotensin, Lotrel (with amlodipine)
S5	Bendroflumethiazide	Diuretic	Naturetin, Corzide
S0	Benorilate	NSAID	Lacks FDA approval
S0	Benoxaprofen	NSAID	Lacks FDA approval
S0	Benperidol	Antipsychotic	Lacks FDA approval
S0	Bentazepam	Anxiolytic	Lacks FDA approval
S0	Benzoctamine	Sedative / Anxiolytic	Lacks FDA approval
S0	Benzonatate	Antitussive	Tessalon
S0	Benzphetamine	Stimulant	Generic DEA Schedule III.
S0	Benzquinamide	Antipsychotic / Anti-emetic	Discontinued, no FDA-approved product commercially available
S0	Benzthiazide	Diuretic	Discontinued, no FDA-approved product commercially available
S0	Benzydamine	NSAID	Lacks FDA approval

1 – SPECIFIED SUBCLASSIFICATION
Specified substances designated with an X

HISA STATUS / SS[1]	SUBSTANCE	ACTION	COMMERCIAL / DEVELOPMENTAL NAME(S) WHERE AVAILABLE
S0	Benzylpiperazine (BZP)	Stimulant	Lacks FDA approval
S0	Bepridil	Antihypertensive	Lacks FDA approval
S0	Betaprodine	Opioid analgesic	Lacks FDA approval. DEA Schedule I.
S0	Betaxolol	Antihypertensive	Betoptic
S0	Bethanidine (Betanidine)	Antihypertensive	Discontinued, no FDA-approved product commercially available
S4	Bimagrumab	Anabolic	Lacks FDA approval (Orphan drug designation with-drawn)
S0	Biperiden	Anticholinergic	Discontinued, no FDA-approved product commercially available
S0	Biphenamine	Local anesthetic	Lacks FDA approval
S0	Biprsoprolol	Antihypertensive	Ziac [with hydrochlorothiazide]
S0	Biriperone (Centbutindole)	Antipsychotic	Lacks FDA approval
S0	Bitolterol	Beta-2 agonist-bronchodilator	Discontinued, no FDA-approved product commercially available
S1	Bolandiol (estr-4-ene3β, 17β-diol)	Anabolic	Lacks FDA approval
S1	Bolasterone (7α, 17α-dimethyltestosterone)	Anabolic	Lacks FDA approval. DEA Schedule III.
S1	*Boldenone	Anabolic	Equipoise DEA Schedule III.

*Threshold: 0.015 mcg free and conjugated boldenone per mL in urine in male horses (other than geldings)

BANNED

HISA STATUS / SS[1]	SUBSTANCE	ACTION	COMMERCIAL / DEVELOPMENTAL NAME(S) WHERE AVAILABLE
S1	Boldione	Anabolic	Lacks FDA approval. DEA Schedule III.
S6	Botulinum toxin	Neurotoxin	Botox, Dysport, Jeuveau
S0	Brallobarbital	Barbiturate	Lacks FDA approval
S0	Brimonidine	Antihypertensive	Alphagan P, Qoliana, Lumify
S0	Bromantan	Psychostimulant	Lacks FDA approval
S0	Bromazepam	Anxiolytic	Lacks FDA approval. DEA Schedule IV.
S0	Bromhexine	Mucolytic	Lacks FDA approval
S0	Bromisovalum	Sedative / Hypnotic	Lacks FDA approval
S0	Bromocriptine	Anticholinergic	Parlodel, Cycloset
S0	Bromophenethylamine	Psychedelic	Lacks FDA approval
S0	Bromperidol	Antipsychotic	Lacks FDA approval
S0	Brotizolam	Sedative / Hypnotic	Lacks FDA approval
S0	Bucetin	NSAID	Lacks FDA approval
S0	Buclizine	Antihistamine / Anti-emetic	Discontinued, no FDA-approved product commercially available
S0	Bufexamac	NSAID	Lacks FDA approval
S0	Buflomedil	Vasodilator	Lacks FDA approval
S0(x)	*Bufotenine	Hallucinogen	Lacks FDA approval. DEA Schedule I.

*Screening Limit: 10 mcg/mL Total (free and conjugated) in urine

1 – SPECIFIED SUBCLASSIFICATION
Specified substances designated with an X

HISA STATUS / SS[1]	SUBSTANCE	ACTION	COMMERCIAL / DEVELOPMENTAL NAME(S) WHERE AVAILABLE
S5	Bumetanide	Diuretic	Bumex
S0	Bunitrolol	Vasodilator	Lacks FDA approval
S0	Bunolol	Antihypertensive	Betagan
S0	Buphenine (Nylidrin)	Vasodilator	Lacks FDA approval
S0	Bupranolol	Antihypertensive	Lacks FDA approval
S0	Bupropion	Antidepressant	Wellbutrin, Zyban
S4	Buserelin	Gonadotropin Releasing Hormone	Lacks FDA approval
S0	Butabarbital (Secbutobarbitone)	Barbiturate	Discontinued, no FDA-approved product commercially available. DEA Schedule III.
S0	Butacaine	Local anesthetic	Lacks FDA approval
S0	Butalbital (Talbutal)	Barbiturate	Esgic, Fioricet DEA Schedule III.
S0	Butanilicaine	Local anesthetic	Lacks FDA approval
S0	Butaperazine	Antipsychotic	Lacks FDA approval
S0	Butoctamide	Serotonin release	Lacks FDA approval
S0	Butofilolol	Antihypertensive	Lacks FDA approval
S0	Butoxycaine	Local anesthetic	Lacks FDA approval

1 – SPECIFIED SUBCLASSIFICATION
Specified substances designated with an X

HISA STATUS / SS[1]	SUBSTANCE	ACTION	COMMERCIAL / DEVELOPMENTAL NAME(S) WHERE AVAILABLE
S0	Cafedrine	Cardiac stimulant	Lacks FDA approval
S0	Calcium dobesilate	Vasoprotective	Lacks FDA approval
S1	Calusterone (Methosarb, Riedemil, NSC-88536, U-22550)	Anabolic	Lacks FDA approval. (NSC-88536, U-22550) DEA Schedule III.
S0	Camazepam	Anxiolytic	Lacks FDA approval. DEA Schedule IV.
S0	Candesartan	Antihypertensive	Atacand
S0(x)	Cannabinoids (natural, synthetic and other cannabimimetics)	Psychotropic	Lack FDA approval
S5	Canrenone	Diuretic	Lacks FDA approval
S1	Capromorelin	Anabolic	Entyce, Elura
S0	Captodiame (Captodiamine)	Antihistamine	Lacks FDA approval
S0	Captopril	Antihypertensive	Generic
S0	Caramiphen	Anticholinergic	Lacks FDA approval
S0	Carazolol	Antihypertensive	Lacks FDA approval
S2	Carbamylated EPO (CEPO)	Erythropoiesis	
S0	Carbazochrome (Adrenochrome monosemicarbazone)	Hemostatic	Lacks FDA approval

1 – SPECIFIED SUBCLASSIFICATION
Specified substances designated with an X

HISA STATUS / SS[1]	SUBSTANCE	ACTION	COMMERCIAL / DEVELOPMENTAL NAME(S) WHERE AVAILABLE
S0	Carbetapentane (Pentoxyverine)	Antitussive	Lacks FDA approval
S0	Carbidopa	Decarboxylase Inhibitor	Lodosyn, Stalevo, Rytary, Duopa, Dhivy, Sinemet (all with levodopa)
S0	Carbimazole	Anti-hyperthyroidism	Lacks FDA approval
S0	Carbocysteine	Mucolytic	Lacks FDA approval
S0	Carbromal	Sedative / Hypnotic	Lacks FDA approval
S0	Carbuterol	Beta-2 agonist-bronchodilator	Lacks FDA approval
S1	Cardarine (GW-501, GW516, GSK-516)	Selective Androgen Receptor Modulator (SARM)	Lacks FDA approval
S0	Carfentanil	Opioid analgesic	Lacks FDA approval. DEA Schedule II.
S0	Carphedon	Psychostimulant	Lacks FDA approval
S0	Carphenazine	Antipsychotic	Discontinued, no FDA-approved product commercially available
S0	Carpipramine	Antipsychotic	Lacks FDA approval
S3	Carteolol	Antihypertensive	Generic
S0	Carvedilol	Antihypertensive	Coreg
S0	Cathinone	Stimulant	Lacks FDA approval. DEA Schedule I.
S0	Celiprolol	Antihypertensive	Lacks FDA approval

1 – SPECIFIED SUBCLASSIFICATION
Specified substances designated with an X

HISA STATUS / SS[1]	SUBSTANCE	ACTION	COMMERCIAL / DEVELOPMENTAL NAME(S) WHERE AVAILABLE
S0	Cephaeline	Emetic, plant alkaloid	Lacks FDA approval
S0	Chlomethiazole	Anticonvulsant	Lacks FDA approval
S0	Chloral (Cloral) betaine	Sedative / Hypnotic	Lacks FDA approval. DEA Schedule IV.
S0	Chloral hydrate	Sedative	Lacks FDA approval. DEA Schedule IV.
S0	Chloralose (AlphaChloralose)	Anxiolytic	Lacks FDA approval
S0	Chlorcyclizine	Antihistamine	Lacks FDA approval
S0	Chlordiazepoxide	Anxiolytic	Librium, Librax (with Chlordiazepoxide Hydrochloride) DEA Schedule IV.
S0	Chlormadinone acetate	Reproductive hormone	Lacks FDA approval
S0	Chlormerodrin	Diuretic	Discontinued, no FDA-approved product commercially available
S0	Chlormezanone	Muscle relaxant	Discontinued, no FDA-approved product commercially available
S0	Chloroform	Anesthetic	Lacks FDA approval
S0	Chlorophenylpiperazine	Psychoactive	Lacks FDA approval
S0	Chloropyramine	Antihistamine	Lacks FDA approval
S5	Chlorothiazide	Diuretic	Diuril
S0	Chlorphenesin	Muscle relaxant	Discontinued, no FDA-approved product commercially available

1 – SPECIFIED SUBCLASSIFICATION
Specified substances designated with an X

HISA STATUS / SS[1]	SUBSTANCE	ACTION	COMMERCIAL / DEVELOPMENTAL NAME(S) WHERE AVAILABLE
S0	Chlorphenoxamine	Antihistamine	Lacks FDA approval
S0	Chlorphentermine	Stimulant	Discontinued, no FDA-approved product commercially available
S0	Chlorproethazine	Muscle relaxant	Lacks FDA approval
S0	Chlorpromazine	Sedative	Generic
S0	Chlorpropamide	Hypoglycemic	Discontinued, no FDA-approved product commercially available
S0	Chlorprothixene	Antipsychotic	Discontinued, no FDA-approved product commercially available
S5	Chlorthalidone	Diuretic	Thalitone
S0	Chlorthenoxazine	NSAID	Lacks FDA approval
S5	Chlorthiazide (Chlorothiazide)	Diuretic	Diuril
S2	Chorionic Gonadotropin (CG) Intact males and geldings	Reproductive hormone	Pregnyl
S0	Cicloprofen	NSAID	Lacks FDA approval
S0	Cilazapril	Antihypertensive	Lacks FDA approval
S0	Cimaterol	Beta-2 agonist-bronchodilator	Lacks FDA approval
S3	Cimbuterol	Beta-2 agonist-bronchodilator	Lacks FDA approval
S0	Cinchocaine	Local anesthetic	Lacks FDA approval
S0	Cinchophen	NSAID	Lacks FDA approval

1 – SPECIFIED SUBCLASSIFICATION
Specified substances designated with an X

HISA STATUS / SS[1]	SUBSTANCE	ACTION	COMMERCIAL / DEVELOPMENTAL NAME(S) WHERE AVAILABLE
S0	Cinnarizine	Antihistamine	Lacks FDA approval
S0	Citalopram	Antidepressant	Celexa
S0	Clanobutin	Cholerectic	Lacks FDA approval
S0	Clemizole	Antihistamine	Lacks FDA approval
S3	Clenpenterol	Beta-2 agonist-bronchodilator	Lacks FDA approval
S0	Clibucaine	Local anesthetic	Lacks FDA approval
S0	Clidinium	Anticholinergic	No FDA-approved product
S0	Clobazam	Anxiolytic	Sympazan, Onfi DEA Schedule IV.
S0	Clobenzorex	Stimulant	Lacks FDA approval
S6	Clodronate (Clodronic acid)	Bisphosphonate	OsPhos
S5	Clofenamid	Carbonic Anhydrase Inhibitor	Lacks FDA approval
S0	Clomethiazole (Chlormethiazole)	Sedative / Hypnotic	Lacks FDA approval
S4	Clomifene	Induce ovulation	Generic
S0	Clomipramine	Antidepressant	Clomicalm
S0	Clonazepam	Anxiolytic	Klonopin DEA Schedule IV.
S0	Clonixin	NSAID	Lacks FDA approval

1 – SPECIFIED SUBCLASSIFICATION
Specified substances designated with an X

HISA STATUS / SS[1]	SUBSTANCE	ACTION	COMMERCIAL / DEVELOPMENTAL NAME(S) WHERE AVAILABLE
S5	Clopamide	Diuretic	Lacks FDA approval
S0	Cloranolol	Antihypertensive	Lacks FDA approval
S0	Clorazepate	Anxiolytic	Lacks FDA approval. DEA Schedule IV.
S0	Clormecaine	Local anesthetic	Lacks FDA approval
S0	Clorprenaline	Bronchodilator	Lacks FDA approval
S1	Clostebol	Anabolic	Lacks FDA approval. DEA Schedule III.
S0	Clotiapine	Antipsychotic	Lacks FDA approval
S0	Clotiazepam	Anxiolytic	Lacks FDA approval
S0	Cloxazolam	Anxiolytic	Lacks FDA approval. DEA Schedule IV.
S0	Clozapine	Antipsychotic	Clozaril, Versacloz
S2	CNTO 530	Erythropoiesis	Lacks FDA approval
S2	*Cobalt Salts (e.g., $CoCl_2$)	Erythropoiesis	
S6	Cobratoxin, alpha	Neurotoxin	Lacks FDA approval
S0	Cocaine (metabolite: benzoylecgonine)	Stimulant	Goprelto, Numbrino DEA Schedule II.
S0	Codeine	Opioid analgesic	Generic (DEA Schedule II) or in combination with NSAIDs, caffeine and other drugs (DEA Schedule III).

*Threshold: 0.1mcg/mL total Cobalt in urine OR 0.025 mcg/mL total (free and protein bound)/ mL in serum or plasma.

1 – SPECIFIED SUBCLASSIFICATION
Specified substances designated with an X

HISA STATUS / SS[1]	SUBSTANCE	ACTION	COMMERCIAL / DEVELOPMENTAL NAME(S) WHERE AVAILABLE
S0	Conorphone	Opioid analgesic	Lacks FDA approval
S2	Corticorelin	Corticosteroid stimulation	Approved Orphan Drug
S0	Cortivazol	Glucocorticoid	Lacks FDA approval
S0	Cotinine (Cotinine is a metabolite of nicotine. If there is credible evidence that the presence of cotinine in a horse's sample is a consequence of nicotine exposure, the classification of cotinine may be revised to S7(A).)	Psychoactive / Anxiolytic	Lacks FDA approval
S0	Cropropamide	Respiratory Stimulant	Lacks FDA approval
S0	Crotethamide	Respiratory Stimulant	Lacks FDA approval
S0	Cyamemazine	Antipsychotic	Lacks FDA approval
S0	Cyclandelate	Vasodilator	Lacks FDA approval
S0	Cyclizine	Antihistamine	Discontinued, no FDA-approved product commercially available
S0	Cyclobarbital	Barbiturate	Lacks FDA approval
S4	Cyclofenil	Selective Estrogen Receptor Modulator (SERM)	Lacks FDA approval
S0	Cycloguanil	Antimalarial	Lacks FDA approval

1 – SPECIFIED SUBCLASSIFICATION
Specified substances designated with an X

HISA STATUS / SS[1]	SUBSTANCE	ACTION	COMMERCIAL / DEVELOPMENTAL NAME(S) WHERE AVAILABLE
S0	Cyclomethycaine	Local anesthetic	Lacks FDA approval
S0	Cyclopentamine	Vasoconstrictor	Lacks FDA approval
S0	Cyclophenil	Selective Estrogen Receptor Modulator (SERM)	Lacks FDA approval
S0	Cyclothiazide	Diuretic	Discontinued, no FDA-approved product commercially available
S0	Cycrimine	Anticholinergic	Discontinued, no FDA-approved product commercially available
S4	Dalantercept (ACE-041)	Anti-neoplastic	Lacks FDA approval
S1	Danazol	Anabolic	Generic
S2	Darbepoetin (dEPO)	Erythropoiesis	Aranesp
S0	Decamethonium	Muscle relaxant	Discontinued, no FDA-approved product commercially available
S1	Dehydrochloromethyltestosterone	Anabolic	Turinabol DEA Schedule III.
S0	Delmadinone acetate	Reproductive hormone	Lacks FDA approval
S0	Delorazepam	Anxiolytic	Lacks FDA approval. DEA Schedule IV.
S0	Dembroxol (Dembrexine)	Mucolytic	Lacks FDA approval
S0(x)	Demecolcine	Anti-neoplastic / Immunomodulator	Lacks FDA approval
S0	Demoxepam	Anxiolytic	Lacks FDA approval

1 – SPECIFIED SUBCLASSIFICATION
Specified substances designated with an X

HISA STATUS / SS[1]	SUBSTANCE	ACTION	COMMERCIAL / DEVELOPMENTAL NAME(S) WHERE AVAILABLE
S0	Deoxycorticosterone	Minerlocorticoid	Lacks FDA approval
S0	Deptropine	Antihistamine	Lacks FDA approval
S6	Dermorphin	Opioid Receptor Agonist	Lacks FDA approval
S0	Deserpidine	Antihypertensive	Discontinued, no FDA-approved product commercially available
S0	Desipramine	Antidepressant	Norpramin
S4	Deslorelin Intact males and geldings	Reproductive hormone	Ovuplant, SucroMate, Suprelorin
S5	Desmopressin	Anti-diuretic	DDAVP, Nocdurna
S1	Desoxymethyltestosterone	Anabolic	Lacks FDA approval. DEA Schedule III.
S1	Desoxyvinyl-testosterone	Anabolic	Lacks FDA approval
S0	Dextromoramide	Opioid analgesic	Lacks FDA approval. DEA Schedule I.
S0	Dextropropoxyphene	Opioid analgesic	Discontinued, no FDA-approved product commercially available. DEA Schedule IV.
S0	Dextrorphan (Dextrorphan may be present as a metabolite of dextromethorphan. If there is credible evidence that the presence of dextrorphan in the horse's sample is the consequence of dextromethorphan administration, the classification of dextrorphan may be revised to S7(A).)	Psychoactive / Antitussive	Lacks FDA approval

1 – SPECIFIED SUBCLASSIFICATION
Specified substances designated with an X

HISA STATUS / SS[1]	SUBSTANCE	ACTION	COMMERCIAL / DEVELOPMENTAL NAME(S) WHERE AVAILABLE
S0	Dezocine	Opioid analgesic	Discontinued, no FDA-approved product commercially available
S0	Diacerein	Anti-osteoarthritic	Lacks FDA approval
S0	Diamorphine (Diacetylmorphine)	Opioid analgesic	Lacks FDA approval. DEA Schedule I.
S0	Diazoxide	Antihypertensive / Hyperglycemic	Proglycem
S0	Dibenzepin	Antidepressant	Lacks FDA approval
S0	Dibucaine	Local anesthetic	Discontinued, no FDA-approved product commercially available
S0	Dichlorisone	Corticosteroid	Lacks FDA approval
S0	Dichloroacetate	Anti-neoplastic	Lacks FDA approval
S0	Dicumarol	Anticoagulant	Discontinued, no FDA-approved product commercially available
S0	Diethylpropion	Stimulant	Lacks FDA approval. DEA Schedule IV.
S0	Diethylthiambutene	Opioid analgesic	Lacks FDA approval. DEA Schedule I.
S0	Diethyltryptamine (DET)	Hallucinogen	Lacks FDA approval
S0	Diflucortolone	Corticosteroid	Lacks FDA approval
S0	Diflunisal	NSAID	Generic
S0	Digitoxin	Antiarrhythmic	Discontinued, no FDA-approved product commercially available
S0	Dihydrocodeine	Opioid analgesic	Trezix (with acetaminophen and caffeine) DEA Schedule III.

1 – SPECIFIED SUBCLASSIFICATION
Specified substances designated with an X

HISA STATUS / SS[1]	SUBSTANCE	ACTION	COMMERCIAL / DEVELOPMENTAL NAME(S) WHERE AVAILABLE
S0	Dihydrocodeinone	Opioid analgesic	Lacks FDA approval
S0	Dihydromorphine	Opioid analgesic	Lacks FDA approval. DEA Schedule I.
S1	Dihydrotestosterone (17β-hydroxy- 5a androstan-3-one, Androstanolone)	Anabolic	Anabolex, Andractimm, Pesomax, Stanolone DEA Schedule III.
S0	Diisopropylamine	Vasodilator	Lacks FDA approval
S0	Diltiazem	Antihypertensive	Cardizem CD, Taztia XT, Tiazac
S0	Dimefline	Respiratory stimulant	Lacks FDA approval
S0	Dimethindene	Antihistamine	Lacks FDA approval
S0	Dimethisoquin (Quinocaine)	Local anesthetic	Lacks FDA approval
S0	Dimethylamphetamine	Stimulant	Lacks FDA approval
S0	Dimethylphenidate	Stimulant	Lacks FDA approval
S0(x)	*Dimethyltryptamine (DMT)	Hallucinogen	Lacks FDA approval. DEA Schedule I.
S0	Diphenadione	Anticoagulant	No FDA-approved product. Rodenticide
S0	Diphenylpyraline	Antihistamine	Discontinued, no FDA-approved product commercially available
S0	Dipipanone	Opioid analgesic	Lacks FDA approval. DEA Schedule I.
S0	Diprenorphine	Narcotic antagonist	M50-50

*Threshold: 10 mcg/mL total (free and conjugated) in urine

1 – SPECIFIED SUBCLASSIFICATION
Specified substances designated with an X

HISA STATUS / SS[1]	SUBSTANCE	ACTION	COMMERCIAL / DEVELOPMENTAL NAME(S) WHERE AVAILABLE
S0	Diprophylline	Bronchodilator	Lacks FDA approval
S0	Disulfiram	Alcohol antagonist	Generic
S0	Divalproex	Anticonvulsant	Depakote
S0	Dixyrazine	Antipsychotic	Lacks FDA approval
S4	Domagrozumab	Anabolic	Lacks FDA approval
S0	Donepezil	Behavior and cognitive modifier	Adilarity, Aricept
S0	Dopexamine	Vasodilator	Lacks FDA approval
S0	Dothiepin	Antidepressant	Lacks FDA approval
S0	Doxacurium	Muscle relaxant	Discontinued, no FDA-approved product commercially available
S0	Doxazosin	Antihypertensive	Cardura
S0	Doxefazepam	Anxiolytic	Lacks FDA approval
S1	Dromostanolone (Drostanolone)	Anabolic	Lacks FDA approval
S0	Droperidol	Antipsychotic	Inapsine
S0	Drospirenone	Reproductive hormone	Slynd, Nextstellis, Angeliq, Lo-Zumandimine, Loryna, Elamisa, Nikki, Yaz
S0	Duloxetine	Antidepressant	Cymbalta, Drizalma
S0	Dyphylline (Diphylline)	Antipsychotic / Anti-emetic	Discontinued, no FDA-approved product commercially available

1 – SPECIFIED SUBCLASSIFICATION
Specified substances designated with an X

HISA STATUS / SS[1]	SUBSTANCE	ACTION	COMMERCIAL / DEVELOPMENTAL NAME(S) WHERE AVAILABLE
S0	Edrophonium	Muscle strengthener	Discontinued, no FDA-approved product commercially available
S2	Efaproxiral (RSR13)	Hemoglobin modifier	FDA orphan drug
S0	Eletriptan	Selective Serotonin Receptor Agonist	Relpax
S0	Eltenac	NSAID	Lacks FDA approval
S0	Embramine	Antihistamine	Lacks FDA approval
S0	Embutramide	Opioid analgesic	Lacks FDA approval. DEA Schedule III.
S0	Emepronium	Antispasmodic	Lacks FDA approval
S0	Emidonol	Anti-inflammatory	Lacks FDA approval
S0	Enalapril (metabolite enaloprilat)	Angiotensin-converting enzyme inhibitor	Vasotec
S0	Enciprazine	Anxiolytic / Antipsychotic	Lacks FDA approval
S6	Epibatidine	Analgesic	Lacks FDA approval
S1	Epi-dihydrotestosterone	Anabolic	Lacks FDA approval. DEA Schedule III.
S1	Epitestosterone	Anabolic	Lacks FDA approval. DEA Schedule III.
S0	Eplerenone	Antihypertensive	Inspra
S2	EPO-based constructs (e.g. EPO-Fc)	Erythropoiesis	Lacks FDA approval
S2	EPO-mimetic agents (e.g. CNTO-530, peginesatide)	Erythropoiesis	

1 – SPECIFIED SUBCLASSIFICATION
Specified substances designated with an X

HISA STATUS / SS[1]	SUBSTANCE	ACTION	COMMERCIAL / DEVELOPMENTAL NAME(S) WHERE AVAILABLE
S0	Ergonovine	Ergot alkaloid	Lacks FDA approval
S0(x)	Ergotamine	Ergot alkaloid	Ergomar, Migergot (with caffeine)
S0	Erythritol tetranitrate	Vasodilator	Lacks FDA approval
S2	Erythropoietin (EPO)	Erythropoiesis	
S0	Esmolol	Antihypertensive	Brevibloc
S0	Estazolam	Sedative / Anticonvulsant	Prosom DE Schedule IV.
S0	Eszopiclone	Hypnotic	Lunesta
S0	Etafedrine	Bronchodilator	Lacks FDA approval
S0	Etamiphylline	Respiratory Stimulant	Lacks FDA approval
S0	Ethamivan (Etamivan)	Respiratory Stimulant	Lacks FDA approval
S5	Ethacrynic acid (Etacrynic acid)	Diuretic	Edecrin
S0	Ethamsylate	Antihemorrhagic	Lacks FDA approval
S0	Ethanol	Depressant	Grain alcohol, Everclear
S0	Ethaverine	Vasodilator	Lacks FDA approval
S0	Ethchlorvynol	Sedative / Hypnotic	Discontinued, no FDA-approved product commercially available. DEA Schedule IV.
S0	Ethiazide	Diuretic	Lacks FDA approval

1 – SPECIFIED SUBCLASSIFICATION
Specified substances designated with an X

HISA STATUS / SS[1]	SUBSTANCE	ACTION	COMMERCIAL / DEVELOPMENTAL NAME(S) WHERE AVAILABLE
S0	Ethinamate	Sedative / Hypnotic	Discontinued, no FDA-approved product commercially available. DEA Schedule IV.
S0	Ethinylestradiol	Reproductive hormone	Lacks FDA approval
S0	Ethoheptazine	Analgesic	Lacks FDA approval
S0	Ethopropazine	Anticholinergic	Discontinued, no FDA-approved product commercially available
S0	Ethosuximide	Anticonvulsant	Zarontin
S0	Ethotoin	Anticonvulsant	Discontinued, no FDA-approved product commercially available
S0	Ethoxzolamide	Carbonic Anhydrase Inhibitor	Discontinued, no FDA-approved product commercially available
S0	Ethyl isobutrazine	Sedative	Lacks FDA approval
S0	Ethyl Loflazepate	Sedative / Anxiolytic	Discontinued, no FDA-approved product commercially available. DEA Schedule IV.
S0	Ethylamphetamine	Stimulant	Lacks FDA approval
S1	Ethylestrenol	Anabolic	Discontinued, no FDA-approved product commercially available. DEA Schedule III.
S0	Ethylmorphine	Opioid analgesic	Lacks FDA approval. DEA Schedule II.
S0	Ethylnorepinephrine	Stimulant	Lacks FDA approval
S0	Ethylphenidate	Stimulant	Lacks FDA approval
S0	Etidocaine	Local anesthetic	Discontinued, no FDA-approved product commercially available

1 – SPECIFIED SUBCLASSIFICATION
Specified substances designated with an X

HISA STATUS / SS[1]	SUBSTANCE	ACTION	COMMERCIAL / DEVELOPMENTAL NAME(S) WHERE AVAILABLE
S0	Etifoxine (Etafenoxine)	Anticonvulsant	Lacks FDA approval
S0	Etilefrine	Stimulant	Lacks FDA approval
S0	Etiocholanolone	Anabolic	Lacks FDA approval
S0	Etizolam	Anxiolytic	Lacks FDA approval
S0	Etodroxizine	Antihistamine	Lacks FDA approval
S0	Etofenamate	NSAID	Lacks FDA approval
S0	Etomidate	Anesthetic	Amidate
S0	Etoricoxib	NSAID	Lacks FDA approval
S0	Etorphine HCl	Opioid analgesic	M99 DEA Schedule II.
S2	Examorelin (Hexarelin)	Growth hormone	Lacks FDA approval
S4	Exemestane	Aromatase inhibitor	Aromasin
S0	Famprofazone	NSAID	Lacks FDA approval
S0	Febarbamate	Anxiolytic	Lacks FDA approval
S0	Felbamate	Anticonvulsant	Trezix, Tuxari, Triacin-C
S0	Felbinac	NSAID	Lacks FDA approval
S0	Felodipine	Antihypertensive	Generic
S0	Fenbufen	NSAID	Felbatol

1 – SPECIFIED SUBCLASSIFICATION
Specified substances designated with an X

HISA STATUS / SS[1]	SUBSTANCE	ACTION	COMMERCIAL / DEVELOPMENTAL NAME(S) WHERE AVAILABLE
S0	Fenbutrazate	Psychostimulant	Lacks FDA approval
S0	Fencamfamine	Stimulant	Lacks FDA approval. DEA Schedule IV.
S0	Fencamine	Psychostimulant	Lacks FDA approval
S0	Fenclofenac	NSAID	Lacks FDA approval
S0	Fenclozic acid	NSAID	Lacks FDA approval
S0	Fenetylline (Fenetylline, Phenethylline, Phenetylline)	Psychostimulant	Lacks FDA approval
S0	Fenfluramine	Stimulant	Fintepla. DEA Schedule IV.
S3	Fenoterol	Beta-2 agonist-bronchodilator	Lacks FDA approval
S0	Fenozolone	Psychostimulant	Lacks FDA approval
S0	Fenpiprane	Antispasmodic	Lacks FDA approval
S0	Fenproporex	Stimulant	Lacks FDA approval. DEA Schedule IV.
S0	Fenspiride	Bronchodilator	Lacks FDA approval
S0	Fentiazac	NSAID	Lacks FDA approval
S0	Feprazone	NSAID	Lacks FDA approval
S2	Fibroblast Growth Factors (FGFs)	Growth Hormone	
S0	Flavoxate	Anticholinergic	Generic
S0	Floctafenine	NSAID	Lacks FDA approval

1 – SPECIFIED SUBCLASSIFICATION
Specified substances designated with an X

HISA STATUS / SS[1]	SUBSTANCE	ACTION	COMMERCIAL / DEVELOPMENTAL NAME(S) WHERE AVAILABLE
S0	Fluanisone	Antipsychotic	Lacks FDA approval
S0	Fludiazepam	Anxiolytic	Lacks FDA approval. DEA Schedule IV.
S0	Fludrocortisone	Corticosteroid	Generic
S0	Flufenamic acid	NSAID	Lacks FDA approval
S5	Flumethiazide	Diuretic	Discontinued, no FDA-approved product commercially available
S0	Flunarizine	Calcium channel blocker	Lacks FDA approval
S0	Flunisolide	Corticosteroid	Generic
S0	Flunitrazepam	Sedative / Anxiolytic	Lacks FDA approval. DEA Schedule IV.
S0	Fluocortolone	Corticosteroid	Lacks FDA approval
S0	Fluopromazine (Triflupromazine)	Antipsychotic	Lacks FDA approval
S0	Fluoresone	Anticonvulsant	Lacks FDA approval
S0	Fluorocortisone	Corticosteroid	Lacks FDA approval
S0	Fluorophenethylamine	Stimulant	Lacks FDA approval
S0	Fluoroprednisolone	Corticosteroid	Discontinued, no FDA-approved product commercially available
S0	Fluoxetine	Antidepressant	Prozac
S1	Fluoxymesterone	Anabolic	Discontinued, no FDA-approved product commercially available. DEA Schedule III.

1 – SPECIFIED SUBCLASSIFICATION
Specified substances designated with an X

HISA STATUS / SS[1]	SUBSTANCE	ACTION	COMMERCIAL / DEVELOPMENTAL NAME(S) WHERE AVAILABLE
S0	Flupenthixol (Flupentixol)	Antipsychotic	Lacks FDA approval
S0	Flupirtine	Analgesic	Lacks FDA approval
S0	Fluprednisolone	Corticosteroid	Discontinued, no FDA-approved product commercially available
S0	Flurazepam	Sedative / Anxiolytic	Generic. DEA Schedule IV.
S0	Fluspirilene	Antipsychotic	Lacks FDA approval
S0	Flutoprazepam	Sedative / Anxiolytic	Lacks FDA approval
S0	Fluvoxamine	Antidepressant	Luvox
S4	Follistatin	Myostatin inhibitor	
S1	Formebolone	Anabolic	Lacks FDA approval. DEA Schedule III.
S4	Formestane	Aromatase inhibitor	Lacks FDA approval
S3	Formoterol (Aformoterol)	Beta-2 agonist-bronchodilator	Brovana; Breyna (with budesonide); Duaklir Pressair (with aclidinium)
S0	Fosinopril	Antihypertensive	Generic
S0	Fosphenytoin	Anticonvulsant	Cerebyx
S4	Fulvestrant	Estrogen antagonist	Falsodex
S1	Furazabol	Anabolic	Lacks FDA approval. DEA Schedule III.
S0	Furazadrol	Anabolic	Lacks FDA approval

1 – SPECIFIED SUBCLASSIFICATION
Specified substances designated with an X

HISA STATUS / SS[1]	SUBSTANCE	ACTION	COMMERCIAL / DEVELOPMENTAL NAME(S) WHERE AVAILABLE
S0	Furfenorex	Stimulant	Lacks FDA approval
S0	Galantamine	Acetylcholinesterase inhibitor	Razadyne
S0	Gallamine	Muscle relaxant	Discontinued, no FDA-approved product commercially available
S0	Gamma Aminobutyric Acid (GABA)	Neurotransmitter	Endogenous substance
S0	Gamma-butyrolactone (GBL)	Neurohormone	Lacks FDA approval
S0	Gamma-hydroxybutyrate (GHB)	CNS depressant	Lacks FDA approval
S0	Gepirone	Antidepressant	Lacks FDA approval
S1	Gestrinone	Anabolic	Lacks FDA approval. DEA Schedule III.
S1	GH-Releasing Peptides (ghrps), e.g., alexamorelin, GHRP-6, hexarelin and pralmorelin (GHRP-2)	Growth Hormone	
S0(x)	*Glaucine	Antitussive	Lacks FDA approval
S0	Glutethimide (Chlorhexidol)	Sedative	Discontinued, no FDA-approved product commercially available. DEA Schedule II.
S4	Gonadorelin Intact males and geldings	Reproductive hormone modulator	Cystorelin, Factrel, Fertelin, OvaCyst, Fertagyl, Gonabreed
S2	Goserelin	Reproductive hormone modulator	Zoladex

*Screening Limit: 0.5 ng/mL in serum or plasma

1 – SPECIFIED SUBCLASSIFICATION
Specified substances designated with an X

HISA STATUS / SS[1]	SUBSTANCE	ACTION	COMMERCIAL / DEVELOPMENTAL NAME(S) WHERE AVAILABLE
S1	Growth Hormone Releasing Hormone (GHRH)	Anabolic	
S0	Guanadrel	Antihypertensive	Discontinued, no FDA-approved product commercially available
S0	Guanethidine	Antihypertensive	Discontinued, no FDA-approved product commercially available
S0	Guanoclor	Antihypertensive	Lacks FDA approval
S0	Halazepam	Sedative / Anxiolytic	Discontinued, no FDA-approved product commercially available. DEA Schedule IV.
S0	Haldrol	Anabolic	Lacks FDA approval
S0	Haloperidol	Antipsychotic	Haldol
S0	Haloxazolam	Sedative / Anxiolytic	Lacks FDA approval
S0	Harmaline	Psychoactive	Lacks FDA approval
S2	Hepatocyte Growth Factor (HGF)	Growth Hormone	
S0	Heptaminol	Cardiac stimulant	
S0	Hexafluorenium	Muscle relaxant	Discontinued, no FDA-approved product commercially available
S0	Hexobarbital	Sedative	Lacks FDA approval
S0	Hexocyclium	Anticholinergic	Discontinued, no FDA-approved product commercially available
S0	Hexylcaine	Local anesthetic	Discontinued, no FDA-approved product commercially available

1 – SPECIFIED SUBCLASSIFICATION
Specified substances designated with an X

HISA STATUS / SS[1]	SUBSTANCE	ACTION	COMMERCIAL / DEVELOPMENTAL NAME(S) WHERE AVAILABLE
S3	Higenamine (Norclaurine, Demethylcoclaurine)	Bronchodilator	Constituent of numerous OTC dietary supplements marketed for weight loss or as sports/energy supplements. Lacks FDA approval
S0	Histapyrrodine	Antihistamine	Lacks FDA approval
S4	Histrelin	GnRH agonist	Supprelin LA, Vantas
S0	Homophenazine	Antipsychotic	Lacks FDA approval
S5	Hydrochlorthiazide	Diuretic	Lotensin (with Bisoprolol), Vaseretic (with Enalapril), Avilide (with Irbesartan), Zestoretic (with Lisinopril), Lopressor (with Metoprolol), Micardis (with Telmisartan), and others
S0	Hydrocodone (Dihydrocodienone)	Opioid analgesic	Hysingla, Apadaz, Anexsia (with acetaminophen); Hycodan (with homatropine) DEA Schedule II.
S5	Hydroflumethiazide	Diuretic	Discontinued, no FDA-approved product commercially available
S0	Hydromorphinol	Opioid analgesic	Lacks FDA approval
S0	Hydromorphone	Opioid analgesic	Dilaudid DEA Schedule II.
S0	Hydroxyamphetamine	Stimulant	Paremyd (with Tropicamide)
S0	Hydroxy-gamma amino butyric acid	Neurohormone	Lacks FDA approval
S0	Hydroxytestosterone	Anabolic	Lacks FDA approval
S6	Ibandronate	Bisphosphonate	Generic

1 – SPECIFIED SUBCLASSIFICATION
Specified substances designated with an X

HISA STATUS / SS[1]	SUBSTANCE	ACTION	COMMERCIAL / DEVELOPMENTAL NAME(S) WHERE AVAILABLE
S0	Ibogaine	Psychoactive	Lacks FDA approval. DEA Schedule I.
S2	Ibutamoren	Growth Hormone	Investigational New Drug (in clinical trials)
S0	Iloprost	Vasodilator	Ventavis
S3	Indacaterol	Beta-2 agonist-bronchodilator	Discontinued, no FDA-approved product commercially available
S5	Indapamide	Diuretic	Generic
S0	Indoprofen	NSAID	Lacks FDA approval
S0	Indoramin	Antihypertensive	Lacks FDA approval
S2	Insulin- like Growth Factor-1 (IGF-1) and its analogues	Peptide hormone	
S2	Insulins	Anti-hyperglycemics	
S2	IOX-2	Erythropoiesis	Lacks FDA approval
S2	Ipamorelin	Growth Hormone	Lacks FDA approval
S0	Iprindole	Antidepressant	Lacks FDA approval
S0	Iproniazid	Antidepressant	Lacks FDA approval
S0	Ipsapirone	Antidepressant	Lacks FDA approval
S0	Irbesartan	Antihypertensive	Avalide, Avapro
S0	Isoaminile	Antitussive	Lacks FDA approval

1 – SPECIFIED SUBCLASSIFICATION
Specified substances designated with an X

HISA STATUS / SS[1]	SUBSTANCE	ACTION	COMMERCIAL / DEVELOPMENTAL NAME(S) WHERE AVAILABLE
S0	Isocarboxazid	Antidepressant	Marplan
S0	Isoetharine	Bronchodilator	Discontinued, no FDA-approved product commercially available
S0	Isomethadone (Isoamidone)	Synthetic Opioid analgesic	Lacks FDA approval. DEA Schedule II.
S0	Isometheptene	Sympathomimetic	Lacks FDA approval
S0	Isopropamide	Anticholinergic	Discontinued, no FDA-approved product commercially available
S3	Isoproterenol	Beta-2 agonist	Generic
S0	Isopyrin (Raminfenazone)	NSAID	Lacks FDA approval
S0	Isothipendyl	Antihistamine	Lacks FDA approval
S0	Isoxicam	NSAID	Lacks FDA approval
S0	Isoxsuprine	Vasodilator	Lacks FDA approval
S0	Isradipine	Antihypertensive	Generic
S0	Kebuzone	NSAID	Lacks FDA approval
S0	Ketazolam	Sedative / Anxiolytic	Lacks FDA approval. DEA Schedule IV.
S2	Krypton	Hypoxia Inducible Factor activating	
S0	Labetalol	Antihypertensive	Trandate
S4	Landogrozumab	Myostatin inhibitor	Lacks FDA approval

1 – SPECIFIED SUBCLASSIFICATION
Specified substances designated with an X

HISA STATUS / SS[1]	SUBSTANCE	ACTION	COMMERCIAL / DEVELOPMENTAL NAME(S) WHERE AVAILABLE
S2	Lenomorelin (Ghrelin)	Growth Hormone	Lacks FDA approval
S0	Lenperone	Antipsychotic	Lacks FDA approval
S0	Leptazole (Pentylenetetrazole)	Stimulant	Lacks FDA approval
S0	Letosteine	Mucolytic	Lacks FDA approval
S2	Letrozole	Aromatase inhibitor	Femara
S2	Leuprorelin (Leuprolide)	Reproductive hormone modulator	Eligard Kit, Fensolvi Kit, Camcevi Kit
S0	Levallorphan	Opioid Antagonist	Discontinued, no FDA-approved product commercially available
S0	Levobunolol	Antihypertensive	Betagan
S0	Levocabastine	Antihistamine	Discontinued, no FDA-approved product commercially available
S0	Levodopa	Decarboxylase Inhibitor	Inbrija, Stalevo, Rytary, Duopa, Dhivy, Sinemet (all with carbidopa)
S0	Levomethadone	Opioid analgesic	Lacks FDA approval
S0	Levomethorphan	Opioid analgesic	Lacks FDA approval. DEA Schedule II.
S0	Levophacetoperane	Psychostimulant	Lacks FDA approval
S0	Levorphanol	Opioid analgesic	Generic DEA Schedule II.
S3	Levosalbutamol (Levalbuterol)	Beta-2 agonist-bronchodilator	Xopenex

1 – SPECIFIED SUBCLASSIFICATION
Specified substances designated with an X

HISA STATUS / SS[1]	SUBSTANCE	ACTION	COMMERCIAL / DEVELOPMENTAL NAME(S) WHERE AVAILABLE
S4	Levothyroxine	Metabolic hormone	Thyro-Tabs, ThyroKare, Tirosint, Ermeza,Euthyrox, Levolet, Synthroid, Levoxyl, Unithroid
S0	Lidoflazine	Vasodilator	Lacks FDA approval
S4	Ligandrol (LGD-4033)	Selective Androgen Receptor Modulator (SARM)	Lacks FDA approval
S0	Lisinopril	Antihypertensive	Zestoretic, Qbrelis
S0	Lithium	Mood Stabilizer	Lithobid
S0(x)	*Lobeline	Respiratory Stimulant	Plant alkaloid (Lobelia, Indian Tobacco) Environmental substance. Lacks FDA approval.
S0	Lofentanil	Opioid analgesic	Lacks FDA approval
S0	Lofepramine	Antidepressant	Lacks FDA approval
S0	Loflazepate, Ethyl	Anxiolytic	Lacks FDA approval
S2	Lonapegsomatropin	Growth Hormone	FDA Orphan Drug
S0	Loprazolam	Anxiolytic	Lacks FDA approval. DEA Schedule IV.
S0	Lorazepam	Anxiolytic	Ativan DEA Schedule IV.
S0	Lormetazepam	Sedative / Anxiolytic	Lacks FDA approval
S0	Lornoxicam	NSAID	Lacks FDA approval. DEA Schedule IV.
S0	Losartan	Antihypertensive	Cozaar, Hyzaar [with hydrochlorothiazide]

*Screening Limit: 2 ng/mL in serum or plasma

1 – SPECIFIED SUBCLASSIFICATION
Specified substances designated with an X

BANNED

HISA STATUS / SS[1]	SUBSTANCE	ACTION	COMMERCIAL / DEVELOPMENTAL NAME(S) WHERE AVAILABLE
S0	Loxapine	Antipsychotic	Adasuve
S3	Lubabegron	Beta adrenergic modulator	Experior
S0	Lumiracoxib	NSAID	Lacks FDA approval
S2	Luspatercept	Erythropoiesis	FDA Orphan Drug
S2	Luteinizing Hormone (LH)	Reproductive hormone modulator	
S3	Mabuterol	Beta-2 agonist-bronchodilator	Lacks FDA approval
S2	Macimorelin	Growth Hormone	Macrilen
S0	Maprotiline	Antidepressant	Discontinued, no FDA-approved product commercially available
S0	Mazindol	Stimulant	Discontinued, no FDA-approved product commercially available. DEA Schedule IV.
S0	Mebanazine	Antidepressant	Lacks FDA approval
S0	Mebeverine	Antispasmodic	Lacks FDA approval
S0	Mebhyroline (Mebhydrolin)	Antihistamine	Lacks FDA approval
S0	Mebutamate	Sedative / Anxiolytic	Discontinued, no FDA-approved product commercially available. DEA Schedule IV.
S0	Mecamylamine	Vasodilator	Generic
S2	Mechano Growth Factors (MGFs)	Growth Hormone	
S0	Meclizine	Antihistamine	Antivert

1 – SPECIFIED SUBCLASSIFICATION
Specified substances designated with an X

HISA STATUS / SS[1]	SUBSTANCE	ACTION	COMMERCIAL / DEVELOPMENTAL NAME(S) WHERE AVAILABLE
S1	Meclofenoxate	Cholinergic nootropic	Lacks FDA approval
S0(x)	Meconine	Opioid	Lacks FDA approval
S0	Medazepam	Sedative / Anxiolytic	Lacks FDA approval. DEA Schedule IV.
S0	Medrylamine	Antihistamine	Lacks FDA approval
S0	Medrysone	Corticosteroid	Discontinued, no FDA-approved product commercially available
S0	Mefenamic acid	NSAID	Ponstel
S0	Mefenorex	Stimulant	Lacks FDA approval. DEA Schedule IV.
S0	Mefexamide	Stimulant	Lacks FDA approval
S0	Mefruside	Diuretic	Lacks FDA approval
S2	Meldonium	Anti-ischemic	Lacks FDA approval
S0	Melperone	Antipsychotic	Lacks FDA approval
S0	Memantine	Alzheimer's treatment	Namenda, Namzaric (with donepezil)
S0	Meparfynol (Methylpentynol)	Sedative	Lacks FDA approval
S0	Mepazine	Antipsychotic	Lacks FDA approval

1 – SPECIFIED SUBCLASSIFICATION
Specified substances designated with an X

HISA STATUS / SS[1]	SUBSTANCE	ACTION	COMMERCIAL / DEVELOPMENTAL NAME(S) WHERE AVAILABLE
S0	Mepednisone	Corticosteroid	Lacks FDA approval
S0	Mepenzolate	Anti-ulcer	Discontinued, no FDA-approved product commercially available
S0	Meperidine	Opioid analgesic	Demerol DEA Schedule II.
S0	Mephenesin	Muscle relaxant	Lacks FDA approval
S0	Mephenoxalone	Muscle relaxant	Lacks FDA approval
S0	Mephentermine	Cardiac stimulant	Discontinued, no FDA-approved product commercially available
S0	Mephenytoin	Anticonvulsant	Discontinued, no FDA-approved product commercially available
S0	Mephobarbital (Methylphenobarbital)	Sedative / Anxiolytic	Lacks FDA approval
S0	Mepindolol	Beta blocker	Lacks FDA approval
S0	Meprobamate (Meprobamate is a metabolite of cariso-prodol. If there is credible evidence that the presence of meprobamate in a horse's sample is the consequence of carisoprodol administration, the classification of meprobamate may be revised to S7(A).)	Anxiolytic	Generic DEA Schedule IV.
S0	Meprylcaine	Local anesthetic	Lacks FDA approval

1 – SPECIFIED SUBCLASSIFICATION
Specified substances designated with an X

HISA STATUS / SS[1]	SUBSTANCE	ACTION	COMMERCIAL / DEVELOPMENTAL NAME(S) WHERE AVAILABLE
S0	Meptazinol	Narcotic	Lacks FDA approval
S5	Meralluride	Diuretic	Lacks FDA approval
S5	Merbaphen	Diuretic	Lacks FDA approval
S5	Mercaptomerin	Diuretic	Lacks FDA approval
S0	Mersalyl	Diuretic	Discontinued, no FDA-approved product commercially available
S0	Mesocarb	Stimulant	Lacks FDA approval
S0	Mesoridazine	Antipsychotic	Discontinued, no FDA-approved product commercially available
S1	Mestanolone	Anabolic	Lacks FDA approval. DEA Schedule III.
S1	Mesterolone	Anabolic	Lacks FDA approval. DEA Schedule III.
S0	Metaclazepam	Anxiolytic	Lacks FDA approval
S1	Metandienone	Anabolic	Lacks FDA approval
S3	Metaproterenol (Orciprenaline)	Beta-2 agonist-bronchodilator	Generic
S0	Metaraminol	Antihypotensive	Generic
S0	Metaxalone	Muscle relaxant	Skelaxin

1 – SPECIFIED SUBCLASSIFICATION
Specified substances designated with an X

HISA STATUS / SS[1]	SUBSTANCE	ACTION	COMMERCIAL / DEVELOPMENTAL NAME(S) WHERE AVAILABLE
S0	Metazocine	Opioid analgesic	Lacks FDA approval. DEA Schedule II.
S1	Metenolone	Anabolic	Lacks FDA approval. DEA Schedule III.
S0	Metformin	Anti-hyperglycemic	Fortamet, Glumetza, Glucophage
S0	Methacholine	Bronchoconstrictor	Provocholine
S0(x)	Methadone	Synthetic opioid agonist	Methadose DEA Schedule II.
S0	Methallenestril	Synthetic estrogen	Lacks FDA approval
S0	Methamphetamine	Stimulant	Desoxyn DEA Schedule II.
S1	Methandienone	Anabolic	Lacks FDA approval. DEA Schedule III.
S1	Methandriol (Methylandrostenediol)	Anabolic	Lacks FDA approval. DEA Schedule III.
S1	Methandrostenolone	Anabolic	Lacks FDA approval
S0	Methantheline	Anticholinergic	Discontinued, no FDA-approved product commercially available
S0	Methapyrilene	Antihistamine	Lacks FDA approval
S0	Methaqualone	Sedative	Lacks FDA approval. DEA Schedule I.
S0	Metharbital	Sedative	Discontinued, no FDA-approved product commercially available
S1	Methasterone	Anabolic	Lacks FDA approval. DEA Schedule III.

1 – SPECIFIED SUBCLASSIFICATION
Specified substances designated with an X

BANNED

HISA STATUS / SS[1]	SUBSTANCE	ACTION	COMMERCIAL / DEVELOPMENTAL NAME(S) WHERE AVAILABLE
S0	Methazolamide	Carbonic Anhydrase Inhibitor	Generic
S0	Methcathinone	Stimulant	Lacks FDA approval. DEA Schedule I.
S0	Methdilazine	Antihistamine	Discontinued, no FDA-approved product commercially available
S1	Methenolone	Anabolic	Lacks FDA approval. DEA Schedule III.
S0	Methimazole	Anti-thyroid	Generic
S0	Methixene	Anticholinergic	Discontinued, no FDA-approved product commercially available
S0	Methohexital	Sedative	Brevital
S0	Methotrimeprazine	Antipsychotic	Lacks FDA approval
S0	Methoxamine	Stimulant	Discontinued, no FDA-approved product commercially available
S3	Methoxyphenamine	Bronchodilator	Lacks FDA approval
S2	Methoxypolyethylene glycol-epoetin beta (CERA)	Erythropoiesis	Micera
S0	*Methoxytyramine (3-)	Neuromodulator	Endogenous substance
S0	Methscopolamine (Methyl scopolamine)	Anticholinergic	Generic
S0	Methsuximide	Anticonvulsant	Celontin

Threshold: 4 mcg/mL total (free and conjugated) 3-methoxytyramine per mL in urine

1 – SPECIFIED SUBCLASSIFICATION
Specified substances designated with an X

HISA STATUS / SS[1]	SUBSTANCE	ACTION	COMMERCIAL / DEVELOPMENTAL NAME(S) WHERE AVAILABLE
S1	Methyl-1-testosterone	Anabolic	Android 25 DEA Schedule III.
S0	Methylaminorex	Stimulant	Lacks FDA approval. DEA schedule I.
S0	Methylatropine	Anticholinergic	Lacks FDA approval
S0	Methylchlorthiazide (Methylclothiazide)	Diuretic	Lacks FDA approval
S1	Methylclostebol	Anabolic	Lacks FDA approval. DEA Schedule III.
S5	Methylclothiazide	Diuretic	Discontinued, no FDA-approved product commercially available
S1	Methyldienolone	Anabolic	Lacks FDA approval. DEA Schedule III.
S0	Methyldopa	Antihypertensive	Generic
S0	Methylenedioxyamphetamine (MDA)	Stimulant	Lacks FDA approval. DEA Schedule I.
S0	Methylenedioxyethylamphetamine (MDEA)	Stimulant	Lacks FDA approval. DEA Schedule I.
S0	Methylenedioxymethamphetamine (MDMA)	Stimulant	Lacks FDA approval. DEA Schedule I.
S0	Methylephedrine	Stimulant	Lacks FDA approval
S0	Methylhexanamine (Methylhexaneamine)	Stimulant	Lacks FDA approval
S0	Methylmethcathinone	Stimulant	Lacks FDA approval

1 – SPECIFIED SUBCLASSIFICATION
Specified substances designated with an X

HISA STATUS / SS[1]	SUBSTANCE	ACTION	COMMERCIAL / DEVELOPMENTAL NAME(S) WHERE AVAILABLE
S1	Methylnortestosterone (Trestolone)	Anabolic	Lacks FDA approval. DEA Schedule III.
S0	Methylphenidate	Stimulant	Ritalin DEA Schedule II.
S0	Methylprylone (methprylon)	Sedative	Lacks FDA approval
S0	Methylpseudoephedrine	Stimulant	Lacks FDA approval
S1	Methyltestosterone	Anabolic	Android 25 DEA Schedule III.
S1	Methyltrienolone (metribolone)	Anabolic	Lacks FDA approval. DEA Schedule III.
S0	Methyprylon	Sedative	Discontinued, no FDA-approved product commercially available. DEA Schedule III.
S0	Methysergide	Ergot alkaloid	Discontinued, no FDA-approved product commercially available
S0	Metiamide	Antihistamine	Lacks FDA approval
S5	Meticrane	Diuretic	Lacks FDA approval
S0	Metipranolol	Antihypertensive	Lacks FDA approval
S0	Metocurine	Muscle relaxant	Discontinued, no FDA-approved product commercially available
S5	Metolazone	Diuretic	Generic
S0	Metomidate	Sedative / Hypnotic	Lacks FDA approval

1 – SPECIFIED SUBCLASSIFICATION
Specified substances designated with an X

HISA STATUS / SS[1]	SUBSTANCE	ACTION	COMMERCIAL / DEVELOPMENTAL NAME(S) WHERE AVAILABLE
S0	Metopon (Methydihydromorphinone)	Opioid analgesic	Lacks FDA approval. DEA Schedule II.
S0	Metoprolol	Antihypertensive	Lopressor
S0	Metrenperone	Myositis preventative	Lacks FDA approval
S1	Metribolone	Anabolic	Lacks FDA approval. DEA Schedule III.
S0	Metyrapone	Hydrocortisone synthesis inhibitor	Metopirone
S0	Mexazolam	Anxiolytic	Lacks FDA approval
S0	Mianserin	Antidepressant	Lacks FDA approval
S0	Mibefradil	Antihypertensive	Lacks FDA approval
S1	Mibolerone	Anabolic	Cheque Drops DEA Schedule III.
S0	Midodrine	Antihypotensive	Orvaten
S0	Minoxidil	Antihypertensive	Rogaine
S0	Mirtazapine	Antidepressant	Remeron
S0	Mitragynine	Stimulant	Lacks FDA approval
S0	Mivacurium	Muscle relaxant	Generic

1 – SPECIFIED SUBCLASSIFICATION
Specified substances designated with an X

HISA STATUS / SS[1]	SUBSTANCE	ACTION	COMMERCIAL / DEVELOPMENTAL NAME(S) WHERE AVAILABLE
S0	Modafinil	Stimulant	Provigil DEA Schedule IV.
S0	Moexipril (Metabolite: Moexiprilat)	Antihypertensive	Generic
S0	Mofebutazone	NSAID	Lacks FDA approval
S2	Molidustat (BAY 85-3934)	Erythropoiesis	Lacks FDA approval
S0	Molindone	Antipsychotic	Generic
S0	Moperone	Antipsychotic	Lacks FDA approval
S0	Moprolol	Antihypertensive	Lacks FDA approval
S0	Morpheridine	Analgesic	Lacks FDA approval
S0	Mosapramine	Antipsychotic	Lacks FDA approval
S0	Moxaverine	Vasodilator	Lacks FDA approval
S0(x)	Muscarine	Cholinergic	Plant alkaloid
S0	Myo-inositol trispyrophosphate (ITPP, OXY111A)	Oxygen transfer	Lacks FDA approval
S0	Nadolol	Antihypertensive	Corgard

1 – SPECIFIED SUBCLASSIFICATION
Specified substances designated with an X

HISA STATUS / SS[1]	SUBSTANCE	ACTION	COMMERCIAL / DEVELOPMENTAL NAME(S) WHERE AVAILABLE
S0	Nadoxolol	Antihypertensive	Lacks FDA approval
S0	Naepaine	Local anesthetic	Lacks FDA approval
S2	Nafarelin	Reproductive hormone modulator	Synarel
S0	Naftidrofuryl	Vasodilator	Lacks FDA approval
S0	Nalbuphine	Opioid receptor agonist and antagonist	Generic
S0	Nalorphine	Opioid receptor agonist and antagonist	Nalline DEA Schedule III.
S1	Nandrolone (19-nortestosterone) Fillies, mares and geldings	Anabolic	Discontinued, no FDA-approved product commercially available. DEA schedule III.
S0	Naratriptan	Selective Serotonin Receptor Agonist	Amerge
S0	Nebivolol	Antihypertensive	Bystolic
S0	Nefazodone	Antidepressant	Generic
S0	Nefopam	Analgesic	Lacks FDA approval
S6	Neridronate	Bisphosphonate	Lacks FDA approval
S0	Nialamide	Antidepressant	Lacks FDA approval
S0	Nicardipine	Antihypertensive	Generic

1 – SPECIFIED SUBCLASSIFICATION
Specified substances designated with an X

HISA STATUS / SS[1]	SUBSTANCE	ACTION	COMMERCIAL / DEVELOPMENTAL NAME(S) WHERE AVAILABLE
S0	Nicoumalone	Anticoagulant	Lacks FDA approval
S0	Nifedipine	Antihypertensive	Procardia
S0	Nifenalol	Antihypertensive / Antiarrhythmic	Lacks FDA approval
S0	Niflumic acid	NSAID	Lacks FDA approval
S0	Nikethamide	Stimulant	Lacks FDA approval
S0	Nimesulide	NSAID	Lacks FDA approval
S0	Nimetazepam	Hypnotic	Lacks FDA approval. DEA Schedule IV.
S0	Nimodipine	Calcium channel blocker	Generic
S0	Nitrazepam	Sedative / Anxiolytic	Lacks FDA approval. DEA Schedule IV.
S0	Nitroglycerin	Vasodilator	Nitromist, Nitro-Dur, Nitrostat
S0	Nomifensine	Antidepressant	Lacks FDA approval
S1	Norandrostenediol	Anabolic	Lacks FDA approval
S1	Norandrostenedione	Anabolic	Lacks FDA approval
S1	Norandrosterone	Anabolic	Lacks FDA approval. DEA Schedule III.

1 – SPECIFIED SUBCLASSIFICATION
Specified substances designated with an X

HISA STATUS / SS[1]	SUBSTANCE	ACTION	COMMERCIAL / DEVELOPMENTAL NAME(S) WHERE AVAILABLE
S1	Norbolethone / Norboletone	Anabolic	Lacks FDA approval. DEA Schedule III.
S1	Norclostebol	Anabolic	Lacks FDA approval. DEA Schedule III.
S0	Nordiazepam / Nordazepam (Nordiazepam is a metabolite of diazepam. If there is credible evidence that the presence of nordiazepam in a horse's sample is the consequence of exposure to diazepam, the classification of nordiazepam may be revised to S7(A).)	Sedative / Anxiolytic	Lacks FDA approval. DEA Schedule IV.
S1	Norethandrolone	Anabolic	Lacks FDA approval. DEA Schedule III.
S1	Norethisterone (norethindrone)	Anabolic	Combipatch, Activella, Amabelz, Nortrel, Alyacen, Aranelle and multiple others (with estradiol)
S0	Norfenefrine	Antihypotensive	Lacks FDA approval
S0	Norfenfluramine	Stimulant	Lacks FDA approval
S0	Norfluoxetine (Seproxetine)	Antidepressant	Lacks FDA approval
S0	Norpseudoephedrine (Cathine)	Stimulant	Lacks FDA approval. DEA Schedule IV.
S0	Nortriptyline	Antidepressant	Pamelor

1 – SPECIFIED SUBCLASSIFICATION
Specified substances designated with an X

HISA STATUS / SS[1]	SUBSTANCE	ACTION	COMMERCIAL / DEVELOPMENTAL NAME(S) WHERE AVAILABLE
S0	Noscapine	Antitussive	Lacks FDA approval
S0	Nylidrin (Buphenine)	Vasodilator	Lacks FDA approval
S0	Octopamine (Octopamine is a metabolite of ephedrine. If there is credible evidence that the presence of octopamine is a consequence of exposure to ephedrine, the classification of octopamine may be revised to S7(A).)	Stimulant	Lacks FDC approval
S0	Olanzapine	Antipsychotic	Zyprexa
S0	Oliceridine	Opioid agonist	Olinvk DEA Schedule II.
S0	Olmesartan	Antihypertensive	Benicar (with medoxomil)
S3	Olodaterol	Beta-2 agonist-bronchodilator	Striverdi Respimat, Stiolto Respimat (with tiotropium bromide)
S6	Olpadronate	Bisphosphonate	Lacks FDA approval
S0	Opipramol	Antidepressant	Lacks FDA approval
S3	Orciprenaline (Metaproterenol)	Beta-2 agonist-bronchodilator	Generic
S0(x)	Oripavine	Opioid	Plant alkaloid DEA schedule II.

1 – SPECIFIED SUBCLASSIFICATION
Specified substances designated with an X

HISA STATUS / SS[1]	SUBSTANCE	ACTION	COMMERCIAL / DEVELOPMENTAL NAME(S) WHERE AVAILABLE
S0	Orphenadrine	Muscle relaxant	Generic
S4	Ospemifene	Selective Estrogen Receptor Modulator (SERM)	Osphena
S1	Ostarine (Enobosarm)	Selective Androgen Receptor Modulator (SARM)	Lacks FDA approval
S1	Oxabolone	Anabolic	Lacks FDA approval
S0	Oxaflumazine	Psychosedative	Lacks FDA approval
S1	Oxandrolone	Anabolic	Generic DEA Schedule III.
S0	Oxaprozin	NSAID	Daypro
S0	Oxazepam (Oxazepam is a metabolite of diazepam. If there is credible evidence that the presence of oxazepam in a horse's sample is the consequence of exposure to diazepam, the classification of oxazepam may be revised to S7(A).)	Anxiolytic	Generic DEA Schedule IV.
S0	Oxazolam	Sedative / Anxiolytic	Lacks FDA approval. DEA Schedule IV.
S0	Oxcarbazepine	Anticonvulsant	Generic
S0	Oxethazaine (Oxetacaine)	Local anesthetic	Lacks FDA approval

1 – SPECIFIED SUBCLASSIFICATION
Specified substances designated with an X

HISA STATUS / SS[1]	SUBSTANCE	ACTION	COMMERCIAL / DEVELOPMENTAL NAME(S) WHERE AVAILABLE
S0	Oxilofrine (Hydroxyephedrine)	Stimulant	Lacks FDA approval
S0	Oxolamine	Antitussive	Lacks FDA approval
S0	Oxprenolol	Antihypertensive	Lacks FDA approval
S0	Oxycodone	Opioid analgesic	Oxycontin, Roxybond, Roxicodone, Oxaydo, Xtampza, Percocet, Percodan, Oxycet (with NSAID). DEA Schedule II.
S1	Oxyguno	Anabolic	Lacks FDA approval
S1	Oxymesterone	Anabolic	Lacks FDA approval. DEA Schedule III.
S1	Oxymetholone	Anabolic	Discontinued, no FDA-approved product commercially available. DEA Schedule III.
S0	Oxymorphone	Opioid analgesic	Generic. DEA Schedule II.
S0	Oxypertine	Antipsychotic	Lacks FDA approval
S0	Oxyphencyclimine	Anticholinergic	Discontinued, no FDA-approved product commercially available
S0	Oxyphenonium	Anticholinergic	Discontinued, no FDA-approved product commercially available
S0	Paliperidone	Antipsychotic	Invega
S0	Palmitoylethanolamid	Anti-inflammatory	Lacks FDA approval

1 – SPECIFIED SUBCLASSIFICATION
Specified substances designated with an X

HISA STATUS / SS[1]	SUBSTANCE	ACTION	COMMERCIAL / DEVELOPMENTAL NAME(S) WHERE AVAILABLE
S6	Pamidronate	Bisphosphonate	Generic
S0(x)	Papaverine	Vasodilator	Plant alkaloid
S0	Paraldehyde	Anticonvulsant	Lacks FDA approval. DEA Schedule IV.
S0	Paramethadione	Anticonvulsant	Discontinued, no FDA-approved product commercially available
S0	Paramethasone	Corticosteroid	Discontinued, no FDA-approved product commercially available
S0(x)	Paraxanthine (Paraxanthine is a metabolite of caffeine. If there is credible evidence that the presence of paraxanthine in a horse's sample is the consequence of exposure to caffeine, the classification of paraxanthine may be revised to S7(B).)	Stimulant	Lacks FDA approval
S0	Parecoxib	NSAID	Lacks FDA approval
S0	Pargyline	Antihypertensive	Discontinued, no FDA-approved product commercially available.
S0	Paroxetine	Antidepressant	Paxil
S2	Pegepoietin	Erythropoiesis	Micera
S2	Peginesatide	Erythropoiesis	Discontinued, no FDA-approved product commercially available.

1 – SPECIFIED SUBCLASSIFICATION
Specified substances designated with an X

HISA STATUS / SS[1]	SUBSTANCE	ACTION	COMMERCIAL / DEVELOPMENTAL NAME(S) WHERE AVAILABLE
S0	Pemoline (Pemoline is a metabolite of aminorex, which is a metabolite of levamisole. If there is credible evidence that the detection of pemoline in a horse's sample is the consequence of exposure to levamisole, the classification of pemoline may be revised to S7(B).)	Stimulant	Discontinued, no FDA-approved product commercially available. DEA Schedule IV.
S0	Pempidine	Ganglion blocker / antihypertensive	Lacks FDA approval
S0	Penbutolol	Antihypertensive	Discontinued, no FDA-approved product commercially available
S0	Penfluridol	Antipsychotic	Lacks FDA approval
S0	Pentaerythritol tetranitrate	Vasodilator	Lacks FDA approval
S0	Pentetrazol	Stimulant	Lacks FDA approval
S0	Pentifylline	Vasodilator	Lacks FDA approval
S0	Pentylenetetrazol	Stimulant	Lacks FDA approval
S2	Perfluorodecahydronophthalene	Oxygen transfer	Lacks FDA approval
S0	Perfluorodecalin (Octadecafluoronaphthalene)	Oxygen transport	Lacks FDA approval

1 – SPECIFIED SUBCLASSIFICATION
Specified substances designated with an X

HISA STATUS / SS[1]	SUBSTANCE	ACTION	COMMERCIAL / DEVELOPMENTAL NAME(S) WHERE AVAILABLE
S2	Perfluorooctyl bromide	Oxygen transfer	Discontinued, no FDA-approved product commercially available.
S2	Perfluorotripropylamine	Oxygen transfer	Discontinued, no FDA-approved product commercially available
S0	Periciazine	Antipsychotic	Lacks FDA approval
S0	Perindopril	Antihypertensive	Generic, Prestalia (with amlodipine besylate)
S0	Perlapine	Sedative / Hypnotic	Lacks FDA approval
S0	Perphenazine	Antipsychotic	Generic
S0	Phenacemide	Anticonvulsant	Discontinued, no FDA-approved product commercially available
S0	Phenaglycodol	Sedative / Anxiolytic	Lacks FDA approval
S0	Phenazocine	Opioid analgesic	Lacks FDA approval. DEA Schedule II.
S0	Phenazone	NSAID	Lacks FDA approval
S0	Phencyclidine (PCP)	Dissociative hallucinogen	Lacks FDA approval. DEA Schedule I.
S0	Phendimetrazine	Stimulant	Bontril DEA Schedule III.
S0	Phenelzine	Antidepressant	Nardil
S0	Phenibut	Anxiolytic	Lacks FDA approval

1 – SPECIFIED SUBCLASSIFICATION
Specified substances designated with an X

HISA STATUS / SS[1]	SUBSTANCE	ACTION	COMMERCIAL / DEVELOPMENTAL NAME(S) WHERE AVAILABLE
S0	Phenindamine	Antihistamine	Lacks FDA approval
S0	Phenindione	Anticoagulant	Discontinued, no FDA-approved product commercially available
S0	Pheniramine	Antihistamine	Bromfed-DM (with dextromethorphan and pseudoephedrine)
S0	Phenmetrazine	Stimulant	Discontinued, no FDA-approved product commercially available
S0	Phenoxybenzamine	Antihypertensive	Dibenzyline
S0	Phenprocoumon	Anticoagulant	Discontinued, no FDA-approved product commercially available
S0	Phenpromethamine	Stimulant	Lacks FDA approval
S0	Phensuximide	Anticonvulsant	Discontinued, no FDA-approved product commercially available
S0	Phentermine	Stimulant	Adipex-P, Lomaira, Qsymia DEA Schedule IV.
S0	Phenylpiracetam (Carphedon)	Stimulant	Lacks FDA approval
S0	Phenylpropanolamine	Stimulant	Proin
S0	Phenyltoloxamine	Antihistamine	Lacks FDA approval
S0	Pholcodine	Opioid antitussive	Lacks FDA approval. DEA Schedule I.
S0	Pholedrine	Stimulant	Lacks FDA approval

1 – SPECIFIED SUBCLASSIFICATION
Specified substances designated with an X

HISA STATUS / SS[1]	SUBSTANCE	ACTION	COMMERCIAL / DEVELOPMENTAL NAME(S) WHERE AVAILABLE
S0	Picrotoxin	Stimulant	Lacks FDA approval
S0	Piminodine	Opioid analgesic	Lacks FDA approval. DEA Schedule II.
S1	Pimobendan	Cardiac stimulant	Vetmedin
S0	Pimozide	Antipsychotic	Generic
S0	Pinazepam	Anxiolytic	Lacks FDA approval. DEA Schedule IV.
S0	Pinazepam	Sedative / Anxiolytic	Lacks FDA approval
S0	Pindolol	Antihypertensive	Generic
S0	Pipamazine	Anti-emetic	Lacks FDA approval
S0	Pipamperone	Antipsychotic	Lacks FDA approval
S0	Pipecuronium	Muscle relaxant	Discontinued, no FDA-approved product commercially available
S0	Pipequaline	Anxiolytic	Lacks FDA approval
S0	Piper Methysticum (Kava)	Anxiolytic / Anti-inflammatory	Lacks FDA approval
S0	Piperacetazine	Antipsychotic	Lacks FDA approval
S0	Piperidione	Sedative	Lacks FDA approval

1 – SPECIFIED SUBCLASSIFICATION
Specified substances designated with an X

HISA STATUS / SS[1]	SUBSTANCE	ACTION	COMMERCIAL / DEVELOPMENTAL NAME(S) WHERE AVAILABLE
S0	Piperidolate	Antispasmodic	Lacks FDA approval
S0	Piperocaine	Local anesthetic	Lacks FDA approval
S0	Piperoxan	Antihistamine / Antihypertensive	Lacks FDA approval
S0	Pipotiazine	Antipsychotic	Lacks FDA approval
S0	Pipradrol	Stimulant	Lacks FDA approval
S0	Piquindone	Antipsychotic	Lacks FDA approval
S0	Piracetam	Stimulant	Lacks FDA approval
S0	Pirbuterol	Beta-2 agonist-bronchodilator	Discontinued, no FDA-approved product commercially available
S0	Pirenzepine	Anticholinergic	Lacks FDA approval
S5	Piretanide	Diuretic	Lacks FDA approval
S0	Piritramide	Synthetic opioid analgesic	Lacks FDA approval
S0	Pirprofen	NSAID	Lacks FDA approval
S6	Pitcher Plant Extract	Analgesic	Sarapin
S0	Pizotifen (Pizotylline)	Antimigraine	Lacks FDA approval

1 – SPECIFIED SUBCLASSIFICATION
Specified substances designated with an X

HISA STATUS / SS[1]	SUBSTANCE	ACTION	COMMERCIAL / DEVELOPMENTAL NAME(S) WHERE AVAILABLE
S2	Platelet-Derived Growth Factor (PDGF)	Growth Hormone	
S5	Polythiazide	Diuretic	Discontinued, no FDA-approved product commercially available
S0	Practolol	Antiarrhythmic	Lacks FDA approval
S2	Pralmorelin	Growth Hormone	Lacks FDA approval
S1	Prasterone (Dehydroepiandrosterone, DHEA, 3βhydroxyandrost-5-en17-one)	Anabolic	Intrarosa
S0	Prazepam	Sedative / Anxiolytic	Discontinued, no FDA-approved product commercially available. DEA Schedule IV.
S0	Prazosin	Antihypertensive	Minipress
S0	Prenylamine	Vasodilator	Lacks FDA approval
S0	Pridinol	Anticholinergic	Lacks FDA approval
S0	Prifinium Bromide	Antispasmodic	Lacks FDA approval
S5	Probenecid	Anti-gout	Probalan
S0	Procarbazine	Anti-neoplastic	Matulane
S3	Procaterol	Beta-2 agonist-bronchodilator	Lacks FDA approval

1 – SPECIFIED SUBCLASSIFICATION
Specified substances designated with an X

HISA STATUS / SS[1]	SUBSTANCE	ACTION	COMMERCIAL / DEVELOPMENTAL NAME(S) WHERE AVAILABLE
S0	Prochlorperazine	Anti-nausea	Compro, Procomp, Compazine
S0	Procyclidine	Anticholinergic	Discontinued, no FDA-approved product commercially available
S0	Proglumide	Anti-ulcer	Lacks FDA approval
S0	Pronethalol	Antiarrhythmic	Lacks FDA approval
S0	Propallylonal	Sedative / Hypnotic	Lacks FDA approval
S0	Propanidid	Anesthetic	Lacks FDA approval
S0	Propantheline	Anticholinergic	Discontinued, no FDA-approved product commercially available
S0	Propentophylline (Propentofylline)	Phosphodiesterase inhibitor	Lacks FDA approval
S0	Propiomazine	Antipsychotic	Discontinued, no FDA-approved product commercially available
S0	Propionylpromazine	Sedative	Lacks FDA approval
S0	Propiram	Opioid analgesic	Lacks FDA approval. DEA Schedule I.
S0	Propoxycaine	Local anesthetic	Discontinued, no FDA-approved product commercially available
S0	Propoxyphene	Opioid analgesic	Discontinued, no FDA-approved product commercially available
S0	Propylhexedrine	Stimulant	Benzedrex

1 – SPECIFIED SUBCLASSIFICATION
Specified substances designated with an X

HISA STATUS / SS[1]	SUBSTANCE	ACTION	COMMERCIAL / DEVELOPMENTAL NAME(S) WHERE AVAILABLE
S0	Propyphenazone	NSAID	Lacks FDA approval
S0	Proquazone	NSAID	Lacks FDA approval
S1	Prostanazol	Anabolic	Lacks FDA approval. DEA Schedule III.
S0	Prothipendyl	Anxiolytic / Antihistamine	Lacks FDA approval
S0	Protokylol	Bronchodilator	Discontinued, no FDA-approved product commercially available
S0	Protriptyline	Antidepressant	Generic
S0	Proxibarbital	Sedative / Anxiolytic	Lacks FDA approval
S0	Proxyphylline	Bronchodilator	Lacks FDA approval
S0	Psilocin (Psilocyn)	Hallucinogen	Lacks FDA approval. DEA Schedule I.
S0	Pyrithyldione	Sedative / Hypnotic	Lacks FDA approval
S0	Pyrrobutamine	Antihistamine	Lacks FDA approval
S0	Quazepam	Sedative	Doral DEA Schedule IV.
S0	Quetiapine	Antipsychotic	Lacks FDA approval
S0	Quinapril, Quinaprilat	Antihypertensive	Accuretic

1 – SPECIFIED SUBCLASSIFICATION
Specified substances designated with an X

HISA STATUS / SS[1]	SUBSTANCE	ACTION	COMMERCIAL / DEVELOPMENTAL NAME(S) WHERE AVAILABLE
S1	Quinbolone	Anabolic	Lacks FDA approval
S5	Quinethazone	Diuretic	Discontinued, no FDA-approved product commercially available
S0	Quinisocaine	Local anesthetic	Lacks FDA approval
S0	Racemethorphan	Anti-Alzheimers	Lacks FDA approval. DEA Schedule II.
S0	Racemorphan	Opioid agonist	Lacks FDA approval. DEA Schedule II.
S0	Raclopride	Antipsychotic	Lacks FDA approval
S1(x)	Ractopamine	Anabolic	Paylean, Optaflexx, Topmax
S4	Raloxifene	Selective Estrogen Receptor Modulator (SERM)	Evista
S4	Ramatercept (ACE-031)	Myostatin inhibitor	Lacks FDA approval
S0	Ramifenazone (Isopyrin)	NSAID	Lacks FDA approval
S0	Ramipril, Metabolite Ramiprilat	Antihypertensive	Altace
S0	Regadenoson	Cardiac stimulant	Lexiscan
S0	Remifentanil	Synthetic opioid analgesic	Ultiva DEA Schedule II.
S0	Remimazolam	Anesthetic	Byfavo DEA schedule IV.

1 – SPECIFIED SUBCLASSIFICATION
Specified substances designated with an X

HISA STATUS / SS[1]	SUBSTANCE	ACTION	COMMERCIAL / DEVELOPMENTAL NAME(S) WHERE AVAILABLE
S0	Remoxipride	Antipsychotic	Lacks FDA approval
S3	Reproterol	Beta-2 agonist-bronchodilator	Lacks FDA approval
S0	Rilmazafone	Sedative / Hypnotic	Lacks FDA approval
S3	Rimiterol	Beta-2 agonist-bronchodilator	Lacks FDA approval
S6	Risedronate	Bisphosphonate	Actonel
S0	Risperidone	Antipsychotic	Perseris Kit, Risperdal Consta, Risperdal
S0	Ritanserin	Antidepressant	Lacks FDA approval
S3	Ritodrine	Beta-2 agonist	Lacks FDA approval
S0	Rivastigmine	Cholinesterase Inhibitor	Exelon
S0	Rizatriptan	Selective Serotonin Receptor Agonist	Maxalt
S0	Rofecoxib	NSAID	Discontinued, no FDA-approved product commercially available
S2	Roxadustat (FG-4592)	Erythropoiesis	Lacks FDA approval
S0	Salicylamide	Analgesic	Lacks FDA approval
S1	SARM YK-11	Anabolic	Lacks FDA approval

1 – SPECIFIED SUBCLASSIFICATION
Specified substances designated with an X

HISA STATUS / SS[1]	SUBSTANCE	ACTION	COMMERCIAL / DEVELOPMENTAL NAME(S) WHERE AVAILABLE
S0	Selegiline	Antidepressant	Emsam, Zelapar
S2	Sermorelin	Growth Hormone	Discontinued, no FDA-approved product commercially available
S0	Sertraline	Antidepressant	Zoloft
S0	Sibutramine	Stimulant	Discontinued, no FDA-approved product commercially available. DEA Schedule IV
S0	Sildenafil	Phosphodiesterase inhibitor	Viagra
S6	Snake Venoms	Neurotoxin	Lacks FDA approval
S2	Somatrem	Growth Hormone	Protropin
S2	Somatrogon	Growth Hormone	Lacks FDA approval
S2	Somatropin	Growth Hormone	Lacks FDA approval
S2	Sotatercept	Growth Hormone	Lacks FDA approval
S0(x)	Sparteine	Antiarrhythmic	Lacks FDA approval
S0	Spiperone	Antipsychotic	Lacks FDA approval
S0	Spirapril (Metabolite: Spiraprilat)	Antihypertensive	Discontinued, no FDA-approved product commercially available
S5	Spironalactone	Diuretic	Aldactazide, Caarospir, Aldactone

1 – SPECIFIED SUBCLASSIFICATION
Specified substances designated with an X

HISA STATUS / SS[1]	SUBSTANCE	ACTION	COMMERCIAL / DEVELOPMENTAL NAME(S) WHERE AVAILABLE
S4	Stamulumab (Myo-29)	Myostatin inhibitor	Lacks FDA approval
S1	Stanozolol	Anabolic	Lacks FDA approval. DEA Schedule III.
S1	Stenbolone	Anabolic	Lacks FDA approval. DEA Schedule III.
S0	Strychnine	CNS stimulant	Lacks FDA approval. (Has anetodal use as contituent of unregulated appetite stimulants and leg paints. Extreme caution is advised when using these products.)
S0	Styramate	Muscle relaxant	Lacks FDA approval
S0	Sufentanil	Opioid analgesic	Sufenta, Dsuvia DEA Schedule II.
S0	Sulfondiethylmethane	Sedative / Hypnotic	Lacks FDA approval. DEA Schedule III.
S0	Sulfonmethane	Sedative / Hypnotic	Lacks FDA approval. DEA Schedule III.
S0	Sulforidazine	Antipsychotic	Lacks FDA approval
S0	Sulindac	NSAID	Generic
S0	Sulpiride	Antipsychotic	Lacks FDA approval
S0	Sultopride	Antipsychotic	Lacks FDA approval
S0	Sumatriptan	Selective Serotonin Receptor Agonist	Imitrex, Treximet [with Naproxen]

1 – SPECIFIED SUBCLASSIFICATION
Specified substances designated with an X

HISA STATUS / SS[1]	SUBSTANCE	ACTION	COMMERCIAL / DEVELOPMENTAL NAME(S) WHERE AVAILABLE
S0	Suxibuzone	NSAID	Lacks FDA approval
S0(x)	Synephrine	Stimulant	Lacks FDA approval
S4	T3 (Triiodothyronine)	Metabolic hormone	Lacks FDA approval
S4	T4 (Tetraiodothyronine / Thyroxine)	Metabolic hormone	Thyro-Tabs Canine, Thyrokare
S2	Tabimorelin	Growth Hormone	Lacks FDA approval
S0	Tadalafil	Phosphodiesterase inhibitor	Cialis
S0	Talbutal	CNS depressant	Discontinued, no FDA-approved product commercially available. DEA Schedule III.
S4	Tamoxifen	Selective Estrogen Receptor Modulator (SERM)	Soltamox
S0	Tandospirone	Anxiolytic	Lacks FDA approval
S0	Tapentadol	Opioid analgesic	Nucynta DEA Schedule II.
S0	Telmisartan	Antihypertensive	Micardis
S0	Tenoxicam	NSAID	Lacks FDA approval
S0	Terazosin	Antihypertensive	Generic
S3	Terbutaline	Bronchodilator	Brethine

1 – SPECIFIED SUBCLASSIFICATION
Specified substances designated with an X

HISA STATUS / SS[1]	SUBSTANCE	ACTION	COMMERCIAL / DEVELOPMENTAL NAME(S) WHERE AVAILABLE
S0	Terfenadine	Antihistamine	Lacks FDA approval
S2	Tesamorelin	Growth Hormone	Egrifta
S4	Testolactone	Aromatase inhibitor	Teslac DEA Schedule III
S2	Testolone	Selective Androgen Receptor Modulator (SARM)	Lacks FDA approval
S1	*Testosterone Fillies and mares unless in foal	Anabolic	Androderm, Testm, Vogelxo, Testopel, Aveed, Kyzatrex, Jatenzo, Xyosted DEA Schedule III.
S1	**Testosterone Geldings	Anabolic	Androderm, Testm, Vogelxo, Testopel, Aveed, Kyzatrex, Jatenzo, Xyosted. DEA Schedule III.
S0	Tetrabenazine (Deutetrabenazine)	Neurotransmitter modulator	Xenazine, Austedo
S1	Tetrahydrogestrinone	Anabolic	Lacks FDA approval. DEA Schedule III.
S0	Tetrazepam	Anxiolytic	Lacks FDA approval. DEA Schedule IV.
S1	Tetrahydrocannabinol (THC)	Psychoactive	Lacks FDA approval. DEA Schedule I.
S0(x)	Thebaine	Opioid analgesic	Lacks FDA approval. DEA Schedule II.
S0	Thialbarbital	Sedative / Hypnotic	Lacks FDA approval

*Threshold: 55 ng/mL total (free and conjugated) testosterone in urine OR 0.1 n/mL free testosterone in serum or plasma

**Threshold: 20 ng/mL total (free and conjugated) testosterone in urine OR 0.1 ng/mL free testosterone in serum or plasma

1 – SPECIFIED SUBCLASSIFICATION
Specified substances designated with an X

HISA STATUS / SS[1]	SUBSTANCE	ACTION	COMMERCIAL / DEVELOPMENTAL NAME(S) WHERE AVAILABLE
S0	Thiethylperazine	Antipsychotic	Discontinued, no FDA-approved product commercially available
S0	Thiopropazate	Antipsychotic	Lacks FDA approval
S0	Thioproperazine	Antipsychotic	Lacks FDA approval
S0	Thioridazine	Antipsychotic	Generic
S0	Thiothixene	Antipsychotic	Generic
S0	Thiphenamil (Tifenamil)	Antispasmodic / Local anesthetic	Lacks FDA approval
S0	Thonzylamine	Antihistamine / anticholinergic	Lacks FDA approval
S0	Thozalinone	Antidepressant	Lacks FDA approval
S2	Thymosin	Peptide hormone	Lacks FDA approval
S0	Tiapride	Antipsychotic	Lacks FDA approval
S0	Tiaprofenic acid	NSAID	Lacks FDA approval
S1	Tibolone	Anabolic	Lacks FDA approval
S6	Tildronate (Tiludronic Acid)	Bisphosphonate	Tildren
S0	Timiperone	Antipsychotic	Lacks FDA approval

1 – SPECIFIED SUBCLASSIFICATION
Specified substances designated with an X

HISA STATUS / SS[1]	SUBSTANCE	ACTION	COMMERCIAL / DEVELOPMENTAL NAME(S) WHERE AVAILABLE
S3	Timolol	Antihypertensive	Istalol, Betimol, Timoptic
S0	Tocainide	Antiarrhythmic	Discontinued, no FDA-approved product commercially available
S0	Tofenacin	Antidepressant	Lacks FDA approval
S0	Tofisopam	Anxiolytic	Lacks FDA approval
S0	Tolfenamic Acid	NSAID	Lacks FDA approval
S0	Tolmetin	NSAID	Discontinued, no FDA-approved product commercially available
S5	Tolvaptan	Diuretic	Jynarque, Samsca
S0	Tolycaine	Local anesthetic	Lacks FDA approval
S0	Topiramate	Anticonvulsant	Topamax, Qsymia (with phentermine hydrochloride)
S4	Toremifene	Selective Estrogen Receptor Modulator (SERM)	Fareston
S5	Torsemide (Torasemide)	Diuretic	Soaanz
S0	Tramazoline	Sympathomimetic	Lacks FDA approval
S0	Trandolapril (and Metabolite, Trandolaprilat)	Antihypertensive	Generic
S0	Tranylcypromine	Antidepressant	Parnate

1 – SPECIFIED SUBCLASSIFICATION
Specified substances designated with an X

HISA STATUS / SS[1]	SUBSTANCE	ACTION	COMMERCIAL / DEVELOPMENTAL NAME(S) WHERE AVAILABLE
S0	Trazodone	Antidepressant	Generic
S1	Trenbolone (Trendione)	Anabolic	Finaplix, Revalor, Synovex (with Estradiol), Component (with Estradiol and Tylosin) DEA Schedule III.
S1	Trendione	Anabolic	Lacks FDA approval
S0	Trestolone	Anabolic	Lacks FDA approval
S3	Tretoquinol (Trimetoquinol)	Beta-2 agonist-bronchodilator	Lacks FDA approval
S5	Triamterene	Diuretic	Dyrenium
S0	Triazolam	CNS depressant	Halcion DEA Schedule IV.
S0	Tribromoethanol	Anesthetic	Lacks FDA approval
S0	Tricaine methanesulfonate	Anesthetic	Syncaine
S0	Trichloroethanol	Sedative / Hypnotic	Lacks FDA approval
S0	Trichloroethylene	Anesthetic	Lacks FDA approval
S0	Triclofos	Sedative	Discontinued, no FDA-approved product commercially available
S0	Tridihexethyl	Anticholinergic	No FDA-approved product
S0	Triflumeprazine	Sedative	Lacks FDA approval

1 – SPECIFIED SUBCLASSIFICATION
Specified substances designated with an X

HISA STATUS / SS[1]	SUBSTANCE	ACTION	COMMERCIAL / DEVELOPMENTAL NAME(S) WHERE AVAILABLE
S0	Trifluoperazine	Antipsychotic	Generic
S0	Trifluoromethylphenyl piperazine	Stimulant	Lacks FDA approval
S0	Trifluperidol	Antipsychotic	Lacks FDA approval
S0	Triflupromazine	Antipsychotic	Discontinued, no FDA-approved product commercially available
S0	Triflupromazine	Antipsychotic	Discontinued, no FDA-approved product commercially available
S0	Trihexyphenidyl	Anticholinergic	Discontinued, no FDA-approved product commercially available
S0	Trimecaine	Local anesthetic	Lacks FDA approval
S0	Trimeprazine (Alimemazine)	Antihistamine	Temaril-P [with Prednisolone]
S4	Trimetazidine	Angina treatment	Lacks FDA approval
S0	Trimethadione	Anticonvulsant	Discontinued, no FDA-approved product commercially available
S0	Trimethaphan	Antihypertensive / Anesthetic	Discontinued, no FDA-approved product commercially available
S0	Trimipramine	Antidepressant	Generic
S0	Triprolidine	Antihistamine	Triacin-C (with codeine phosphate and pseudoephedrine hydrochloride)
S2	Triptorelin	Reproductive hormone modulator	Triptodur, Trelstar

1 – SPECIFIED SUBCLASSIFICATION
Specified substances designated with an X

HISA STATUS / SS[1]	SUBSTANCE	ACTION	COMMERCIAL / DEVELOPMENTAL NAME(S) WHERE AVAILABLE
S0	Trometamol (Tris hydroxymethylaminomethane [THAM])	Alkalinizing agent	Discontinued, no FDA-approved product is commercially available
S0	Tuaminoheptane	Stimulant	Lacks FDA approval
S0	Tubocurarine (Curare)	Muscle relaxant	Plant alkaloid Discontinued, no FDA-approved product commercially available
S3	Tulobuterol	Beta-2 agonist-bronchodilator	Lacks FDA approval
S0	Tybamate	Anxiolytic	Lacks FDA approval
S0	Valdecoxib	NSAID	Discontinued, no FDA-approved product commercially available
S0	Valnoctamide	Sedative / Hypnotic	Lacks FDA approval
S0	Valproate Sodium	Anticonvulsant	Discontinued, no FDA-approved product commercially available
S0	Valsartan	Antihypertensive	Diovan, Entresto (with sacubitril)
S0	Vardenafil	Phosphodiesterase inhibitor	Levitra
S2	Vascular-Endothelial Growth Factor (VEGF)	Growth Hormone	
S0	Vedaprofen	NSAID	Lacks FDA approval
S0	Venlafaxine	Antidepressant	Pristiq

1 – SPECIFIED SUBCLASSIFICATION
Specified substances designated with an X

HISA STATUS / SS[1]	SUBSTANCE	ACTION	COMMERCIAL / DEVELOPMENTAL NAME(S) WHERE AVAILABLE
S0	Veralipride	Antipsychotic	Lacks FDA approval
S0	Verapamil	Antihypertensive	Verelan, Calan
S3	Vilanterol	Beta-2 agonist-bronchodilator	Trelegy, Ellipta
S0	Viloxazine	Antidepressant	Qelbree
S0	Vinbarbital	Hypnotic	Lacks FDA approval. DEA Schedule III.
S0	Vinylbital	Sedative / Hypnotic	Lacks FDA approval
S2	Xenon	Hypoxia Inducible Factor activating agent	
S5	Xipamide	Diuretic	Lacks FDA approval
S0	Xylometazoline	Stimulant	Afrin, Vicks Sinex
S0	Zafirlukast	Asthma prevention	Accolate
S0	Zaleplon	CNS depressant	Sonata DEA Schedule IV.
S1	Zeranol	Anabolic	Ralgro
S6	Ziconotide	Neurotoxin	Prialt
S0	Zileuton	Asthma prevention	Zyflo

1 – SPECIFIED SUBCLASSIFICATION
Specified substances designated with an X

HISA STATUS / SS[1]	SUBSTANCE	ACTION	COMMERCIAL / DEVELOPMENTAL NAME(S) WHERE AVAILABLE
S1(x)	Zilpaterol hydrochloride	Anabolic	Zilmax, Heifermax
S0	Zimeldine	Antidepressant	Lacks FDA approval
S0	Ziprasidone	Antipsychotic	Geodon
S6	Zoledronic acid	Bisphosphonate	Reclast
S0	Zolmitriptan	Selective Serotonin Receptor Agonist	Zomig
S0	Zolpidem	Sedative / Hypnotic	Ambien DEA Schedule IV.
S0	Zomepirac	Anticonvulsant	Lacks FDA approval
S0	Zonisamide	Anticonvulsant	Zonegran
S0	Zopiclone	Sedative / Hypnotic	Lunesta DEA Schedule IV.
S0	Zotepine	Antipsychotic	Lacks FDA approval
S0	Zuclopenthixol	Antipsychotic	Lacks FDA approval

1 – SPECIFIED SUBCLASSIFICATION
Specified substances designated with an X

Exhibit

3

Arnold: Horsemen must speak up — loudly — about testing policies that defy realities of our environment

The following is being distributed by the National HBPA on behalf of trainer Rusty Arnold.

Open letter to horsemen by Rusty Arnold:

I received notification and today am accepting the penalties for a horse in my care testing for a controlled substance under current HISA/HIWU regulations. For the 2-year-old filly Figgy testing for a trace level of a Tramadol metabolite, I will start a seven-day suspension tomorrow, pay a $1,000 fine and have two points on my record. Figgy's owner is out the $40,000 winner's purse.

To be clear: I have no issue that I have a positive post-race test. The problem is why it is a positive.

We can't afford to just stand on the rail expressing outrage whenever one of our training colleagues gets ensnared in HISA and HIWU testing policies and penalties that defy common sense and ignore the realities of our environment.

We need to speak not amongst ourselves but to speak up together to regulators — loudly — that the system in place is unfairly hurting livelihoods and reputations while doing nothing to make our horses and industry safer. I'm not anti-HISA (Horseracing Integrity & Safety Authority). But I'm very much against some of the policies they've put in place — and allowed its enforcement arm HIWU (Horseracing Integrity & Welfare Unit) to put into place — in determining drug and medication violations.

Any trainer who thinks this can't happen to them has their head in the sand. Your turn is coming — as mine just did.

On January 27, 2024, I was informed by email that I was facing a possible controlled-substance violation for Figgy's post-race test following her victory in a December 27 maiden race at Turfway Park. (That's 31 days after the race – so much for HISA and HIWU vowing to be much quicker in turning around lab results.)

Tramadol is a human painkiller for which there are almost 30 million prescriptions in America. In Europe, a survey of 90 wastewater plants in 18 countries found Tramadol in 100 percent of the wastewater samples, according to research published in 2013.

Figgy had a finding of less than 3 nanograms (parts per billion) per milliliter only in urine. Researchers who have studied Tramadol and its metabolites in horses recommend a

conservative no-effect threshold level of 50 ng/mL in urine, determining that anything below that has no impact on performance.

I am accepting my penalty and will move on. However, I do 100 percent deny giving or instructing anyone on my staff to administer Tramadol to Figgy.

We interviewed every person who came in contact with Figgy and no one had a Tramadol prescription. Figgy is under 24/7 state-of-the-art video surveillance supplied by Keeneland. This was offered to HISA, but they never responded to the offer to make that video available. We do not believe Figgy received Tramadol in our care.

It is our belief that Figgy was contaminated with Tramadol either on the van ride to Turfway Park that day or in the receiving barn where she was in her assigned stall for approximately eight hours prior to her race. We have no control over those factors.

HISA has become a dictatorship. They are the judge, jury and executioner. Whether with open eyes or out of ignorance, they have let their enforcement arm throw common sense and fairness out the window.

How is it that the NBA, NFL and MLB all have a players' union and have representation in the rule and penalty policies of their leagues and horsemen have no representation in HISA policy? And don't tell me we have real representation through HISA's horsemen's advisory group.

We need more than seats on a glorified advisory committee. We need actual seats at the table where the shots are called. It's our livelihood we are dealing with, and it's being controlled by people with no idea what we face on a daily basis. HISA/HIWU are holding us to an unattainable standard.

I have no issues with HISA CEO Lisa Lazarus. I think she's a good person. But she is living in a fantasy land when it comes to keeping horses clear of environmental contamination. There is only so much we can do. Horses are grazing animals. They eat dirt. They love to lick smelly wet spots in stalls. They eat manure. They lick the walls of ship-in stalls. It is unreasonable to think we can control this.

The stalls in these receiving barns are used daily for different stables to race out of. Some are used multiple times a day for training in the morning before housing the race horses that night. It's very scary that you could be looking at a week or two-year suspension just because of the receiving-barn stall your horse was assigned.

HIWU undoubtedly knows that many of these positives are due to environmental contamination. The only explanation for acting on them, then, is to justify their existence.

Only one entity has been vocal about this fatally-flawed system for months, even years: The National HBPA, of which tens of thousands of horsemen are members through affiliates

such as Kentucky. It's time individual horsemen collectively join the chorus. Whether you believe HISA is constitutional or not, we should all be able to agree HIWU's system of gotcha chemistry is not helping but instead harming racing.

Horsemen have faced fines and suspensions for the unavoidable. When HISA has to backtrack, the rote response is "we've got to do better" or "see, the system is working!" — leaving behind trainers' trashed reputations and finances.

I was told a long time ago that it's easier to step on an ant than an elephant. Right now we are ants to HISA. But if we stand together, we become an elephant. We have the numbers. It's our own fault if we don't demand our rights and representation on HISA policy-making.

HISA, you are the law of the land. But don't forget how you got here. You didn't get in by majority vote. You didn't come in the front door. You snuck in the basement at midnight. So it's time for you to work with the majority.

Ms. Lazarus says we are better with HISA than without them. I say the same to her: Make us a partner, not an enemy.

It's not a lot of fun being accused and convicted of something you didn't do. However, if the HISA system doesn't get changed, we better get used to it.

Exhibit

4

Horsemen for common-sense drug testing

To the Attention of: Senator Mitch McConnell, Representative Andy Barr, Representative Paul Tonko, Senator Kirsten Gillibrand.

Horse racing is enduring self-inflicted wounds by not having equine testing standards that reflect today's sophistication of testing and the prevalence of environmental contaminants, including inadvertent transfer of human prescription medications causing adverse analytical findings.

The signatories ask the Horseracing Integrity & Safety Authority (HISA) and its testing and enforcement arm, the Horseracing Integrity & Welfare Unit (HIWU), to modify current testing thresholds. We ask that you demand HISA and HIWU to utilize established research and qualified veterinary scientists to set allowable thresholds below which substances have no pharmacological effect on racehorses.

Here's a link to uniformly well-respected thoroughbred trainer Rusty Arnold's open letter, "Horsemen must speak out - loudly - about testing that defies common sense, environmental realities": https://bit.ly/3IElRce

As signed by the following:

Petition Signatures

Contact				
Tom	Abrams		NE	68901
Kathryn	Ackman		OH	44125
Arthur	Adair		NY	11507
Ann M.	Adam		WA	98225
Terry	Adams		AR	71913
Mark	Adams		KY	42330
Blaine	Adams		NE	68528
Dennis	Adams		NE	68831
Anthony	Aguirre	Jr	MD	21046
Ashley	Aguirre		MD	21046
Carmen	Agustin		LA	71006
Gary	Aisquith		MD	21140
Raquel K.	Albright		KY	40361
Diane	Allen		FL	34482
Jay	Allen		IN	47987
Deana	Allen		MN	55044
Tom	Allen		NE	68137
Robert	Allen		PA	19020
Chuck	Allen		TX	76008
Lanna	Allen		WA	98391
Marshall	Allen		WA	98391
Robert	Allensworth		NY	11789
Federico	Alvarez		AZ	86315
Adrianna Cuesta	Amado		FL	33436
Rene	Amescua		AZ	85053
Pierre	Amestoy		NM	87120
Kelli	Amundson		WA	98001
Steven	Andersen		KY	40324
William	Anderson		NE	68854

Kathleen	Anderson		NE	68803
Steve	Anderson	DDS	NE	68803
Susan	Anderson		OH	45150
Jane	Anderson		WV	25414
Georgia	Andreadakis		MD	21093
Samuel	Angelo		NJ	7933
Karen	Angelos		WA	98058
Joseph	Anzalone		FL	33432
David	Applebee		MN	55372
Mary	Arendt		OR	97405
George	Arnold		KY	40513
Sarah	Arnold		KY	40513
Mark	Arnold		TN	37379
Ashley	Arreola		NM	88240
Caden	Arthur		OK	73099
Gatha	Artis		NJ	7712
Logan	Asbury		OH	45106
Norman	Ashauer		AR	71929
H. Ray	Ashford	Jr	OK	73165
Tristan	Ashford		OK	73165
Logan	Ashford		OK	73165
Karon	Ashford		OK	73165
Carl	Asmus		TN	38057
Naomi	Astorita		SC	29577
Tracy	Atkinson		KY	40505
Rob	Atras		NY	11010
Kevin	Atwood		KY	42240
Jose	Avalos		CA	90630
Kevin	Avera		SC	29928
Brian	Babbage		KY	40502
Tracy	Babcock		NE	68801

Kathleen	Babcock		NY	14425
Brandon	Bachle		NE	68803
Bob	Baffert		CA	91066
Jill	Baffert		CA	91066
J Michael	Baird		WV	26047
James	Baker		KY	40243
Sheena	Baker		KY	40324
William	Baker		KY	40383
Katherine	Baker		TN	37067
Lynette	Baldwin		NM	88324
Joseph	Baliga		IN	47334
Ann	Banks		KY	40511
Luis	Barajas		MD	20707
Danielle	Barber		OK	73099
Danielle	Barber		OK	73099
Carol	Barker		OH	45068
Amy	Barker		TN	37179
Jane	Barlament		WI	54155
Debbie	Barnett		KY	40241
Bobby	Barnett		TX	78621
Lon	Baronne		LA	70508
Erin	Barrett		IA	50009
Suzanne	Barrett		IL	60655
William	Basham		VT	5403
Stacy	Bason		NM	88352
Phil	Bauer		KY	40299
Arthur	Baxter		NY	11365
Robert	Baze		WA	98338
Lisa	Baze		WA	98002
Janet	Baze		WA	98092
Ben	Bealmear		KY	40370

Cathy	Beck		VT	5753
Lori	Beiber		NE	68872
Jason	Beissenherz		NE	68803
Thomas	Bell		CA	91007
Headley	Bell		KY	40513
Adele	Bellinger		FL	33446
Fenneka	Bentley		MD	21921
Fred	Benzel		NE	68873
Sean	Berger		NY	12801
Jeff	Berggren		NE	68801
Craig	Bergmann		SC	29572
Jenyka	Bergsma		AZ	85373
Gary	Bergsrud		ND	58367
Mike	Berry		FL	33409
Joann	Bertone		NJ	8527
Michael	Biehler		OK	73049
Mike	Bilbrey		MO	65616
Anna	Binkley		WV	25442
Danny	Bird		OH	44451
Billy	Birkett		OH	45066
Marissa	Black		OK	73051
Erin	Blaisure		PA	18801
Gregory	Bland		IN	46797
Anne	Bland		IN	46797
Scott	Blasi		TX	76011
David	Blickenstaff		KY	40361
Randy	Bloch		KY	40059
Chris	Block		IL	60007
Charles	Boden		KY	40502
Sharon	Boland		KY	40503
Mike	Bolduc		AZ	85260

Renay	Borel		AR	71913
Griffiths	Boreland		MD	21237
Shelby	Bornheimer		WA	98446
Charlie	Bosselman		NE	68801
James	Boulger		NJ	8109
Lynne	Boutte		FL	32686
Mona	Bowley		MD	20904
Roxann	Bowley		MD	20904
Ted	Bowman		AR	71913
Kelly	Bownes		KY	40513
Alan	Bozell		WA	98321
Bradley	Braasch		NE	68467
Patty	Braden		FL	34677
Donna	Bradley		NV	89406
Maury	Brassert		FL	34491
Rachel	Bratton		KY	40508
Ronald	Breed	sr	NY	14469
Kariann	Breed		PA	17601
Terry	Brennan		AR	71964
Kim	Brewer	DVM	FL	33414
Roy	Brewer		WA	98597
Brett	Brinkman		LA	70507
Brooken	Brinsfield		OH	44402
Joseph	Briordy		NJ	8690
Glenn	Bromagen	II	KY	40508
Sandra	Bromagen		KY	40513
Tyler	Bromagen		KY	40502
Caroline	Bromagen		KY	40502
Brad	Brown		KY	40065
Douglas	Brown		MD	21757
Petagaye	Brown		MD	20784

David	Brown		NY	14564
Charles	Brown		TN	37080
Ronney	Brown		WV	25446
Kaitlynn	Buchholz		MO	65605
Danna	Buechler		NM	88008
Bill	Buening		IL	62233
Terri	Burch		KY	40165
Lettie	Burch		KY	41091
Robert	Burgess		OK	73034
Margaret	Burlingham		WI	53156
Rob	Burroughs		FL	34476
Sandra	Busch		NE	68632
Vernon	Bush		FL	34652
George	Bush		KY	41001
Alex	Busker		MN	55318
Anthony	Butler		MA	1844
Jim	Butler		NJ	8053
Gary	Butrim		WA	98272
Vinnie	Buttice		KY	40509
Chris	Cahill	DVM	KY	40505
Danny	Caldwell		OK	74956
Bret	Calhoun		KY	40207
Michael	Callanan		KY	40511
Andrew	Calloway		IL	62025
Robert	Calvert		TX	76116
John	Camele		GA	30188
Mary Ellen	Camele		OH	45140
Rev. Martha	Camele		OH	45213
David	Camele		OH	45140
Michael B.	Campbell		IL	60047
Don	Campbell		KY	42301

Sarah	Campion		KY	40204
Padraig	Campion		KY	40362
Stacy	Campo		AZ	85355
Dick	Cappellucci		TX	79932
Gary	Capuano		MD	21401
Dale	Capuano		MD	21036
Ligia	Caramori		KY	40505
Eduardo	Caramori		KY	40505
Michael	Carinda		FL	33414
Shawna	Carlson		WA	98092
Douglas	Carlson		WA	98092
Angie	Carmona		CA	91007
Helen	Carpenter		IL	62441
Ed	Carr		IL	62865
Joe	Carr		KY	40505
Linda	Carrera		OH	44451
David	Carroll		KY	40207
William	Carson		TX	76109
Michael	Caruso		PA	18015
Tina	Casalinova		OH	44614
Carol	Casella		MA	1451
Traci	Casey		IL	62269
James	Casey	DVM	MD	20724
Maurice	Casey		VA	20115
Kyle	Caspersen		NE	68820
James	Cassidy		CA	91016
Steve	Castagno		WY	83013
Beth	Caster		OK	73049
Boyd	Caster		OK	73049
Laura	Cazares		FL	33414
Joseph	Cervi		FL	32708

Mike	Chambers		AK	99835
Kailey	Chambers		TX	79602
Laura	Champagne		LA	70592
John	Chandler		FL	33914
Vena	Charlton		OK	73093
Andrew	Chavers		CO	80602
Natalie	Chavez		NE	68827
Mario	Chavez		NE	68827
Mark	Cheney	DVM	FL	33435
Patricia	Chinn		WA	98125
Lynn	Chleborad		OK	73111
Mark	Christensen		NE	68803
Pamela	Christopherson		OR	97838
Jane	Cibelli		FL	34695
Joe	Clabes		KY	40513
Caitriona	Clancy		KY	40513
Sean	Clancy		VA	20118
Patrick	Clark		KY	42701
Patricia	Clark		KY	40361
Redge	Clarke		KY	40510
Ronald	Classen		WA	98125
Charlotte	Clavier		LA	70520
Odessa	Clelland		WV	25438
Nelson	Clemmens		KY	40026
Donna	Cline		AR	71913
Robert	Cline		OH	43146
Sheri	Clott		TN	38463
Ed	Cofiño		FL	33178
Tim	Cohen		CA	93040
Alice	Cohn		KY	40513
Ben	Colebrook		KY	40504

Tina	Cole-Davis		NJ	7731
Kelly	Colgan		PA	19350
Rusty	Collins		NE	68788
Marlene	Colvin		SD	57334
Bentley	Combs		KY	40511
Debbie	Connell		WA	98003
Steven	Connolly		IL	62223
Danita	Conte		KY	40218
Deborah	Conway		KY	40601
Susan	Cooney		VA	20144
Patrick	Cooney		VA	20144
Steve	Cooper		MD	21001
Carlotta	Cooper		TN	37743
Kay	Cooper		WA	98372
Casey	Cornwell		NE	68818
Taylor	Cornwell		NE	68818
Jim	Corrigan		KY	40361
Cassie	Corvin		KY	42712
Patrick	Costello		KY	40503
Kenny	Cox		MD	21054
Jeff	Cox		NE	69127
Vernon	Coyle		KY	40510
Mark	Coyle		NY	10956
Kari	Craddock		OK	73112
Sarah	Crane		DE	19709
Dirk	Crane		DE	19709
David	Crawford			33909
Anita	Cripps		FL	33756
David	Crisp		TX	75503
Mindy	Crocker		NE	68801
Jim	Crotts		AZ	85534

Vito	Cucci		NJ	7719
Analisa	Culy		WA	98390
Jeanine	Cumiskey		TN	37075
Debra	Curtis		AZ	85027
Philip	D'Amato		CA	91006
Steven	Daignault		PA	19040
Mike	Dale		WA	98071
Douglas K.	Daniels	DVM	VA	23103
Doug	Danner		KY	41042
Kenneth	Danyluk		OK	74955
Kehinde	Dare		DC	20018
Walter	Daum		FL	34460
Jamen	Davidovich		PA	15666
Blaine	Davidson		IN	47802
Milo	Davidson		OK	74738
Joseph	Davis		IN	46176
Donna	Davis		KY	41048
Gwen	Davis		KY	40245
Shawn	Davis		NE	68873
Robert	Davis		OH	45160
Joe	Davis		TX	76092
Elizabeth	Davis		WV	25443
Tom	Dawson		TX	79912
Lori	Dawydiuk		AZ	86315
Karla	De Jesus		FL	34695
Francesca	de la Flor		KY	40383
Rich	Decker		KY	40383
Mark	Deckert		LA	70001
Joe	Deegan		KY	40207
Glenn	Delahoussaye		LA	70520
Lyndsay	Delello		KY	40509

Mark	Delello		NY	13838
Meridith	Delello		NY	13730
Vincent	DellaMura		NY	10541
Peggy	Dellheim		FL	34477
Nancy	Delony		AL	35243
George	DeLuca		NJ	8748
Susan	Denning		AZ	85086
Peter	Deraitus		WA	98038
Cherie	DeVaux		KY	40507
Mark	Devereaux		CA	94904
William	Diamant		CAN	L5M 1E5
Keith	Dickey		FL	32668
Dr JoAnn	DiFillipo		AZ	85735
Joseph	Digeronimo		NJ	7444
Erin	Dilger		KY	40515
Damon	Dilodovico		MD	20625
Sarah	Dilodovico		MD	21136
Scott	Dilworth		KY	40383
Mike	Dini		FL	34677
Richard	Diss		FL	34434
Frank	Distefano		NJ	7083
James	DiVito		IL	60067
Linda	Dixon		FL	34473
Rob	Dobbs		IN	46239
Steven	Dobles		NY	14424
Lou	Donato		FL	34236
Carl	Doran		MD	21921
Alan	Dorton		KY	40383
Peter	Doth		KY	40511
Jeff	Douglas		NE	68901
Sue	Dowling		TX	77445

Evan	Downing		KY	40342
Michael J.	Doyle		FL	33316
Selmer Sam	Dronen		WA	98604
Richard	Dseveri		CO	80720
Ryan	Duffy		TX	75033
Henry	Dula		WA	98373
Larry	Duncan		IL	62010
Lisa	Duncanson		ME	4071
Richard	Dunn		CA	92861
Charles	Dunn		ID	83340
Stephen	Dunn		KY	40050
Harrison	Dunn		KY	40356
Pat	Dupuy		KY	40214
Danele	Durham		TX	76118
Janet	Durso		NY	10941
John	Dyer		KY	40383
Bryan	Easley		NE	68516
Tommy	Eastham		KY	40503
Terri	Eaton		CA	95320
Gretchen	Eaton		MN	55065
William	Edwards		IN	47164
Clemika	Edwards		NY	10553
Lee	Eferstein		KY	42757
Tammy	Ellerman		CO	80621
Ronald	Ellis		CA	91024
Cecilia	Ellis		KY	40361
Lisa	Ellison		FL	32668
John	Ellison		KY	40502
Brian	Elmore		IN	46163
Ganbat	Enebish		CA	91006
Greg	Engle		KY	40514

Chris	Englehart		NY	14548
Danielle	Eppard-Obert		KY	40383
Mary	Eppler		FL	33414
Richard	Erickson		WI	53012
Shellye	Essenpreis		IL	62254
Christina	Estvanko		KY	41048
Jeff	Evans		KY	40241
William	Evans		PA	19130
Michael	Ewing		KY	40510
Michael	Faber		IL	60031
Shannon	Fadden		WA	98828
Megan	Fadlovich		OH	44511
Robert	Falcone	Jr.	NY	11003
Carolyn	Fales		AZ	85128
Randal	Falldorf		NE	68803
Len	Fangmeyer		NE	68901
Mark	Farrar		OK	73750
Michael	Farwick		OH	45140
Louis	Fattorusso		NJ	7927
Brian	Fausch		NE	68801
Tim	Fella		IL	60091
Phil	Fendt		KY	40361
Clara	Fenger	DVM	KY	40324
Michael	Feriole		FL	32626
Denise	Ferrari		OH	44241
Kyle	Ferraro		CA	92657
Michael S.	Ferraro		NY	14425
Brandi	Fett		IA	50009
Todd	Fincher		TX	79932
Denny	Firestone		PA	17028
Michele	Fischer		KY	40205

Rick A.	Fischer	DVM	KY	40291
John	Fischetti		PA	17728
Ed	Fisher		AR	71901
Carol	Fisher		NY	12866
James	Fisher		OH	44512
Corby	Flagle		NE	68803
Roger	Fletcher		WA	98112
Tim	Fletcher		WI	53227
Danielle	Flores		IN	46176
Christian	Flores		OH	44509
David	Flynn		CT	6812
Greg	Foley		KY	40059
Sheree	Foley		KY	40059
Alex	Foley		KY	40059
Sean	Foley		NE	68028
Charles	Forrest		FL	34711
Grant	Forster		KY	40208
Steve	Fosdick		IN	47561
Eric	Foster		KY	42376
Tiffani	Fouks		WI	54023
Bambi	Fox		KY	42002
Gregory	Fox	DVM	KY	40511
John	Fradkin		CA	92706
Joel	Frances		NJ	8816
Margie	French		OK	74501
Kevin	Fries		NE	68801
David	Fronterhouse		TX	76258
Dennis	Fruchtl		NE	68865
Drew	Fulmer		AZ	85298
Joe	Funkhouser		WV	25414
Gregg	Gagliardi		FL	33618

Carla	Gaines		CA	91107
Olive	Gallagher		KY	40510
Brian	Gallagher		NE	68801
Fergus	Galvin		KY	40383
Genaro	Garcia		IN	46143
Denise	Garee		FL	32967
Michelle	Gargan		KY	40214
Susan	Garrett		OK	73003
Nicholas	Gay		NE	68864
Crystal	Gay		NE	68651
Abbie	Gdowski		NE	68801
Tom	Gdowski		NE	68801
Andrew	Gdowski		NE	68801
Martin	Genee		NJ	8816
Patricia	George		OK	74525
Tim	Geraci		OH	45245
Mike	Geralis		MD	21044
Michelle	Giangiulio		NY	11003
Bonnie	Gibbs		UT	84321
Mary Jo	Gilmore		IL	62958
Christina	Gindt		CA	92075
Tim	Girten		PA	16509
Kassie	Gladd		OK	74464
Ralph	Glass		KY	40383
Mark	Glatt		CA	91016
Christy	Gnadt		NE	68801
Alan	Goldberg		NJ	7722
Curt	Golden		WA	99214
Mickey	Goldfine		IL	60067
Katherine	Gollner		KY	40324
David	Gomez		LA	71064

Peter	Gonella		NJ	8742
Kylie	Gonzalez		IA	50047
José R.	Gonzalez	Jr.	NM	87105
Kathy	Goodale		TX	76087
Robert	Goodin		KY	40207
Dan	Govier		NE	68801
IdaMae	Gracia		TX	76088
Dudley	Graham		TX	76240
Judith	Grams		WV	25430
Anthony	Granitz		IN	46237
Michelle	Grant		NY	10452
Barbara	Graves		NE	68803
John	Greathouse		KY	40511
Andrea	Greathouse		KY	40347
Allen	Greathouse		KY	40504
Dorothy	Greeley		MD	21160
Amelia	Green		NY	12866
DeLinda	Green		TX	79922
Jeff	Greenhill		KY	41042
Sherri	Greenhill		KY	41042
Alfred	Greenwald	Jr	KY	40065
Carl	Grether		KY	40502
Ted	Grevelis		MN	55904
Martha	Griffin		KY	40513
Roger	Griffin		KY	40513
Ronald	Griffin		TX	79083
Patrick	Grimmett		OK	73075
Mike	Grossman		TX	78624
Kathleen	Guciardo		FL	33556
Jacque	Guerra		AZ	85022
Jody	Guida		KY	40068

Henry	Guillory	Jr	IA	50166
Victoria	Guinn		KY	40361
Randall	Gullatt		KY	40383
Glenn	Gunter		FL	32708
Tony	Guyette		NE	68872
Billie	Guyette		NE	68901
Daniel	Guzie		PA	18104
Beverly	Ha		OH	44406
John	Hackman		AL	36561
Kurt	Haecker		NE	68801
Edward	Hagan		OR	97216
Jimmie	Hale		AR	72936
Gloria	Haley		CA	94801
Aimee	Hall		MD	21921
Debra	Hamelback		KY	40601
William	Hamilton		KY	42303
Christy	Hamilton		LA	71064
Brian	Hamilton		NE	68848
Danny	Hamilton		OH	44420
John	Hamm		PA	17019
Kate	Hammer		AR	71901
Deloris	Hammer		WA	98292
Pat	Hammond		FL	34481
Belinda	Hancock		IN	47119
Joe	Hancock		KY	40601
Scott	Hansen		KY	40513
Stephen	Hansen		NE	68801
Todd	Hansen		WA	98589
George	Hanson		WA	99328
George	Harmening		MD	21158
Chuck	Harper		KY	40207

William	Harrigan		KY	40324
David	Harrington		AL	35115
William	Harris		AR	72450
Lisa	Harris		MO	65707
Doni	Harris		NE	68803
Jeff	Harris		WA	98937
Liane	Hart		IN	46140
Gary	Hartlage		KY	40216
Chris	Hartman		KY	40214
Hillary	Hartman		KY	40214
Eoin	Harty		CA	91024
Margaret	Hass		AR	72128
George	Hathaway		PA	19020
James	Hatley	Jr	TX	75115
Donnovan	Haughton		MD	20723
Shahrazad	Haughton		MD	21093
Philip	Hauswald		NY	11001
Mike	Hawbaker		MO	63383
Bryan	Hawk		OK	74804
John	Hawn		OH	43065
Michael	Hayes		IN	47401
Ann	Hayes		KY	40383
Randolph	Hayes	DVM	LA	71111
John	Hayes			1000
Neal	Hayias		NY	11590
James	Hays		TX	78621
Robert	Hearne		AR	72116
Tony	Hegarty		IL	60439
Monte	Hehnke		NE	68803
Norbert	Heider		TX	77354
Terry	Heinrich		NE	68901

H. Jack	Hendricks		KY	40422
Jack	Henry		NE	68801
John	Herbert		NJ	8730
Miguel	Hernandez		AZ	85345
Deborah	Hernandez		OH	45219
Marilee	Herrera		KY	40347
Rick	Hiles		KY	40223
Ronald	Hillerich		KY	40245
Annie	Hines		NE	68040
David	Hinton		OK	73025
Claudia	Hinz		NJ	8234
Bill	Hipp		PA	15905
Donna	Hjort		AZ	85382
Harry	Hoch		NE	68154
Max	Hodge		KY	40339
Penny	Hoeft		OR	97801
Ken	Hoffman		NJ	8809
Joerg	Hoffmann		NJ	8234
David	Hofmans		CA	91006
Teri	Hogan		KY	42431
Patricia	Hogan		NJ	8514
Jay	Holden		IN	46140
Arven	Holden		IN	46140
Hutch	Holsapple		KY	40383
Brandon	Holstein		KY	41042
Bart	Hone		AZ	85304
Thomas	Horan		OH	45014
Terry	Houghton		OH	44515
Mona	Hour		WA	98006
Neil J.	Howard		KY	40241
Hadley	Howard		MN	55721

Jared	Howard		MN	55721
George	Howard		NE	68901
Zizi	Howell		CA	91320
Jessica	Howell		KY	40513
Lesley	Howes		KY	40601
Michele	Hubbs		OH	44615
Nick	Hudak		PA	15001
Andrew	Hudson		NC	28036
Nancy	Hudson		OH	45368
Larry	Huffman		IN	47130
Byron	Hughes		KY	40509
Doyle	Hulme		NE	68521
Richard	Hummer		WV	25438
Donald	Hunt		FL	32696
Georgia	Hunt		WY	82410
Susan	Hunter		NM	88201
Michelle	Hurd		NE	68801
Sallyellen	Hurst		MD	21014
Rohan	Hutchinson		MD	20782
Robert	Hutt		PA	18977
Robert	Hutton		AZ	85395
Larry	Huwaldt		NE	68118
Robert	Ike		CA	91016
Dottie	Ingordo-Shirreffs		CA	91007
Karen	Jacks		TX	76140
Richard	Jackson		AR	71956
Laura	Jackson		MI	48356
George	Jacobs		CA	92649
Maggie	Jacobs		KY	40324
Piet	Jacobs		MA	1840
Gene	Jacquot		AR	71913

Susan	Jakovac		IL	62236
Mike	Jakubowski		NE	68824
Bredin	James		KY	40513
Jerry	Jamgotchian		CA	90267
Patricia S.	Jamgotchian		CA	90250
Keith	Jardine		NE	68803
Katherine	Jarvis		OH	45103
Troy	Javorsky		NE	68803
Bonnie	Jenne		WA	98002
Kandie	Jennings		WA	98266
Carmelina	Jerome		NJ	8753
Bob	Jobson		NY	14425
Davis	Jody		WI	53139
Kirsten	Johnson		KY	40383
Steve	Johnson		MD	20735
Diane	Johnson		NE	68801
Karen	Johnson		OH	45160
Jon	Johnson		OH	45036
Jamey	Johnson		WV	25427
Michelle	Johnson		WV	25405
Jennifer	Johnson		WV	26047
R. Wade	Johnson		WY	83111
Elizabeth	Johnson		WY	83111
Ginny	Johnston		AZ	85375
Rebecca	Johnston		OH	45157
Edward	Jones		CA	91750
Jim	Jones		CA	92010
Jeff	Jones		CA	92101
Mary	Jones		MN	55721
Tom	Jones		MN	55721
Ken	Jones		OH	45242

Timothy	Joseph		GA	30305
Ann	Juarez		WA	98903
John	Julia		PA	18925
Matt	Jurgena		NE	68901
Janette	Kahles		KY	41042
Ron	Kahles		KY	41042
Mark	Kane		MN	55302
Patrick	Kane		PA	19422
Eric	Kates		NJ	7726
George	Katrouzos		NE	68801
George	Katzenberger		NJ	7750
Kristine	Kaufeld		KY	40361
Marlene	Kedl		FL	32092
Cyndi	Kelley		CA	91007
Blake	Kelly		FL	33472
Shyann	Kelly		OH	44231
Eddie	Kenneally		KY	40502
Dan	Kennedy		NE	68803
Jocelyne	Kenny		FL	33019
Iveta	Kerber		IA	50266
Allan	Ketteler		NE	68801
Larry	Kettles		OK	73044
Edwyn	Kiely		KY	40383
Peter	Kiely		KY	40507
Barry	King		KY	40272
Sabrina	Kirton		OK	73950
Dan	Kjorsvik		MN	55416
Brian	Klatsky		NJ	7722
Luke	Kleen		NE	68325
Sam	Klein		OH	44060
Steve	Klesaris		DE	19808

Michael	Klingler		IN	47802
Liz	Klopp		IN	46173
Cori	Knapp		CA	92629
Paul	Knapper		KY	40330
Heather	Knisley		PA	16509
Nancy	Knott		IL	61235
Suzette	Koch		CO	80825
Brian	Koriner		CA	91010
John	Kosciak		MA	1757
Chris	Kotulak		NE	68818
Kaymarie	Kreidel		MD	20724
Joseph Eric	Kruljac		CA	92067
Dennis	Kruse		NE	68802
Don	Kruse		NE	68803
Brian	Kruse		NE	68801
Travis	Kuchar		NE	68826
Travis	Kuenning		NE	69101
Brandon	Kulp		PA	17078
James	kuntz		NE	68510
Steve	Kunzman		NE	68801
Rachel	LaCount		MN	55378
Michael	Lacy		OK	73034
Perry	Ladd		CO	80741
Robert	Ladd		KY	40502
MaryEllen	Laird		WA	98198
Barry	Lake		NE	68506
Scott	Lake		PA	18940
Tom	Lambro		WA	98038
Jill	Lammey		NJ	7035
Sara	Landon		CA	93463
Bo	Landry	DVM	KY	40502

Kara Paige	Landry		KY	40502
Don	Lang		NE	68950
John	Langemeier		KY	40324
Cheryl	Lankford		CA	92069
Crystal	Lanum		FL	34482
Robert	Lapine		NJ	8879
Kathy	Large		AZ	86327
Paul	Larocca		NJ	8050
Justin	Larsen		NE	68138
Frances	Latimer		TX	79755
Penny	Lauer		KY	40022
Michael	Lauer		KY	40022
Brian	Laux		NE	68901
Troy	Lavene		NE	68927
Bonnie	Lawlor		PA	19087
Dalton	Lawrence		FL	33625
Bob	Lawrence		WA	99323
Heath	Lawrence		WA	99323
Brooke	Lawrence		WA	99336
Luke	Lawrence		WA	99323
Michael	Lecesse		NY	14425
Cynthia	Leigh		LA	70520
Daniel	Leitch		KY	40347
George	Leonard	III	IN	46176
Patrick	LePley		WA	98006
Mary	Leppo		KY	40509
Michael	Lerman		FL	34474
Sergio	Levenzon		CA	92620
Kany	Levine		WA	98342
Petra	Lewin		WA	98531
Jim	Lewis		AZ	85624

Patrick	Lewis		TN	37205
Juan	Leyva		CA	91016
Steven	Linden		NY	11801
Jon	Lindo		CA	92011
Annette	Lindstrom		TX	76088
Christopher	Linke		NE	68803
Danny	Linville		FL	33594
Thomas	Little	DVM	IN	47119
Pam	Little		IN	47119
Kelsey	Littleton		IA	50009
Joseph	Locicero		NY	12477
Nancy	Lock		NM	88203
David	Lock		NM	88203
Adam	Lockard		MD	20724
Stacy	Locke		CA	92003
Jimmie	Lockhart		KY	40583
Dustin	Lofing		NE	68801
Linda	Loftin		CO	80908
Richard	Loftus		NJ	7013
Charles	Logsdon		KY	40223
Naomi	Long		VA	22611
Jacinto	Lopez		FL	32668
Angel	Lopez		KY	40515
Michelle	Lovell		KY	40208
Rod	Lowe		OR	97537
Tobi	Lowe		PA	17078
Kenneth	Lowe		WV	25443
Vito	Lucarelli		WA	98296
Ted	Lucas		KY	40476
Joe	Lucas		OK	73064
James	Lueken		IA	52042

Valorie	Lund		KY	40511
Brett	Lundin		NE	68124
Daniel	Lusk		MO	65725
Keith	Luzar		IL	60467
Lee	Lyle		KY	40059
Cal	Lynch		DE	19711
Daniela	Lynch		DE	19711
Brian	Lynch		FL	33472
Ann	Lynch		KY	40361
Mary	Lynch		MI	48103
Virgil	Maas		WA	98055
Wayne	Mackey		KY	40505
Mark	Maddox		OH	44288
Rachael	Maddox		OH	44288
Zach	Mader		NE	68803
Max	Mader		NE	68803
Jeremy	Mader		NE	68803
Todd	Mader		NE	68803
Paul	Magee		KY	40207
Ronald	Magrady		AZ	85747
Neal	Maharaj		FL	32668
Rebecca	Maker		KY	40383
Jonathan	Maldonado		MD	21144
Vonn	Mallery		TX	75232
Brenda	Malmgren		PA	16505
Brent	Malmstrom		KY	40515
Sue	Mancilla		MD	21042
Roy	Manfredi		NM	87740
Maria	Mann		FL	34482
Michael	Mann		IN	46239
John	Manning		KY	40475

Chip	Mansfield		TX	76110
John	Marceda		NY	11580
Timothy	Marchant		MD	21234
Henry	Marchetti		PA	18224
Richard	Marchiondo		NM	87113
Frank	Marcum	DVM	KY	40383
Anthony	Margotta		FL	33139
Melissa	Marinello		VA	20175
David	Marinello		VA	20175
Joel	Marr		NM	88352
Michele	Marshall		NJ	8098
Audrey	Martel		CA	93611
Adam	Marthaler		KY	40383
Paul	Martin		MO	63304
Lisa	Martin		PA	18431
Clarence	Martin		PA	18431
Lisa	Martin		WA	98908
Ralph	Martinez		IL	62062
Erica	Martinez		IL	62062
Ramon	Martino		FL	33445
Matthew	Maser		NE	68801
Ingrid	Mason		AR	46176
Ron	Massaro		ID	83634
Patrick	Masson		KY	40511
Richard	Masson		KY	40511
Randy	Matthews		IN	47124
Pete	Mattson		FL	34135
Alan	Maus		MO	65802
April	Mayberry		FL	34482
Donna	McArthur		TX	79912
Raymond	McAtee		OH	44512

Kim	McCarthy		CA	91017
Sean	Mccarthy		CA	91017
Jack	McCartney		ID	83616
Nathan	Mccauley		KY	40508
Erin	McClellan		PA	17033
Emilee	McClun		MD	20708
Diana	Mcclure		VA	22611
Faron	Mccubbins		KY	40047
Melanie	McDonald		NV	89447
Teresa	Mcfarlin		AR	71933
Paul	McGee		KY	40205
Jim	McGreevy		PA	19363
Mark	McGregor		CO	80104
Melissa	McInturf		TN	37138
Leona	Mckanas		MA	2180
James	McKay		TN	37027
Andrew	Mckeever		KY	40504
Kenneth	McKeever	DVM	NJ	8527
Joe	McKellar		TX	75603
Tom	McKenna		NM	88119
Andrew	McKenzie		FL	34113
Brian	McKenzie		FL	32806
Daniel	Mckenzie		MD	20723
Mary	McKinley		PA	16142
Hugh	McMahon		MD	20776
David	Mcshane		IA	50161
Daniel	Meagher		WV	25414
Jan	Meehan		FL	33556
Fawn	Meehan		FL	33556
Cynthia	Megariz		AZ	85713
Martha	Megredy		KY	40511

Monty	Meier		CA	94566
Nicholas	Meittinis	DVM	MD	21152
Charles	Meredith		KY	40214
Joe	Merrick		OK	73662
Edwin	Merryman		MD	21904
Lisa	Meyer		IL	62062
Dan	Meyer		PA	19087
Madison	Meyers		VA	20117
Judy	Mickelson		KY	40383
Michael	Mickens		NJ	7457
Thomas	Middleton		NE	68802
Matt	Middleton		NE	68801
Camme	Miles		KY	40207
Peter	Miller		CA	92024
Maurleen	Miller		FL	32696
Patti	Miller		IL	60067
Kenneth	Miller		IN	47713
James	Miller		MD	21742
Mark	Miller		NE	68801
James	Miller		NJ	7755
Arizona	Miller		OH	44515
Ashley	Miller		OH	44515
James	Miller		SC	29803
Allen	Milligan		AR	71913
John	Milliron		DE	19808
Milan	Milosevic		MD	21234
Walstra-Hummel	Mindy		MO	63038
Patt	Mineer		IL	62260
Shelly	Minnick		IA	52726
Allen	Mintzmyer		NE	69138
Leonard	Miranda		CA	91908

Geraldyne	Mitchell		NJ	8241
Sally	Mixon		MN	55379
Aaron	Moderow		NE	68801
Maria	Molnar		OH	44139
Julie	Molt		NE	68803
Lauren	Monnet		KY	40383
Ron	Monnig		MO	65641
Joseph	Montano		KY	40118
Jon	Moore		AZ	85085
Peter	Moore		NY	10527
Daniel	Morales		TX	76682
Kathleen	Mordenti		LA	71037
D.E.	Moreland	Jr	NJ	8846
William	Morey		KY	40324
Terry	Morgan		IL	62260
Athol	Morgan		MD	21044
John	Moroney		MO	63084
Michael	Morris		OR	97070
Chris	Morrow		TX	79118
Frank	Mosca		FL	33180
Jon	Moss		IA	50009
Carolyn	Moss		TN	37137
William	Mott		NY	12020
Jeremy	Mueller		NE	68803
William	Mullenberg		WA	98611
Jeff	Mullins		CA	91773
Ben	Mumaw		OH	44412
James	Munch		OR	97325
Bruce	Murphy		IN	47933
Christopher	Murphy		KY	40059
Karen	Murphy		NY	12136

Cindy	Murphy		OK	73049
Mike	Murphy		TX	75080
Stacy	Muskopf		IL	62234
Gary	Myers		NE	68876
Edwward	Nabakowski		OH	43123
Ellis	Naifeh		OK	74701
Tyler	Neal		NE	68803
Roger	Neff		OK	73084
Jay	Nehf		CA	91024
Albert	Nelson		MS	38619
Kimberly	Nesbitt		IN	47710
Mandy	Ness		MD	21915
Craig	Netwig		CA	92198
Joan	Nevin		OK	73049
Denise	Newsome		PA	19348
Matthew	Nichol		NY	10310
Mindy	Nichols		CA	93514
Angela	Nichols Leventhal		NY	11542
Brandi	Nicholson		KY	40383
Michelle	Nihei		FL	33472
Sarah	Nims-Seaman		KY	40505
Nancy	Noble		KY	40504
Henry	Nothhaft		TX	78732
Jennifer	Nunnally		WA	98360
William	O’Donnell		MA	1801
Doug	O’Neill		CA	91403
Taricia	Obeng		MD	21144
Chris	Obermeier		NE	68803
Rolf	Obrecht		OH	45230
Ronald	Occhino		CT	6903
Maryann	O'Connell		WA	98001

Robert	O'Connor		KY	40243
Frankie	O'Connor		KY	40509
Matthew	O'Connor		WI	54941
Immanuel	Odhiambo		MD	20794
Olivia	Odle		IL	62040
Carin	Offerman		MN	55364
Tom	O'Keefe		LA	70005
Randy	Okimoto		WA	98031
Kim	Oliver		CO	81652
Bill	Oltean		NE	68801
Joanne	Oltean		OK	73013
James	O'Malley		FL	34235
Tressa	O'Neill		NE	68801
Pat	O'Neill		NE	68810
Steven	O'neill		VA	22735
Ed	Orr		CO	80634
Susie	Orr		CO	80634
Frank	Ortiz		AZ	85027
John	Ortiz		KY	40511
Kevin	Owens		AZ	85306
Debbie	Owens		AZ	85306
RKory	Owens		AZ	85306
Mark	Owens		KY	40513
Matt	Owens		MO	63051
Debra	Pabst		WA	98321
Michelle	Padgett		FL	34470
Juan	Padilla		OK	73049
Helokay	Palmer		MD	20705
David	Palmer		MD	21136
Brian	Paras		IN	46260
Jacqueline	Paris		MO	65084

Billy Joe	Park		KY	42456
Jerry	Parker		CA	94804
Francis	Parker		KY	40502
Riley	Parsons		KS	67801
Joyce	Patci		KY	40511
Thomas	Patella		OH	44511
Kyrie	Patino		WA	98022
Faustino	Patino Lopez		WA	98022
Robert	Patrick		IN	46064
Kevin	Patterson		AZ	85255
Joni	Patterson		IN	46140
Darrin	Paul		WA	98012
Chadwick	Peach		KY	40601
Debbie	Peery		WA	98002
Jody	Peetz		WA	98027
Joe	Pell		NY	11729
Amparo	Pena		TX	78520
Michael	Penna		KY	40383
Gene	Penrose		NE	68803
John	Perkins		TN	38060
Mike	Perkins		TX	76462
Gina	Perri		MD	21163
Jim	Perry		KY	40517
Joe	Petalino		AR	71903
Val	Peterson		IL	60067
Andy	Philopulos		IL	60453
Tom	Phipps		MA	2561
Jason	Phipps		NE	68803
Jody	Pieper		AR	71956
Scott	Pierce		KY	40511
Holly	Pierson		NE	68876

Sabina	Pish		TX	76462
Danny	Pish		TX	76462
Joseph	Pish		TX	78108
Daniel	Pita		FL	32668
Michael	Pitcher		NE	68801
Helen	Pitts		KY	40022
Peter	Pizzo		KY	40577
Kenneth	Pleasant		VA	22602
Paulette	Pogue		OK	74343
Christian	Polzin		IN	46163
Catherine	Pool		CA	94605
Joe	Poole		OH	44266
Michael	Porcaro		IL	60618
Shane	Porter		AR	72176
Michael	Porter		KY	40207
Eugene	Post		AR	72903
Vicki	Potter		WA	98001
Michelle	Potter		WI	53015
Wayne	Potts		NY	11413
Dee	Poulos		IL	60067
Jimmy	Poulos		MD	20901
Leonard	Powell		CA	91024
Elena	Pradenas		MD	20707
Mike	Prah		MD	21144
Susie	Prather		NM	88240
Randy	Preston		KY	40505
Rick	Proctor		MD	21093
Dwight	Puckett		FL	34482
Slither	Pudding		KY	40208
Kim	Puhl		OH	43515
Victor	Pulido		WA	98512

Lori	Pullen		TN	37033
David	Purcell		TN	37204
Orv	Qualsett		NE	68803
Jennifer	Quinones		FL	34482
Richard	Rader		KY	40513
Scott	Rake		MN	55020
Jose	Ramirez		DE	19808
Larry	Ramnarine		WA	98092
Cruz	Ramos		NE	68803
Anthony	Randazzo		IL	62040
Tony	Randone		NE	68803
Melissa	Randone		NE	68803
Philip	Raymond		PA	19425
Philip	Reale		WV	25311
John	Rednour		IL	62832
Laurie	Rednour		IL	62832
Richard	Reed		KY	40330
Kenneth	Reed	DVM	NC	28571
Jeffrey	Reed		NE	68865
Austin	Reed		NY	14424
Jennie	Rees		KY	40214
Jeffery	Reeves	Sr.	LA	70711
John	Regan		FL	34689
Adrian	Regan		KY	40383
Mark	Reilly		MA	2367
Peter	Reilly		NJ	8846
Peter	Reiman		IL	62916
Russell	Reinsch		NE	68436
Rock	Renning		CA	92082
Russ	Rerucha		NE	68803
AJ	Rerucha		NE	68801

Ramiro	Restrepo		FL	33129
Richard	Rettele		MI	48168
Justin	Revak		MN	55020
Patrick	Rhodes		FL	34688
Bernell	Rhone		FL	34677
Alan	Ribble		AR	71754
Alfred	Riccio		NY	12118
Albert	Richman		NJ	7039
Tricia	Rickert		NE	68601
Darrel	Rickert		NE	68601
Pamela	Rider		KY	40361
Caitlin	Riland		MO	65804
Janet	Ritchey		MD	21921
Larry	Rivelli		IL	60010
Mark	Rivolta		MO	63049
Gina	Robb		MD	21797
Linda	Robbins	DVM	AR	71913
Denis	Roberson		KY	40059
Andy	Roberts	DVM	KY	40515
Tommy	Roberts		NE	68853
Joseph	Roberts		NM	88352
Jim	Roberts		OK	73012
Pam	Roberts		TX	77018
Kevin	Roberts		TX	77363
Amber	Rockwell		WV	25427
Gerry	Rodak		OH	43204
Michele	Rodriguez		LA	70435
Scott	Rollins		AZ	86333
Jerry L.	Romans	Jr.	NC	27043
Robert	Rondeau		WA	98166
Michael	Rone		OH	43146

Katrina	Rother		NE	68803
Martin	Rouck		IN	47172
David	Rouzee		NE	68803
Mary	Rowan		CA	91016
John	Royer		OH	43215
Cynthia	Rush		IL	62234
Jared	Ruskin		MN	55108
Randall	Russell		FL	34608
Tim	Russell		KY	40324
William	Ryan		KY	40206
Bruce	Ryan		OH	45152
Richard	Sackett		UT	84713
Stan	Sadler		KY	40383
Marilyn	Sadler		KY	40383
Jenine	Sahadi		CA	92014
Michelle	Salazar		NM	87123
Teresa	Salazar		NM	87123
Melissa	Saldana		CA	91106
Karen	Salisbury		IN	47136
Mark	Salvaggio		PA	17078
Michael	Salvaggio		PA	17042
Timmy	Salzman			21158
Erven	Samsel		MA	2021
Carlos	Sanabria		FL	33027
Christine	Sanchez		CA	92630
Phillip	Sanchez		NM	87047
Wade	Sanderson		WV	25414
Barry	Sandstrom		NE	68845
Brenda	Sandy		KY	40391
Bonnie	Santanello		IL	62260
Beth	Saul		NY	14513

Thomas	Saunders		Ont	1000
James	Sautter		KY	40511
Scott	Sawyer		NJ	7067
James	Sayler		OR	97367
Kim	Schaffer Marrs		CA	92003
Hal	Schager		NE	68803
Randy	Scheen		KY	40206
Ellen	Schenk		FL	33472
Wayne	Scherr		SD	57380
Shana	Schiemann		IL	60033
Bradley	Schliefert		NE	68405
Janine	Schmitt		KY	41042
Phil	Schoenthal		PA	19352
Constance	Schuble		KY	40383
Sheryl	Schuette		SD	57345
Mark	Schultz		CO	81505
John	Schultz		IN	47427
Roger	Schuster		NE	68840
Fred	Schwartz		WI	53558
Denise	Scoles		IA	50166
Jason	Scott		NM	88312
Cecil	Seaman		KY	40505
Tony	Seel		NY	13850
Mark	Sell		WV	25430
John	Sellmeyer		NJ	8857
Pamela	Sena		NM	87068
Dawn	Serey		NJ	8037
Shari	Sewell		WA	98146
Joanne	Shankle		MD	21755
Tim	Sharp		TX	75433
John	Sharpe		NJ	7733

John	Shaw		FL	34471
Jack	Sheard		NE	68803
Clark	Shepherd		KY	40503
Trisha	Shepherd		OH	44647
Nikki	Sherman		PA	19090
Michael	Sherr		FL	33993
Mort	Shirazi		KY	40361
John	Shirreffs		KY	40588
Charles	Shive		KY	40299
Tommy	Short		KY	40385
Aaron	Shorter		IN	47882
Mark	Shuman		MD	21921
Adam	Sidlo		NE	68973
Samantha	Siegel		CA	90210
Grace E.	Siegwarth		WA	98003
John	Sikura		KY	40361
Maryellen	Silva		CA	94710
Rosie	Simkins		WA	98360
JB	Simmons		TX	75751
Andrew	Simoff		PA	19351
Sharon	Simons-Passmore		TN	37774
Tracy	Simpson		OK	74864
Matthew	Sims		KY	40347
Philip	Sims		KY	40544
John	Sivick		MD	21771
Danielle	Skorniak		NE	68803
Kerry	Skowronsky		OH	44880
Ron	Skowronsky		OH	44880
Donald	Smith		CA	94804
Robin	Smith		CO	80117
Holli	Smith		KY	40245

Mary Jane	Smith		KY	40356
Connie	Smith		MD	21158
Valrie	Smith		MD	20723
Robert	Smith		MO	65803
Kirk	Smith		NE	68803
Laurie	Smith		TX	75751
Jaqueline	Smith		WA	99337
Heather	Smullen		FL	32766
John	Smyth		IL	60076
Margaret	Smyth		OH	44022
Brayden	Snell		NE	68801
Charles	Sniffen		MD	21054
John	Snow		WA	98022
Ken	Snyder		KY	42055
Martin	Somelofske		NJ	7920
Anthony	Spadea	Jr	MA	2184
R. Townsend	Sparks		MO	63857
Quinten	Spivey		IL	62398
Sue	Spooner		WA	98513
Jordan	Springer		KY	40291
Fred	Sprinkle	DVM	KY	40010
Kenneth	Staab		NE	68803
Keith	Stafford		SC	29670
Albert	Stall		LA	70401
Janice	Stefanik		PA	18222
Don	Stemmans		LA	70520
William	Stenberg		WA	98058
Chris	Stenslie		WA	98387
James	Stephenson		AZ	85308
Christopher	Sterbenz		VA	22183
Josh	Stevens		KY	40324

Chad	Stewart		FL	34482
Dallas	Stewart		KY	40245
William	Stewart		WV	26047
Michael	Stidham		CA	92692
Kenneth	Stiff		IL	60008
Damon	Stinson		FL	33138
Flint	Stites		PA	17028
Nichole	Stoltenberg		NE	68803
Beverly	Strauss		PA	19352
Robert	Sturgeon		KY	40361
Clark	Sturgeon		KY	40361
Megan	Sturgeon		KY	40361
Shawn	Sudo		WA	98057
Lance	Sullenberger		AR	72211
Erin	Sullivan		NE	68803
Nancy	Summers		NM	87105
KC	Sundermeier		NE	68871
Pamela	Sveinson		TX	76462
Keith	Swagerty		WA	98092
Steven	Swartz		OH	44515
J. Kent	Sweezey		KY	40511
Candice	Sweitzer Deubel		WA	99114
Bruce	Swihart		NE	68803
Stephanie	Swiontek		AZ	85085
Jon	Tainow		FL	33070
Francisco	Tapia		AZ	85641
Albert	Tassone		MA	1862
Everett	Taylor		IL	62236
Eduardo	Terrazas		KY	40511
Lawrence	Terrell		CO	81524
William	Terrill		FL	33019

Valora	Testerman		MD	21093
Daniel	Thayer		NE	68801
Ruby	Thomas		CA	93675
James F.	Thomas		KY	40205
Philip	Thommen		MD	21701
Valerie	Thompson		CA	92088
Lynnette	Thompson		SD	57638
Jeffrey	Thornbury		KY	40362
Keith	Throm		IL	62062
Brian	Tibbels		PA	19330
David	Tibbitts		IA	50658
Ramona	Tingdale		WA	98022
Patty	Tipton		KY	40513
Thomas	Tobin	DVM	KY	40511
Lacy	Toennies		IL	62830
Vicki	Tomlinson		KY	40065
Jennifer	Tooley		KY	40511
Michael	Toro		NY	11772
Joe	Toye		WA	98901
Karl	Toye		WA	98901
Joan	Tracy		TX	78624
Bill	Tracy		TX	78624
Ben	Trask		FL	33470
Terri	Trimble		VA	22620
Connell	Trout		OK	73034
Robert	Tucker		AR	71901
Joy	Tucker		TX	78617
Rhonda	Tuley		TX	76044
Terry	Tuley		TX	76044
Frederick	Tunks		KY	42211
Aidan	Turnage-Barney		VA	23805

Connie	Turner		AZ	86323
Jesse	Ullery		KY	40383
Eugene	Ulmer		NE	68803
Kim	Valerio		KY	40383
Stacy	Van Horn		OK	73051
Debbie	Van Horne		OR	97305
Becky	Van Housen		NE	68654
Terry	Van Housen		NE	68666
Marylee	Vanderpool		IA	50125
Linda	Vannoster		WA	99328
Dianne	VanScoyk		IL	62001
Cathy	Varian		WV	25123
Treva	Vazquez		FL	34609
Ochoy	Virgo		CT	6704
Derek	Vogt		NE	68831
Kelly	Von Hemel		IA	50327
Donnie K.	Von Hemel		OK	73078
Pamela	Vonhemel		IA	50327
Kelsey	VonHemel		IA	50315
Katharine	Voss		MD	21794
John	Wade		IL	60462
Karin	Wagner		DE	19711
Mona	Wahlert		CO	80723
John	Wainwright		IL	62260
Robert	Wald		NE	68803
Doug	Wall		OK	73069
Jerry	Wallace		CA	92570
Andrew	Wallace		VA	24017
Jennifer	Wallace		VA	24017
Norhett	Walls		CA	92570
Bill	Walmsley		AR	71903

Chris	Walsh		IN	47201
Brian	Waltz		OH	45244
Dorreen	Wanitschke		NE	68801
David	Warchol		IL	62221
Wesley	Ward		KY	40383
Riley	Ward		KY	40502
Patrick	Waresk		KY	40361
Mary	Warner		VA	22556
Herbert	Warren	DVM	CA	92817
Peter	Wasiluk		FL	34677
Patrick	Watkins		IL	62269
Pamela	Watterson		WA	98038
George	Weaver		FL	33414
Whitney	Webb		FL	33696
Deb	Weber		NE	68872
Larry	Weeden		KY	40026
Ray	Weis		KY	40207
Christina	Welker		KY	40383
Bayne	Welker		KY	40383
Kelly	Wellington		IA	50054
Sarah	Wells		KY	42303
Joseph	Wells		KY	40361
Brooks	Wells		NE	68803
Barton	Wells		NE	68803
Charles	Wermuth		PA	19136
Patricia	Wermuth		PA	19136
Ethan	West		KY	41042
Terry	Westemeir		OK	74014
Kelly	Wheeler		KY	40383
Richard	White		IN	47720
Charles	White		KY	40291

Gary	White		OK	74955
Robert	White		PA	16509
Patricia	White Johnson		KY	40513
James	Whitler		LA	71115
Ralph	Whitney		FL	34482
Lon	Wiggins		KY	40245
John	Wilborn		IL	62234
Jimmie	Wilbur		FL	33576
Stacia	Wiliams Minshew		FL	32643
David	Wilkett	DO	OK	74114
Jeff	Willard		KY	40059
Rick	Williams		FL	33327
Robert	Williams		FL	32643
James	Williams		KY	40223
Melanie	Williams		MD	21921
Charlie	Williams		MI	48224
Casey	Williams		NE	68801
Misti	Williams		NE	68801
Laura	Williams		NE	68801
Tyler	Williams		NE	68801
Tom	Williams		NE	68801
Roger	Williams		OK	73059
Russell	Williams		PA	17331
Justin	Williams		TX	78114
Steven	Wills		KY	42303
Robert	Wilmot		WA	98059
Vicki	Wilson		WA	98584
Don	Winfree		KY	40214
Michael	Winings		OH	44691
Christen	Winton		NE	68818
Tracey	Wisner		MI	48829

Ernie	Witt		AR	71913
Keith	Witting		MI	48038
William	Wofford		KY	40511
John	Wolf		NE	68801
Margaret	Wolfe		MD	21227
Garald	Wollesen		NE	68136
Larry	Womack		AR	72128
Patricia	Wonderley		CO	80129
Jonathan	Wong		CA	91741
Linda	Wood		CO	81403
Virginia	Wood		IL	62254
A	Woodford		PA	19311
Sarah	Woodland		CO	80615
Ryan	Woodland		CO	80615
Robert	Workman		KY	41042
Hume	Wornall		KY	40361
Fred	Worsham		KY	40513
Carol	Worsham		KY	40513
Jennie	Wright		NE	68801
Tammy	Wright		OK	73003
Cherie	Wright		WA	98058
Blaine	Wright		WA	98022
Susan	Wright		WA	98901
Mark	Wroblewski		IL	60517
Robert	Y		FL	34677
Anita	Yacoub		CA	90630
Tim	Yakteen		CA	91066
Joseph	Yarberry		OK	73044
William	Yarbrough	DVM	FL	33414
Daniel	Yarbrough		FL	33414
Charles	Yeldezian		GA	30223

James Lloyd	Yother		AZ	85331
Scott	Young		OK	74361
Erin	Younger		KY	40165
Susan	Zaleski		PA	19348
Marla	Zanelli		CA	92014
Matthew	Zehr		KY	42437
Whitney	Zeringue		LA	70094
Kirsten	Zielinski		OH	44039
Richard	Zielinski		OH	44039
Mark	Zoller		NJ	8742
Cristina	Zunino		WA	98071

Exhibit

5

U.S. Court of Appeals for the Fifth Circuit

No. 23-10520

NATIONAL HORSEMEN'S BENEVOLENT AND PROTECTIVE ASSOCIATION; ARIZONA HORSEMEN'S BENEVOLENT AND PROTECTIVE ASSOCIATION; ARKANSAS HORSEMEN'S BENEVOLENT AND PROTECTIVE ASSOCIATION; INDIANA HORSEMEN'S BENEVOLENT AND PROTECTIVE ASSOCIATION; ILLINOIS HORSEMEN'S BENEVOLENT AND PROTECTIVE ASSOCIATION; LOUISIANA HORSEMEN'S BENEVOLENT AND PROTECTIVE ASSOCIATION; MOUNTAINEER PARK HORSEMEN'S BENEVOLENT AND PROTECTIVE ASSOCIATION; NEBRASKA HORSEMEN'S BENEVOLENT AND PROTECTIVE ASSOCIATION; OKLAHOMA HORSEMEN'S BENEVOLENT AND PROTECTIVE ASSOCIATION; OREGON HORSEMEN'S BENEVOLENT AND PROTECTIVE ASSOCIATION; PENNSYLVANIA HORSEMEN'S BENEVOLENT AND PROTECTIVE ASSOCIATION; WASHINGTON HORSEMEN'S BENEVOLENT AND PROTECTIVE ASSOCIATION; TAMPA BAY HORSEMEN'S BENEVOLENT AND PROTECTIVE ASSOCIATION; GULF COAST RACING, L.L.C.; LRP GROUP, LIMITED; VALLE DE LOS TESOROS, LIMITED; GLOBAL GAMING LSP, L.L.C.; TEXAS HORSEMEN'S PARTNERSHIP, L.L.P.,

Plaintiffs – Appellants

STATE OF TEXAS; TEXAS RACING COMMISSION,

Intervenor Plaintiffs – Appellants

v.

JERRY BLACK; KATRINA ADAMS; LEONARD COLEMAN; MD NANCY COX; JOSEPH DUNFORD; FRANK KEATING; KENNETH SCHANZER; HORSERACING INTEGRITY AND SAFETY AUTHORITY, INCORPORATED; FEDERAL TRADE COMMISSION; COMMISSIONER NOAH PHILLIPS; COMMISSIONER CHRISTINE WILSON; LISA LAZARUS; STEVE BESHEAR; ADOLPHO BIRCH; ELLEN MCCLAIN; CHARLES SCHEELER; JOSEPH DEFRANCIS; SUSAN STOVER; BILL THOMASON; LINA KHAN, CHAIR; REBECCA SLAUGHTER, COMMISSIONER; ALVARO BEDOYA, COMMISSIONER; D. G. VAN CLIEF,

Defendants – Appellees

On Appeal from the United States District Court
for the Northern District of Texas
Case No. 5:21-cv-00071-H
Honorable James Wesley Hendrix

NHBPA MOTION TO SUPPLEMENT THE RECORD

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MOTION TO SUPPLEMENT THE RECORD

Pursuant to Federal Rule of Appellate Procedure 10(e)(3), the NHBPA Appellants move to supplement the record to add Appendix A, a press release from the Horseracing Integrity & Safety Authority dated June 4, 2024, and state as follows:

1. In the record at the trial court, the NHBPA Appellants introduced numerous examples of the Horseracing Integrity and Safety Authority acting to rewrite the rules of horseracing through guidance documents such as operational bulletins, press releases, and newsletters. *See* ROA 3228, 3668, 3672, 3674, 3675, 3676, 3678, 3682, 3685, 3687, and 3690. The Defendants did not object to the inclusion of these documents in the record at trial.
2. When the Authority issued additional guidance documents rewriting the rules of horseracing during the appellate briefing process, the NHBPA Appellants asked the Court to take judicial notice or supplement the record with them. *See* Principal Br. p. 37 n.31; Reply Br. p. 8 n.2.
3. When the Authority issued another guidance-type document after briefing closed, the NHBPA Appellants submitted it as a letter to

the Court pursuant to Federal Rule of Appellate Procedure 28(j), but were advised by the Clerk that this was not the appropriate rule for such submissions (docket note 9/15/23).

4. The new guidance document issued June 4, 2024 (Exhibit A), is a current example in addition to the score of existing examples provided by the NHBPA Appellants at page 36-39 of their principal brief and pages 8-9 of their reply brief. The newest document again demonstrates the NHBPA Appellants' point that HISA, not the FTC, has "the last word" and "final say over horseracing rules." Principal Br. 42 (*quoting Nat'l Horsemen's Benevolent & Protective Ass'n v. Black*, 53 F.4th 869, 890 & 872 (5th Cir. 2022)). Though the new policy, announced through a press release, is in response to concerns raised by horsemen, it again represents an exercise of the Authority's self-claimed "enforcement discretion" (Principal Br. 40) to rewrite a rule from what the FTC approved to what the Authority now wants.
5. In this instance, the document is doubly damning because in it the Authority admits it drafted the rule with a dubious scientific basis for the substance's inclusion in the banned substances list, a

problem that perhaps would have been avoided if the FTC had exercised more robust initial review than mere "consistency," *i.e.*, rubber-stamping. Principal Br. 29-30.

6. Therefore, the NHBPA Appellants respectfully move this Court to exercise its discretion to supplement the record with this additional document, *see United States v. Anderson*, 712 Fed. Appx. 383, 387 (5th Cir. 2017), which is a true and correct copy downloaded from the Authority's website. This is one more of twenty previous examples; remand now to ensure its inclusion and consideration by the district court "would be contrary to both the interests of justice and the efficient use of judicial resources." *Gibson v. Blackburn*, 744 F.2d 403, 405 n.3 (5th Cir. 1984).
7. In the alternative, the Court can take judicial notice of this document. *See Halo Wireless, Inc. v. Alenco Communs. Inc. (In re Halo Wireless, Inc.)*, 684 F.3d 581, 597 (5th Cir. 2012).
8. Either way, consideration is appropriate: "The Supreme Court has in numerous instances noticed facts long after both parties, including the government, rested in the trial court." *United States v. Berrojo*, 628 F.2d 368, 370 (5th Cir. 1980).

9. By either supplementation or judicial notice, inclusion of this document gives the Court a clear, continuing, and current picture of the reality on the ground: that the Authority continues to use the statutory scheme's guidance provision to have "the last word" and "final say" on the rules. Inclusion of this document in the record shows that HISA remains the real lawmaking authority until this Court decides otherwise.

10. The NHBPA Appellants have sought the views of the other parties, which are as follows: the State of Texas and Gulf Coast Racing do not oppose the motion. The FTC opposes the motion. The Horseracing Integrity and Safety Authority opposes the motion and reserves the right to file a response.

Respectfully submitted,

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June 6, 2024

CERTIFICATE OF COMPLIANCE

This document complies with the type-volume limit of Fed. R. App. P. 27(d)(2)(A) because, excluding the parts of the document exempted by Fed. R. App. P. 32(f), this document contains 675 words.

This document complies with the typeface requirements of Fed. R. App. P. 32(a)(5) and the type-style requirements of Fed. R. App. P. 32(a)(6) because this document has been prepared in a proportionally spaced typeface using Century Schoolbook, Microsoft Word, 14 point font.

CERTIFICATE OF SERVICE

This motion was served on all parties' counsel via ECF.

CERTIFICATE OF CONSULTATION

Counsel for movants consulted all parties' counsel via email prior to filing this motion, and the positions of the other parties are stated above in paragraph 10.

Certificates signed: /s/ Daniel R. Suhr

Dated: June 6, 2024

HORSERACING INTEGRITY AND SAFETY AUTHORITY

Doping and Medication Control ("ADMC")
Standing Committee, the Board of Directors of the Horseracing Integrity and Safety Authority ("HISA")

"In line with HISA's Anti

available regarding the substance's use in horses.
pic is necessary to determine if any refinement of HISA's rules is appropriate," said Charles Scheeler,

Consequently, HISA and HIWU have asked the Racing Medication & Testing Consortium's (RMTC) Scientific

RMTC's Board of Director's the SAC's
with HISA's ADMC

HISA is grateful for the feedback from all industry constituents, especially the Horsemen's Advisory Group, as we continue to refine our regulatory program to make horse racing safer and fairer for all.

Exhibit

6

HORSERACING INTEGRITY AND SAFETY AUTHORITY

HISA Announcement Regarding Metformin

JUNE 4, 2024 - Following the recommendation of the Anti-Doping and Medication Control ("ADMC") Standing Committee, the Board of Directors of the Horseracing Integrity and Safety Authority ("HISA") announces the following policy change effective immediately regarding the ADMC Program with respect to the Banned Substance metformin. **Commencing immediately, the HISA Board has requested that HIWU defer active Provisional Suspensions for metformin positives**.

"In line with HISA's Anti-Doping and Medication Control requirements, metformin is included in the Prohibited Substances List because there is no recognized therapeutic use for the substance in horses involved in Thoroughbred racing. In addition, HIWU has intelligence that some horsemen may be using metformin to try and gain a performance advantage. However, there is limited scientific information available regarding the substance's use in horses. The HISA Board feels that further expert analysis on the topic is necessary to determine if any refinement of HISA's rules is appropriate," said Charles Scheeler, Chairman of the HISA Board.

Consequently, HISA and HIWU have asked the Racing Medication & Testing Consortium's (RMTC) Scientific Advisory Committee (SAC) to conduct a review of the available science relating to metformin. Following RMTC's Board of Director's approval, any conclusions drawn, and guidance produced regarding the SAC's review will be shared with HISA's ADMC Committee. Once the RMTC Scientific Advisory Committee completes its work, which we expect will take a few months, the ADMC Committee and the HISA Board will determine whether any policy changes regarding metformin should be submitted to the Federal Trade Commission for approval.

In this interim period, metformin remains on the Prohibited Substances List and, as such, regardless of the source and/or the existence of any mitigating circumstances, any ADMC Program violation resulting from an Adverse Analytical Finding for metformin will result in the automatic disqualification of race results of the relevant Covered Horse. The deferral of Provisional Suspensions is only applicable to the Responsible Person; Covered Horses that test positive for metformin are still subject to a Provisional Suspension.

HISA is grateful for the feedback from all industry constituents, especially the Horsemen's Advisory Group, as we continue to refine our regulatory program to make horse racing safer and fairer for all.

Exhibit

7

HORSERACING INTEGRITY AND SAFETY AUTHORITY

HISA Prohibited Substances List

Controlled Medications

Last updated on March 1st, 2023

CONTROLLED - S7

HORSERACING INTEGRITY AND SAFETY AUTHORITY

SS[1]	SUBSTANCE	ACTION	COMMERCIAL / DEVELOPMENTAL NAME(S)	DETECTION TIME	SCREENING LIMIT Unless otherwise designated as a Threshold
B	Acepromazine	Sedative	PromAce, Aceproject	72 hrs: 0.15 mg/kg single oral dose (6 horses). 48 hrs: 0.05 mg/kg single IV dose (20 horses).	10 ng/mL as 2-(1-hydroxyethyl) promazine sulfoxide (HEPS) in urine; 0.02 ng/mL in serum or plasma
C	Acetaminophen (Paracetamol)	NSAID	Tylenol		
C	Acetylcysteine	Mucolytic	Mucomyst, Parvolex		
C	Acetylsalicylic acid (Aspirin)	NSAID	Generic		
B	Albuterol (Salbutamol)	Bronchodilator	FDA-approved equine product Torpex no longer commercially available. Available as FDA-approved for human use via inhalation as Proair HFA, Ventolin HFA, and generic formulations.	72 hours: 5 x 100 µg actuations per dose for 2 days dosed every 4 hours. Note: Albuterol administered by any route other than inhalation is a Banned Substance. Evidence that albuterol was administered by a route other than inhalation, regardless of the albuterol concentration in a urine sample, constitutes a Doping Violation.	0.5 ng/mL in urine

1 – SPECIFIED SUBCLASSIFICATION
Specified substances designated with an X

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HORSERACING INTEGRITY AND SAFETY AUTHORITY

SS[1]	SUBSTANCE	ACTION	COMMERCIAL / DEVELOPMENTAL NAME(S)	DETECTION TIME	SCREENING LIMIT Unless otherwise designated as a Threshold
C	Alclometasone	Corticosteroid	Generic		
A	Alfentanil	Opioid Analgesic	Alfenta. DEA Schedule II.		
B	Allopurinol	Xanthine oxidase inhibitor	Lopurin, Zyloprim, Aloprim		
A	Alprazolam	Sedative / Anxiolytic	Xanax DEA Schedule IV.		
C	Altrenogest Fillies and Mares	Progestogen / Estrus Suppression	Regumate		
C	Amcinonide	Corticosteroid	Generic		
C	Aminocaproic acid	Anti-fibrinolytic	Amicar		
B	Aminophylline	Bronchodilator	Generic		
B	Amiodarone	Antiarrhythmic	Nexterone, Pacerone		
A	Amitraz	Stimulant	Mitaban		
A	Amitriptyline	Antidepressant	Elavil		
B	Amrinone (Inamrinone)	Vasodilator	Amicor, Cardiotone		
B	Antazoline	Antihistamine (ophthalmic)	Discontinued, no FDA-approved product commercially available		
A	Aripiprazole	Antipsychotic	Abilify		
B(x)	Arsenic	Stimulant	Environmental substance		0.3 mcg.mL total (free and conjugated) in urine
B	Articaine	Local anesthetic	Orabloc, Septocaine		
A	Atipamezole	Alpha adrenergic antagonist	Antisedan, Revertidine		

1 – SPECIFIED SUBCLASSIFICATION
Specified substances designated with an X

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HORSERACING INTEGRITY AND SAFETY AUTHORITY

SS[1]	SUBSTANCE	ACTION	COMMERCIAL / DEVELOPMENTAL NAME(S)	DETECTION TIME	SCREENING LIMIT Unless otherwise designated as a Threshold
A	Atracurium	Muscle relaxant	Generic		
B(x)	Atropine	Anticholinergic	Atropen		60 ng/mL total (free and conjugated) in urine
B	Baclofen	Muscle relaxant	Lyvispah, Gablofen, Lioresal, Ozobax, Fleqsuvy		
C	Beclomethasone	Corticosteroid	Qvar, Qnasl, Beclovent		
C	Benoxinate (Oxybucaine, Oxybuprocaine)	Local anesthetic	Altafluor Benox [with fluorescein stain]		
B	Benzocaine	Local anesthetic	Orajel, Anbesol, Lanacane		
A	Benztropine	Anticholinergic	Generic		
C	Betamethasone	Corticosteroid	Betavet, Celestone		0.2 ng/mL in urine
C	Bethanechol	Cholinergic	Duovoid		
B	Bretylium	Antiarrhythmic	Generic		
B	Brinzolamide	Carbonic Anhydrase Inhibitor	Simbrinza, Azopt		
A	Bromfenac	NSAID	Prolensa, Bromsite		
B	Bromodiphenhydramine	Antihistamine	Ambodryl, Ambrodil		
B	Brompheniramine	Antihistamine	Dimetapp		
C	Budesonide	Corticosteroid	Uceris, Entocort, Tarpeyo, Ortikos, Pulmicor Flexhaler, Symbicort (with formoterol), Rhinocort Allergy		
B	Bupivacaine	Local anesthetic	Marcaine, Sensorcaine, Exparel		

1 – SPECIFIED SUBCLASSIFICATION
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HORSERACING INTEGRITY AND SAFETY AUTHORITY

SS[1]	SUBSTANCE	ACTION	COMMERCIAL / DEVELOPMENTAL NAME(S)	DETECTION TIME	SCREENING LIMIT Unless otherwise designated as a Threshold
A	Buprenorphine	Analgesic	Simbadol, Zorbium,Butrans, Sublocade, Belbuca, Buprenex, Zubsolv DEA Schedule III.		
A	Buspirone	Anxiolytic	Generic		
C	Butamben (Butyl aminobenzoate)	Local anesthetic	Cetacaine		
B	Butorphanol	Sedative	Torbugesic, Tobutrol, Stadol, Dolorex DEA Schedule IV.	72 hrs: 0.1 mg/kg single IV dose (6 horses)	1 ng/mL in hydrolyzed urine or 0.01 ng/mL plasma or serum
B(x)	Caffeine	Stimulant	Cafcit, Migergot (with ergotamine), combined with NSAIDs in OTC formulations. Recognized by IFHA as Feed Contaminant		50 ng/mL (free and conjugated) in urine
C	Camphor	Local anesthetic	Vicks VapoRub		
B	Cannabidiol (CBD)	Analgesic / anti-inflammatory	Epidiolex		
B	Capsaicin	Topical analgesic / irritant	Zostrix, Salonpas Hot		
B	Carbachol	Cholinergic	Miostat		
B	Carbamazepine	Anticonvulsant	Tegretol, Carbatrol, Equetro, Teril		
B	Carbinoxamine	Antihistamine	Karbinal ER		
B	Carisoprodol	Muscle relaxant	Soma DEA Schedule IV.		
B	Carprofen	NSAID	Rimadyl		
B	Carticaine (see Articaine)	Local anesthetic	Septocaine, Orbloc		

1 – SPECIFIED SUBCLASSIFICATION
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HORSERACING INTEGRITY AND SAFETY AUTHORITY

SS[1]	SUBSTANCE	ACTION	COMMERCIAL / DEVELOPMENTAL NAME(S)	DETECTION TIME	SCREENING LIMIT Unless otherwise designated as a Threshold
B	Celecoxib	NSAID	Celebrex		
C	Cetirizine	Antihistamine	Quzyttir, Zerviate, Zyrtec	48 hours: 0.4 mg/kg twice daily for 5 doses. (9 horses)	3 ng/mL in serum or plasma
A	Chloroprocaine	Local anesthetic	Nesacaine		
B	Chlorpheniramine	Antihistamine	ChlorTrimeton		
B	Chlorzoxazone	Muscle relaxant	Generic		
B	Chorionic Gonadotropin (CG) Fillies and Mares	Reproductive hormone	Pregnyl-biologic, does not require FDA approval.		
C	Ciclesonide	Corticosteroid	Aservo EquiHaler, Alvesco	48 hours: 5.5 mg/day x 5 days, then 4.1 mg/day x 5 days via inhalation (Aservo Equihaler). (8 horses)	
B	Cilostazol	Vasodilator	Pletal		

1 - SPECIFIED SUBCLASSIFICATION
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HORSERACING INTEGRITY AND SAFETY AUTHORITY

SS[1]	SUBSTANCE	ACTION	COMMERCIAL / DEVELOPMENTAL NAME(S)	DETECTION TIME	SCREENING LIMIT Unless otherwise designated as a Threshold
C	Cimetidine	Anti-ulcer	Tagamet	Restricted administration time: 24 hours. 20 mg/kg orally twice daily for a total of 7 doses (9 horses)	400 ng/mL in serum or plasma
B	Clemastine	Antihistamine	Tavist, Dayhist		
B	Clenbuterol	Beta-2 agonist-bronchodilator	Ventipulmin	Treated horse Vet Listed for minimum 21 days after last treatment. Official Workout and Clearance Testing (blood and urine) required to re-establish eligibility to race. Dosing specification: 0.8 mcg/kg orally twice daily for up to 30 days total in a 6 month period.	
C	Clobetasol	Corticosteroid	Olux, Cormax, Embeline, Impoyz, Clobex, Impeklo		
C	Clocortolone	Corticosteroid	Cloderm		
A	Clonidine	Antihypertensive / Analgesic	Catapres-TTS		
B(x)	Colchicine	Anti-gout	Colcrys, Mitigare		

1 - SPECIFIED SUBCLASSIFICATION
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HORSERACING INTEGRITY AND SAFETY AUTHORITY

SS[1]	SUBSTANCE	ACTION	COMMERCIAL / DEVELOPMENTAL NAME(S)	DETECTION TIME	SCREENING LIMIT Unless otherwise designated as a Threshold
B	Corticotrophin	Corticosteroid stimulation	ACTH-80, Acthar Gel		
C	Cromolyn (Cromoglycate)	Mast Cell Stabilizer	Gastrocrom		
B	Cyclobenzaprine	Muscle relaxant	Flexeril, Amrix		
	Cyclopentolate	Mydriatic	Akpentolate, Cyclogyl, Pentolair, Cyclomydril		
B	Cyproheptadine	Antihistamine	Periactin		
C	Dantrolene	Muscle relaxant	Dantrium	48 hours: 500 mg orally once daily for 3 days (12 horses)	3 ng/mL of 5-hydroxy-dantrolene in urine; 0.1 ng/mL in serum or plasma as 3'-hydroxy-dantrolene
B	Deracoxib	NSAID	Deramaxx		
B	Deslorelin Fillies and Mares	Induce ovulation	Ovuplant, SucroMate, Suprelorin		
C	Desonide	Corticosteroid	Verdeso, Desowen		
C	Desoximethasone (Desoxymethasone, Desoximetasone)	Corticosteroid	Topicort		
B	Detomidine	Sedative / Analgesic	Dormosedan	48 hours: 0.02 mg/kg single IV dose (10 horses)	2 ng/mL 3-carboxy-detomidine in urine; 0.02 ng/mL in serum or plasma.
C	Dexamethasone	Corticosteroid	Azium, Dexasone	72 hours: Single 20 mg oral dose (20 horses)	0.2 ng/mL in urine.

1 – SPECIFIED SUBCLASSIFICATION
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CONTROLLED - S7

HORSERACING INTEGRITY AND SAFETY AUTHORITY

SS[1]	SUBSTANCE	ACTION	COMMERCIAL / DEVELOPMENTAL NAME(S)	DETECTION TIME	SCREENING LIMIT Unless otherwise designated as a Threshold
C	Dexamethasone sodium phosphate	Corticosteroid	Generic	72 hours: 0.06 mg/kg single IV dose (6 horses)	
B	Dextromethorphan	Antitussive	Delsym, Robitussin		
B	Diazepam	Anxiolytic	Valium DEA Schedule IV.		
C	Dichlorphenamide	Carbonic Anhydrase Inhibitor	Keveyis		
C	Diclofenac	NSAID	Surpass, Voltaren		50 ng/mL in urine
C	Diflorasone	Corticosteroid	Florone		
B	Digoxin	Antiarrhythmic	Lanoxin		
B	Dihydroergotamine mesylate	Ergot alkaloid	Migranal, Trudhesa		
C	Dimethylsulfoxide (DMSO)	NSAID	Domoso	48 hours: 70 mL 90% DMSO in 500 mL LRS IV single administration (30 horses)	15 mcg/mL in urine or 1,000 ng/mL in serum or plasma. Note: The detection of more than one NSAID in a horse's post-Race or Post-Official Workout blood sample constitutes a Stacking Violation.
B	Diphenhydramine	Antihistamine	Benadryl		
B	Diphenoxylate	Anti-diarrheal	Lomotil (with atropine) DEA Schedule II.		
B	Dipyridamole	Platelet inhibitor	Persantine		

1 - SPECIFIED SUBCLASSIFICATION
Specified substances designated with an X

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HORSERACING INTEGRITY AND SAFETY AUTHORITY

SS[1]	SUBSTANCE	ACTION	COMMERCIAL / DEVELOPMENTAL NAME(S)	DETECTION TIME	SCREENING LIMIT Unless otherwise designated as a Threshold
C	Dipyrone	NSAID / Anti-pyretic	Zimeta	72 hours: 30 mg/kg single IV dose (10 horses)	1,000 ng/mL of 4-methylaminoantipyrine in urine. Note: The detection of more than one NSAID in a horse's post-Race or Post-Official Workout blood sample constitutes a Stacking Violation.
B	Disopyramide	Antiarrhythmic	Norpace, Rythmodan		
B	Dobutamine	Beta-1 agonist	Generic		
A	Dopamine	Neurotransmitter	Generic		
B	Dorzolamide	Carbonic Anhydrase Inhibitor	Cosopt		
A	Doxapram	Respiratory Stimulant	Dopram, Respiram		
A	Doxepin	Antidepressant	Generic		
B	Doxylamine	Antihistamine	Unisom		
C	Dyclonine	Topical anesthetic	Dyclopro		
A	Ephedrine	Stimulant	Akovaz, Corphedra, Emerphed		
A	Epinephrine	Stimulant	Adrenalin, Epipen, Adrenaclick, Auvi-Q, Symjepi, Primatene Mist		
C	Esomeprazole	Anti-ulcer	Nexium		

1 - SPECIFIED SUBCLASSIFICATION
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CONTROLLED - S7

HORSERACING INTEGRITY AND SAFETY AUTHORITY

SS[1]	SUBSTANCE	ACTION	COMMERCIAL / DEVELOPMENTAL NAME(S)	DETECTION TIME	SCREENING LIMIT Unless otherwise designated as a Threshold
B	Estranediol in male horses (other than geldings)	Estrogen			Threshold: 0.045 mcg/mL total (free and conjugated) 5α-estrane-3β, 17α-diol per millilitre in urine when, at screening, the total 5α-estrane-3β, 17α-diol exceeds the total 5,10 estrene-3β,17α-diol in urine
B	Etanercept	NSAID	Enbrel		
C	Ethylaminobenzoate (Benzocaine)	Local anesthetic	Orajel		
B	Etodolac	NSAID	Generic		
C	Famotidine	Anti-ulcer	Duexis, Pepcid		
B	Fenoldopam	Vasodilator	Corlopam		
B	Fenoprofen	NSAID	Nalfon		
A	Fentanyl (Fentanil)	Opioid Analgesic	Actiq, Fentora, Lazanda, Sublimaze, Subsys DEA Schedule II.		
C	Fexofenadine	Antihistamine	Allegra		
C	Firocoxib	NSAID	Equioxx, Previcox	360 hours: 100 mcg/kg orally once daily for total of 7 doses. (20 horses)	2 ng/mL in serum or plasma
B	Flecainide	Antiarrhythmic	Generic		
C	Flumethasone (Flumetasone)	Corticosteroid	Flucort, Anaprime		

1 - SPECIFIED SUBCLASSIFICATION
Specified substances designated with an X

CONTROLLED - S7

SS[1]	SUBSTANCE	ACTION	COMMERCIAL / DEVELOPMENTAL NAME(S)	DETECTION TIME	SCREENING LIMIT Unless otherwise designated as a Threshold
C	Flunixin	NSAID	Banamine, Flunixamine, Equileve, Meflosyl	48 hours: 1.1 mg/kg single IV dose (16 horses); 500 mg single IV dose (12 horses)	4 ng/mL in serum or plasma. Note: The detection of more than one NSAID in a horse's post-Race or Post-Official Workout blood sample constitutes a Stacking Violation. 3 NSAIDs (Flunixin, Ketoprofen, Phenylbutazone) are associated with a Detection Time of 48 hours. Only one of the three may be administered using a Withdrawal Interval based on the 48 hour Detection Time. To avoid a stacking violation (detection of more than 1 NSAID in a blood sample) the following secondary Detection Times should be applied for the following NSAIDs: Flunixin: 144 hours; Ketoprofen 96 hours; Phenylbutazone: 168 hours.
C	Fluocinolone acetonide	Corticosteroid	Flucort-N		

1 – SPECIFIED SUBCLASSIFICATION
Specified substances designated with an X

CONTROLLED - S7

SS[1]	SUBSTANCE	ACTION	COMMERCIAL / DEVELOPMENTAL NAME(S)	DETECTION TIME	SCREENING LIMIT Unless otherwise designated as a Threshold
C	Fluocinonide	Corticosteroid	Fluonex, Lidex, Lonide, Lyderm		
C	Fluorometholone	Corticosteroid	FML Forte		
B	Fluphenazine	Antipsychotic	Generic		
C	Flurandrenolide (Flurandrenolone, Fludroxycortide)	Corticosteroid	Cordran		
B	Flurbiprofen	NSAID	Ansaid, Ocufen, Strepfen		
C	Fluticasone	Corticosteroid	Flovent, Flonase		
C	*Furosemide	Diuretic	Lasix, Salix	Restricted Administration: 48 hours. 1 mg/kg single IV dose (6 horses)	50 ng/mL in urine or 0.1 ng/mL in serum or plasma
C	**Furosemide	Diuretic	Lasix, Salix	Shall not be administered within 4 hours prior to Post-Time	100 ng/mL in serum or plasma AND urine S.G. < 1.010
B	Gabapentin	Anticonvulsant	Horizant, Gralise, Neurontin		

* (Permitted at all times during Workouts, Official Workouts, and other training exercise)

** Where permitted on race day

1 - SPECIFIED SUBCLASSIFICATION
Specified substances designated with an X

CONTROLLED - S7

HORSERACING INTEGRITY AND SAFETY AUTHORITY

SS[1]	SUBSTANCE	ACTION	COMMERCIAL / DEVELOPMENTAL NAME(S)	DETECTION TIME	SCREENING LIMIT Unless otherwise designated as a Threshold
C	Glycopyrrolate	Anticholinergic	Robinul	48 hours: 1 mg single dose IV. (20 horses)	0.003 ng/mL in serum or plasma
B	Gonadorelin Fillies and Mares	Induce ovulation	Cystorelin, Factrel, Fertelin, OvaCyst, Fertagyl, Gonabreed		
C	Guaifenesin (Glycerol guiacolate)	Expectorant	Mucinex	48 hours: 2 grams total body dose, orally twice daily for 5 doses. (9 horses)	1 ng/mL in serum or plasma
C	Halcinonide	Corticosteroid	Halog		
C	Halobetasol	Corticosteroid	Lexette, Bryhali, Ultravate		
B	Harpagoside (Devil's Claw)	Anti-inflammatory	Glycoside of plant origin. No FDA-approved products commercially available. Constituent of multiple, unregulated OTC herbal remedies.		
B	Homatropine	Anticholinergic	Hycodan [with hydrocodone]		

1 - SPECIFIED SUBCLASSIFICATION
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HORSERACING INTEGRITY AND SAFETY AUTHORITY

SS[1]	SUBSTANCE	ACTION	COMMERCIAL / DEVELOPMENTAL NAME(S)	DETECTION TIME	SCREENING LIMIT Unless otherwise designated as a Threshold
A(x)	Hordenine	Stimulant	Plant alkaloid (e.g. barley). Constituent of numerous OTC dietary supplements marketed for weight loss. Lacks FDA approval		80 mcg/mL total (free and conjugated) in urine
B	Hydralazine	Vasodilator	Hydra-Zide, Bidil		
C	Hydrocortisone	Corticosteroid	Cortef. Note: hydrocortisone is a component of numerous products, particularly those for topical, ophthalmic, and otic applications. The Responsible Person is advised to read all medication labels prior to authorizing administration.		Threshold: 1 mcg/mL in urine
C	Hydroxyzine	Antihistamine	Atarax	96 hours: 190 mg twice daily for a total of 9 doses (2 horses)	
C	Ibuprofen	NSAID	Advil, Motrin		
B	Ibutilide	Antiarrhythmic	Corvert		
A	Imipramine	Antidepressant	Tofranil		
B	Indomethacin	NSAID	Indocin		

1 – SPECIFIED SUBCLASSIFICATION
Specified substances designated with an X

CONTROLLED - S7

SS[1]	SUBSTANCE	ACTION	COMMERCIAL / DEVELOPMENTAL NAME(S)	DETECTION TIME	SCREENING LIMIT Unless otherwise designated as a Threshold
B	Infliximab	Immunosuppressor	Remicade		
B	Ipratropium	Bronchodilator	Atrovent	120 hours: 5.5 mcg/ kg once daily via nebulization for 3 total doses (6 horses)	0.25 ng/mL in urine
B	Ipratropium bromide	Bronchodilator	Atrovent, Combivent (with albuterol)		
C	Isoflupredone	Corticosteroid	Predef 2x	14 day stand down for all intra-articular injections. Serum concentrations associated with an experimental dose of 8 mg IA single joint (6 horses) were all below Limit of Detection by 14 days.	
B	Isosorbide dinitrate	Vasodilator	Isordil		
B	Ketamine / Norketamine	Anesthetic	Ketaset, Vetalar DEA Schedule III.		

1 - SPECIFIED SUBCLASSIFICATION
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CONTROLLED - S7

SS[1]	SUBSTANCE	ACTION	COMMERCIAL / DEVELOPMENTAL NAME(S)	DETECTION TIME	SCREENING LIMIT Unless otherwise designated as a Threshold
C	Ketoprofen	NSAID	Ketofen	48 hours: 2.2 mg/kg single IV dose. (24 horses)	2 ng/mL in serum or plasma. Note: The detection of more than one NSAID in a horse's post-Race or Post-Official Workout blood sample constitutes a Stacking Violation. 3 NSAIDs (Flunixin, Ketoprofen, Phenylbutazone) are associated with a Detection Time of 48 hours. Only one of the three may be administered using a Withdrawal Interval based on the 48 hour Detection Time. To avoid a stacking violation (detection of more than 1 NSAID in a blood sample) the following secondary Detection Times should be applied for the following NSAIDs: Flunixin: 144 hours; Ketoprofen 96 hours; Phenylbutazone: 168 hours.
A	Ketorolac	NSAID	Acular, Acuvail, Sprix, Omidria		
B	Ketotifen	Antihistamine	Alaway, Zaditor		

1 - SPECIFIED SUBCLASSIFICATION
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HORSERACING INTEGRITY AND SAFETY AUTHORITY

SS[1]	SUBSTANCE	ACTION	COMMERCIAL / DEVELOPMENTAL NAME(S)	DETECTION TIME	SCREENING LIMIT Unless otherwise designated as a Threshold
A	Lamotrigine	Anticonvulsant	Lamictal		
C	Lansoprazole	Anti-ulcer	Prevacid		
B	Levamisole	Anthelmintic / Immunostimulant	Ripercol, Tramisol, Levasole, Prohibit, LevaMed		
B	Lidocaine	Local anesthetic	Xylocaine [with epinephrine], Lignospan, Ztlido, Akten	48 hours: 200 mg of lidocaine as its hydrochloride salt administered subcutaneously (6 horses)	10 ng/mL as 3-hydroxylidocaine in urine; 0.02 ng/mL as 3-hydroxylidocaine in serum or plasma
B	Loperamide	Anti-diarrheal	Imodium		
C	Loratidine	Antihistamine	Claritin		
B	Luteinizing Hormone (LH) Fillies and Mares	Reproductive hormone modulator			
B	Magnesium sulfate	Sedative / Laxative	Generic		

1 - SPECIFIED SUBCLASSIFICATION
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HORSERACING INTEGRITY AND SAFETY AUTHORITY

SS[1]	SUBSTANCE	ACTION	COMMERCIAL / DEVELOPMENTAL NAME(S)	DETECTION TIME	SCREENING LIMIT Unless otherwise designated as a Threshold
B	Meclofenamic acid	NSAID	Discontinued, no FDA-approved product commercially available. Routinely compounded.		
B	Medetomidine	Sedative / Analgesic	Domitor, Placadine		5 ng/mL as 3-hydroxymedetomidine in urine
B	Medroxyprogesterone	Reproductive hormone	Depo-Provera		
B	Meloxicam	NSAID	Metacam		
B	Mepivacaine	Local anesthetic	Carbocaine, Polocaine, Scandonest	72 hours: 40 mg (2 ml) single dose SQ distal limb (6 horses)	10 ng/mL as 3-hydroxymepivacaine in urine; 0.05 ng/mL in serum or plasma
C	Mesalamine (Mesalazine)	Anti-inflammatory	Delzicol, Pentasa, Sfrowasa, Canasa, Lialda		
C	Methocarbamol	Muscle relaxant	Robaxin	48 hours: 15 mg/kg single IV dose. (20 horses)	1 ng/mL in serum or plasma
B	Methotrexate	Immunomodulator	Otrexup, Rasuvo, Reditrex, Trexall		
C	Methylergonovine	Ergot alkaloid	Methergine		
C	Methylprednisolone	Corticosteroid	Depo-Medrol		
C	Methylsalicylate	NSAID	Salonpas (with menthol)		

1 - SPECIFIED SUBCLASSIFICATION
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HORSERACING INTEGRITY AND SAFETY AUTHORITY

SS[1]	SUBSTANCE	ACTION	COMMERCIAL / DEVELOPMENTAL NAME(S)	DETECTION TIME	SCREENING LIMIT Unless otherwise designated as a Threshold
C	Methylsulfonylmethane (MSM)	Anti-inflammatory	Feed contaminant per IFHA		1200 mcg/mL in urine
C	Metoclopramide	Anti-emetic / Prokinetic	Gimoti, Reglan		
B	Mexiletine	Antiarrhythmic	Generic		
B	Midazolam	Anticonvulsant	Seizalam DEA Schedule IV		
B	Milrinone	Vasodilator	Generic		
C	Misoprostol	Prostaglandin analog	Cytotec	48 hours: 5 mcg/kg orally twice daily for 14 days. (6 horses)	
C	Mometasone	Corticosteroid	Asmanex, Sinuva, Elocon, Ryaltris, Nasonex		
C	Montelukast	Leukotriene receptor antagonist	Singulair		
A(x)	Morphine	Opioid Analgesic	Duramorph, Infumorph, Mitigo, MS Contin. DEA Schedule II; Dietary substance per IFHA		30 ng/mL total (free and conjugated) in urine
B	Nabumetone	NSAID	Generic		
A	Nalmefene	Opioid antagonist	Revex		

1 - SPECIFIED SUBCLASSIFICATION
Specified substances designated with an X

CONTROLLED - S7

HORSERACING INTEGRITY AND SAFETY AUTHORITY

SS[1]	SUBSTANCE	ACTION	COMMERCIAL / DEVELOPMENTAL NAME(S)	DETECTION TIME	SCREENING LIMIT Unless otherwise designated as a Threshold
B	Naloxone	Opioid antagonist	Narcan, Zimhi, Suboxone (with buprenorphine hydrochloride), Zubsolv (with buprenorphine hydrochloride)		
A	Naltrexone	Opioid antagonist	Trexonil		
B	Naphazoline	Sympathomimetic	Naphcon-A (with pheniramine maleate), Opcon-A (with pheniramine maleate), Visine (with pheniramine maleate)		
C	Naproxen	NSAID	Aleve, Naprosyn, Anaprox		
C	N-Butylscopolammonium	Anticholinergic	Buscopan	48 hours: 0.3 mg/kg single IV dose (6 horses)	25 ng/mL in urine
C	Nedocromil	Mast Cell Stabilizer	Alocril		
B	Neostigmine	Anticholinesterase	Bloxiverz		
C	Nizatidine	Anti-ulcer	Axid		
A	Norepinephrine	Stimulant	Levophed		
C	Olsalazine	Anti-inflammatory	Diipentum		

1 - SPECIFIED SUBCLASSIFICATION
Specified substances designated with an X

CONTROLLED - S7

SS[1]	SUBSTANCE	ACTION	COMMERCIAL / DEVELOPMENTAL NAME(S)	DETECTION TIME	SCREENING LIMIT Unless otherwise designated as a Threshold
C	Omeprazole	Anti-ulcer	Gastrogard	Restricted administration time: 24 hours. 2.2 g orally once daily for 4 doses (9 horses)	10 ng/mL in serum or plasma as omeprazole sulfide
C	Oxybuprocaine (Benoxinate, oxybucaine)	Local anesthetic	Atafluor Benox		
B	Oxymetazoline	Nasal decongestant	Rhofade, Upneq, Visine		
B	Oxytocin	Uterine contraction	Pitocin		
A	Pancuronium	Muscle relaxant	Generic		
C	Pantoprazole	Anti-ulcer	Protonix		
B	Pentazocine	Opioid analgesic	Generic (with naloxone hydrochloride) DEA Schedule IV		
A	Pentobarbital	Barbiturate	Nembutal DEA Schedule II.		
C	Pentoxyfylline	Vasodilator	Discontinued, no FDA-approved product commercially available.		
B	Pergolide	Dopamine agonist	Prascend		

1 – SPECIFIED SUBCLASSIFICATION
Specified substances designated with an X

CONTROLLED - S7

SS[1]	SUBSTANCE	ACTION	COMMERCIAL / DEVELOPMENTAL NAME(S)	DETECTION TIME	SCREENING LIMIT Unless otherwise designated as a Threshold
A	Phenazopyridine	Local anesthetic	Discontinued, no FDA-approved product commercially available		
A	Phenobarbital	Barbiturate	Predates FDA, grandfathered DEA schedule IV.		
B	Phentolamine	Vasodilator	Oraverse		

1 - SPECIFIED SUBCLASSIFICATION
Specified substances designated with an X

CONTROLLED - S7

SS[1]	SUBSTANCE	ACTION	COMMERCIAL / DEVELOPMENTAL NAME(S)	DETECTION TIME	SCREENING LIMIT Unless otherwise designated as a Threshold
C	Phenylbutazone	NSAID	Butazolidin, Butatron, EquiBute, Phen Buta Vet, Bizolin, Butequine, Superiorbute, Pributazone	48 hours: 4.4 mg/kg single IV dose. (17 horses)	0.2 mcg/mL in serum or plasma. Note: The detection of more than one NSAID in a horse's post-Race or post-Official Workout blood and sample constitutes a Stacking Violation. 3 NSAIDs (Flunixin, Ketoprofen, Phenylbutazone) are associated with a Detection Time of 48 hours. Only one of the three may be administered using a Withdrawal Interval based on the 48 hour Detection Time. To avoid a stacking violation (detection of more than 1 NSAID in a blood sample) the following secondary Detection Times should be applied for the following NSAIDs: Flunixin: 144 hours; Ketoprofen 96 hours; Phenylbutazone: 168 hours.
B	Phenylephrine	Stimulant	Biorphen		
B	Phenytoin	Anticonvulsant	Dilantin, Phenytek		

1 - SPECIFIED SUBCLASSIFICATION
Specified substances designated with an X

CONTROLLED - S7

HORSERACING INTEGRITY AND SAFETY AUTHORITY

SS[1]	SUBSTANCE	ACTION	COMMERCIAL / DEVELOPMENTAL NAME(S)	DETECTION TIME	SCREENING LIMIT Unless otherwise designated as a Threshold
A	Physostigmine	Acetylcholinesterase inhibitor	Antilirium		
B	Piroxicam	NSAID	Feldene		
A	Potassium Bromide	Anticonvulsant / Anxiolytic	KBroVet-CA1		
C	Pramoxine	Topical anesthetic	Epifoam (with hydrocortisone acetate), Pramosone (with hydrocortisone acetate)		
C	Prednisolone	Corticosteroid	Endogenous subtance (urine only) per IFHA		Threshold: 0.01 mcg/ mL free prednisolone in urine
C	Prednisone	Corticosteroid	Rayos		
A	Pregabalin	Anticonvulsant / Analgesic	Lyrica DEA Schedule V.		
B	Prilocaine	Local anesthetic	Emla (with lidocaine), Oraqix (with lidocaine), Citanest (with epinephrine)		
B	Primidone	Anticonvulsant	Mysoline		
B	Procainamide	Antiarrhythmic	Generic		
B	Procaine	Local anesthetic	(with Penicillin G)	17 mg (~17,000 IU) per kg IM	25 ng/mL in serum or plasma
B	Promazine	Sedative / Antipsychotic	Promazine Granules		
B	Promethazine	Antihistamine	Promethegan. Note: Component of multiple OTC cough/cold formulations.		

1 – SPECIFIED SUBCLASSIFICATION
Specified substances designated with an X

CONTROLLED - S7

HORSERACING INTEGRITY AND SAFETY AUTHORITY

SS[1]	SUBSTANCE	ACTION	COMMERCIAL / DEVELOPMENTAL NAME(S)	DETECTION TIME	SCREENING LIMIT Unless otherwise designated as a Threshold
B	Propafenone	Antiarrhythmic	Rythmol		
C	Proparacaine (Proxymetacaine)	Local anesthetic	Alcane		
A	Propofol	Anesthetic	PropoFlo, Rapanofal		
A	Propranolol	Antiarrhythmic / Antihypertensive	Inderal, Hemangeol		
B	Pseudoephedrine	Stimulant	Sudafed		
B	Pyridostigmine	Cholinesterase Inhibitor	Mestinon, Regonol		
B	Pyrilamine	Antihistamine	Histavet-P		
B	Quinidine	Antiarrhythmic	Generic		
C	Rabeprazole	Anti-ulcer	Aciphex		
C	Ranitidine	Anti-ulcer	Generic	Restricted administration time: 24 hours. 8 mg/kg orally twice daily for 7 doses. (9 horses)	40 ng/mL in serum or plasma
A	Reserpine	Antihypertensive / Depressant	Serpasil		
A	Rocuronium	Muscle relaxant	Generic		

1 – SPECIFIED SUBCLASSIFICATION
Specified substances designated with an X

CONTROLLED - S7

HORSERACING INTEGRITY AND SAFETY AUTHORITY

SS[1]	SUBSTANCE	ACTION	COMMERCIAL / DEVELOPMENTAL NAME(S)	DETECTION TIME	SCREENING LIMIT Unless otherwise designated as a Threshold
B	Romifidine	Sedative	Sedivet	60 hours: 80 mcg/kg single IV dose (6 horses)	1 ng/mL in urine
A	Ropivacaine	Local anesthetic	Naropin		
C	Salicylic acid	NSAID	Paser		Threshold: 750 mcg/mL in urine or 6.5 mcg/mL in serum or plasma
B	*Salmeterol	Beta-2 agonist-bronchodilator	Serevent, Advair (with fluticasone), Airduo (with fluticasone), Wixela (with fluticasone)		
C(x)	Scopolamine (Hyoscine)	Anticholinergic	Transdermal Scop, Dietary substance per IFHA		60 ng/mL total (free and conjugated) in urine
A	Secobarbital (Quinalbarbitone)	Sedative / Hypnotic	Discontinued, no FDA-approved product commercially available. DEA Schedule II.		
B	Sotalol	Antiarrhythmic	Betapace, Sorine, Sotylize		
A	Succinylcholine	Muscle relaxant	Anectine, Quelicin		
C	Sulfasalazine	Disease-modifying anti-rheumatic	Azulfadine		
C	Suprofen	NSAID	Discontinued, no FDA-approved product commercially available		

* When administered via inhalation

1 - SPECIFIED SUBCLASSIFICATION
Specified substances designated with an X

CONTROLLED - S7

HORSERACING INTEGRITY AND SAFETY AUTHORITY

SS[1]	SUBSTANCE	ACTION	COMMERCIAL / DEVELOPMENTAL NAME(S)	DETECTION TIME	SCREENING LIMIT Unless otherwise designated as a Threshold
B	*Temazepam (Temazepam is a major metabolite of diazepam. If there is credible evidence that the presence of temazepam in a horse's sample is the consequence of exposure to diazepam, the classification of temazepam may be revised to S7(B).)	Anxiolytic	Restoril DEA Schedule IV.		
B	Tepoxalin	NSAID	Zubrin		
B	Tetracaine	Local anesthetic	Pliaglis [with Lidocaine], Synera [with Lidocaine], Kovanze [with Oxymetazoline]		
B	Tetrahydrozoline	Topical Decongestant	Visine		
B(x)	Theobromine	Bronchodilator / Vasodilator	Lacks FDA approval, Dietary substance per IFHA		2 mcg/mL (free and conjugated) in urine OR 0.3 mcg/mL in serum or plasma
B(x)	Theophylline	Bronchodilator	Generic, Dietary substance per IFHA		250 ng/mL (free and conjugated) in urine
A	Thiamylal	Sedative / Hypnotic	Surital, Biotal, Anestatal DEA Schedule III.		
A	Thiopental (Pentothal)	Anesthetic	Combuthal Powder, Xylamed DEA Schedule III		

* Temazepam is incorrectly designated in the Technical Document as a Controlled Substance (S7). The correct designation of Temazepam is a Banned Substance (SO). The Authority will correct this error in future rulemaking. Until the error is corrected, the presence of Temazepam in a horse's system will be enforced as the presence of a Controlled Substance (S7).

1 - SPECIFIED SUBCLASSIFICATION
Specified substances designated with an X

CONTROLLED - S7

HORSERACING INTEGRITY AND SAFETY AUTHORITY

SS[1]	SUBSTANCE	ACTION	COMMERCIAL / DEVELOPMENTAL NAME(S)	DETECTION TIME	SCREENING LIMIT Unless otherwise designated as a Threshold
A	Tiletamine	Anesthetic	Telazol [with zolazepam] DEA Schedule III.		
B	Tiotropium	Bronchodilator	Spiriva		
A	Tolazoline	Vasodilator	Discontinued, no FDA-approved product commercially available		
B	Tramadol	Opioid Analgesic	Ultram DEA Schedule IV.		
C	Tranexamic acid	Anti-fibrinolytic	Cykokapron		
C	Triamcinolone	Corticosteroid	Vetalog, Kenalog		0.5 ng/mL in urine
C	Trichlormethiazide	Diuretic	Discontinued, no FDA-approved product commercially available		
B	Tripelennamine	Antihistamine	Re-Covr		
B	Tropicamide	Ophthalmic Anticholinergic	Mydriacyl		
A	Valerenic acid	Sedative	Plant derived		
A	Vecuronium	Muscle relaxant	Generic		
C	Warfarin	Anticoagulant	Coumadin, Jantoven		

1 – SPECIFIED SUBCLASSIFICATION
Specified substances designated with an X

CONTROLLED - S7

HORSERACING INTEGRITY AND SAFETY AUTHORITY

SS[1]	SUBSTANCE	ACTION	COMMERCIAL / DEVELOPMENTAL NAME(S)	DETECTION TIME	SCREENING LIMIT Unless otherwise designated as a Threshold
B	Xylazine	Sedative / Analgesic	Rompun, Anased	72 hours: 200 mg single IV dose.	10 ng/mL in urine (as 4-OH xylazine); 0.05 ng/mL in serum or plasma
B	Yohimbine	Stimulant	Antagonil		
A	Zolazepam	Sedative / Anxiolytic	Telazol [with Tiletamine]		

1 - SPECIFIED SUBCLASSIFICATION
Specified substances designated with an X

Exhibit

8

Pferdeheilkunde – Equine Medicine 40 (2024) 1 (January February) 10–17 DOI 10.21836/PEM20240102

20-Hydroxyecdysone identification in performance horses – case reports and review

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Summary: This case report presents the first reported identifications of 20-hydroxyecdysone in post-event blood samples from an Endurance horse and a Harness horse racing in New South Wales, Australia. 20-Hydroxyecdysone is a plant secondary metabolite structurally related to testosterone and used by plants to discourage insect predation. 20-Hydroxyecdysone is found in many plants including spinach and is not infrequently identified at low concentrations in mammalian systems including in humans. Given its steroid-related structure, 20-hydroxyecdysone has been reported to enhance athletic performance in humans, although at this time 20-hydroxyecdysone is simply being monitored by the *World Anti-Doping Agency* (WADA) [31]. The Harness horse identification led the Harness Racing New South Wales (HRNSW) authorities to evaluate the home pastures of the horse in question, where they recovered at least three plants containing significant concentrations of 20-hydroxyecdysone. The HRNSW stewards were satisfied that the 20-hydroxyecdysone identification was due to "environmental contamination emanated from plants" in the trainer's establishment and did not impose a penalty on the trainer. These findings show that the pasture plant steroid 20-hydroxyecdysone is found in pasture plants from which it is bioavailable to equines and can present as low part per billion concentrations in equine blood samples, as has also been noted in humans. Based on these Australian identifications of 20-hydroxyecdysone in pasture plants and also in equine blood samples and the HRNSW decision not to penalize the trainer involved and the likelihood of no pharmacological response to pasture plant exposure to this substance we now present 2 parts per billion in equine blood/plasma as an appropriate regulatory cut-off or Screening Limit of Detection (SLOD) for 20-hydroxecdysone in equine blood/plasma.

Keywords: 20-Hydroxyecdysone, environmental contamination, performance horse, steroid-hormone, plant-based diet, insect moulting hormone

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Introduction

20-hydroxyecdysone (20-OH; crustecdysone; beta-ecdysone; ecdysterone, Fig 1) is a naturally occurring insect and plant steroid hormone belonging to the ecdysteroid class whose major biological function is the regulation of molting in insects [22]. These species have specific binding receptors for 20-hydroxyecydysone that are an integral part of their molting mechanism [11]. The function of 20-hydroxyecdysone in plants is its inhibitory effect on insect molting, by which 20-hydroxyecdysone functions as a deterrent to insect predation [27, 9, 10]. 20-Hydroxyecdysone and numerous related plant-derived analogs have been identified and studied for their pharmacological effects on mammalian systems [3, 7]. 20-Hydroxyecdysone and its metabolites differ significantly from mammalian steroid hormones in that they have a cis-fused A/B ring junction essentially giving these insect/plant steroid molecules a relatively "hooked" structure in contrast to the "flatter" mammalian steroids such as testosterone, a significant structural difference between these two groups of steroids (Fig. 1) [7, 8, 9].

20-hydroxyecdysone is synthesized from cholesterol in insect ovaries and is pulsed out at specific developmental timepoints such as during puparium formation and head eversion in preparation for metamorphosis [17, 30]. Plants also synthesize analogs of these compounds as phytoecdysteroids and the review by *Dinan* [9] lists about 200 plant steroids structurally related to 20-hydroxyecdysone which have been reported to occur in over 100 terrestrial plant families including ferns, gymnosperms, and angiosperms with more than 390 different phytoecdysteroids identified [32]. *Báthori* et al. [3] have reviewed many of these structures noting their polyhydroxylated nature. From reports on anabolic effects, they conclude that in mammals ecdysteroids are unlikely to act via classical steroid cytoplasmic receptors and gene transcription regulation but may instead act to accelerate translocation processes or signal transduction pathways. The administration of 20-hy-

droxyecdysone improves growth characteristics in pigs, and exercise performance in both mice [25] and humans [22]. Presumably, this substance could similarly confer performance advantages for endurance and racing performance in horses [22]. Based on these characteristics, 20-hydroxyecdysone is currently on the *World Anti-Doping Agency* (WADA) monitoring list although at this time it has not been placed on the prohibited list [31]. Although the *Fédération Equestre Internationale* (FEI) has it listed under the name 20-hydroxyecdysone as a prohibited substance [14], 20-hydroxyecdysone and ecdysone are not listed in either the *Horseracing Integrity and Safety Authority* (HISA) Prohibited Substances List [18, 19]or the *Association of Racing Commissioners International* (ARCI) Uniform Classification Guidelines for Foreign Substances as of July 18th, 2023 [1].

Because of its widespread presence in plant material, horses are at risk of inadvertent exposure through feedstuffs containing 20-hydroxyecdysone. We report on two such cases, one in an *Australian Endurance Riders Association* Incorporated (AERA) event and the other in Harness Racing New South Wales (HRNSW) [28].

The reported anabolic actions of 20-hydroxyecdysone

Ecdysteroids are naturally occurring in invertebrates and across a wide group of plants and are responsible for growth modulation, development and molting in insects, and function to deter predation in plant species. Mammals do not produce endogenous ecdysteroids, but exogenous administration promotes growth [6]. As a consequence of these effects of ecdysteroids, they are under consideration for addition to the WADA Banned List.

Phytoectosteroids are estimated to be found in high concentrations in over 100 terrestrial plant species, including spinach [29]. The widespread distribution of this class of compounds makes it impractical to establish a complete ban, i.e., by Limit of Detection (LOD) sensitivity testing and regulation.

An unexpected 21st century consequence of these long in place plant/insect biological interactions is that competition horses that inadvertently consume plants containing 20-hydroxyecdysone have the potential to test analytically "positive" for small concentrations of 20-hydroxyecdysone, as apparently also happens in humans [10].

The world anti-doping agency (WADA) status of 20-hydroxyecdysone

An important regulatory consideration is that as a naturally occurring substance produced by plants, including plants such as spinach widely consumed by humans, a diet or cultural or regionally related background level of ecdysterone exposure might reasonably be expected to occur in both humans and horses. With regard to this likelihood, we now specifically draw attention to the serum concentrations of ecdysterone reported detected in the control and pre-administration samples in a 2019 study [22]. In this study *Isenmann* et al. reported the detection of serum concentrations of ecdysone in their control and pre-administration samples in the concentration range of the Limit of Quantitation of their analytical method [22]. As noted in their publication, these detections of ecdysterone in their placebo group serum samples "most likely resulted from regular diet", namely that small concentrations of ecdysterone are not unexpected in the normal human diet which dietary intake gives rise to low concentration identifications of ecdysterone in human serum samples [22]. This background level of ecdysterone in humans originating from plants with variable levels is presumably the reason that 20-hydroxyecdysone has not been placed on the WADA prohibited list [31].

Case 1

At 12.00 am on September 10th, 2022, a 9-year-old Arabian Endurance mare ridden by a minor started in a 160km Endurance ride conducted by AERA held at the Tonimbuk Equestrian Centre in Victoria, Australia. This ride started at point A and

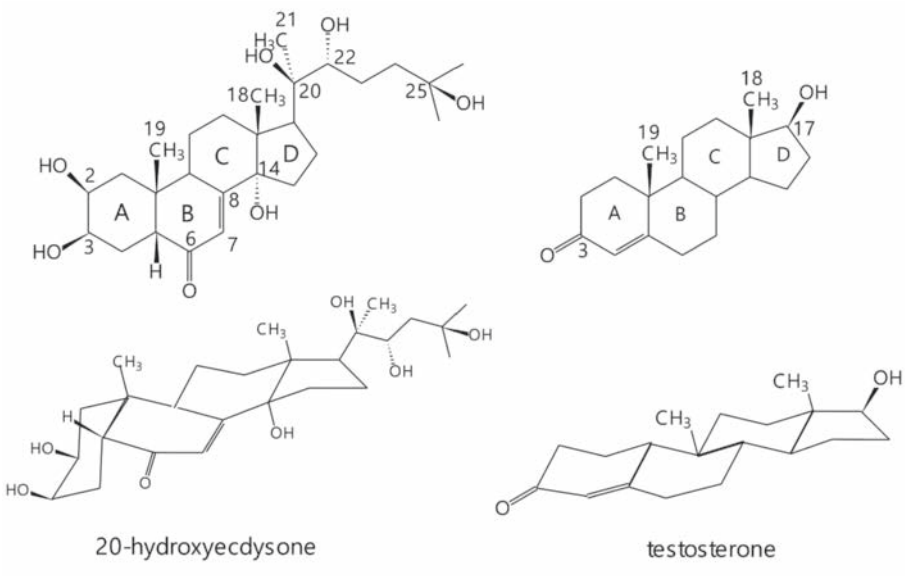

Fig. 1 Left, 20-hydroxyecdysone, (2β, 3β,5β,22R)-2,3,14,20,22,25-Hexa-hydroxychol-est-7-en-6-one, $C_{27}H_{44}O_7$, Molar mass 480.642 g·mol^{-1}, also known as ecdysterone, depicted in structural and configurational diagrams. Right, a typical anabolic hormonal steroid, testosterone, for comparison. Besides significant differences in oxidized substituents, note the contrast in the hooked effect of the 20-hydroxyecdysone cis A/B ring fusion as compared with the planar A/B ring fusion in testosterone. | *Links, 20-Hydroxyecdyson, (2ß,3ß,5ß,22R)-2,3,14,20,22,25-Hexahydroxychol-est-7-en-6-on, $C_{27}H_{44}O_7$, Molmasse 480,642 g·mol^{-1}, auch bekannt als Ecdysteron, dargestellt in Struktur- und Konfigurationsdiagrammen. Rechts, zum Vergleich ein typisches anaboles hormonelles Steroid, Testosteron. Beachten Sie neben signifikanten Unterschieden bei oxidierten Substituenten den Kontrast im Hakeneffekt der cis-A/B-Ringfusion von 20-Hydroxyecdyson im Vergleich zur planaren A/B-Ringfusion in Testosteron.*

traversed a 160 km course to the end point of the event at point B at the event base. At the end of the ride the mare was selected for drug testing and two sets of blood samples, uniquely numbered were taken and submitted for analytical testing. The A sample portion was reported as testing "positive" for the substance 20-hydroxyecdysone in the primary testing laboratory, the Australian Racing Forensic Laboratory (ARFL) in Sydney, as set forth in the January 20th, 2023, ARFL Certificate of Analysis. The remaining portion of this sample, Sample B, was received on February 10th, 2023, by a separate laboratory, Racing Analytical Services Limited (RASL) in Flemington, Victoria, Australia for confirmation of the presence of 20-hydroxyecdysone. The B sample laboratory was selected by Equestrian Australia. The B or split sample analysis was not witnessed [2, 26]. Next, on February 21st, 2023, the RASL Laboratory Director submitted to the Chief Executive Officer of Equestrian Australia (EA) a Certificate of Analysis on RASL letterhead reporting that "The blood sample was shown to contain 20-hydroxyecdysone" [26].

The responsible persons were surprised and concerned by this claimed 20-hydroxyecdysone Adverse Analytical Finding, never having heard of this substance previously in any context whatsoever [23].

Case 2

On Thursday, October 13th, 2022, a 5-year-old Harness horse competed in Race 2 in the ARNALL TROPHIES PACE at Penrith NSW, a western suburb of Sydney. The horse was blood sampled post-race and the samples analyzed at the ARFL in Sydney. The ARFL analysis reported on January 18th, 2023, that 20-hydroxyedecysone was detected in the sample and which identification was confirmed in the B sample analysis reported on February 1st, 2023, by the Racing Analytical Services Laboratory in Flemington, Victoria.

At the March 20th, 2023, Harness Racing New South Wales (HRNSW) inquiry on these matters analytical reports were presented in relation to plant samples obtained by the HRNSW stewards from a paddock in which the horse was located within the trainer's registered training establishment. These analytical reports confirmed that the substance in question 20-hydroxyecdysone was detected in a number of these plant samples taken from his training establishment [28].

These plant samples (Figure 2) identified as containing 20-hydroxyecdysone had been collected by the HRNSW stewards from the training establishment exactly two months after collection of the postrace sample and somewhat unusually approximately one month before 20-hydroxyecdysone was actually certified as being present in the postrace sample. The HRNSW stewards arrived at the training establishment unannounced and informed the trainer that the blood sample ("swab") from the October 13th race had produced an irregularity, and that the substance involved was "a natural steroid and not administered". They also informed the trainer that "they knew it was not a drug administered to the horse". They then proceeded to "sample a number of feed and hay samples as well as the 13 weeds they collected" [26].

Given the above facts, namely that the stewards knew the substance in question was "a natural steroid" and it "was not a drug administered to the horse" and the fact that 13 plant samples were taken from the training establishment on December 13th, and only three other non-plant samples analyzed, it is clear that the HRNSW stewards collecting the samples from the training establishment on December 13th, 2022, had plant sources of 20-hydroxyecdysone high on their list of potential/suspected sources of the 20-hydroxyecdysone identification.

Fig. 2 Photographs of three plant samples taken from the registered training establishment and in which plant samples 20-hydroxyecdysone was reportedly detected. Sample 22/3275-9, left, was colloquially identified as "Salix", Sample 22/3275-6, center, was colloquially identified as "Pigweed" and Sample 22/3275-10, right, has been tentatively identified as a Chenopodium species, colloquially "Fat Hen". These colloquial names are those associated with these plants in New South Wales, Australia and are also the names presented to the stewards in the Harness Racing New South Wales (HRNSW) inquiry. | *Fotos von drei Pflanzenproben, die aus der registrierten Ausbildungseinrichtung entnommen wurden und in denen Berichten zufolge 20-Hydroxyecdyson nachgewiesen wurde. Probe 22/3275-9 (links) wurde umgangssprachlich als eine Art „Salix" identifiziert, Probe 22/3275-6 (Mitte) wurde umgangssprachlich als „Schweinegras" identifiziert und Probe 22/3275-10 (rechts) wurde vorläufig als Chenopodium identifiziert, umgangssprachlich „Fette Henne". Diese umgangssprachlichen Namen sind diejenigen, die mit diesen Pflanzen in New South Wales, Australien, in Verbindung gebracht werden und sind auch die Namen, die den Stewards im Rahmen der Untersuchung von Harness Racing New South Wales (HRNSW) vorgelegt wurden.*

Photographs of the 3 plants positively identified as containing 20-hydroxyecdysone collected on December 13th from the training establishment are presented in Figure #2 above. The amounts of 20-hydroxyecdysone reported detected in these three plant samples were not insignificant: Plant 22/3275-9 reported as 12,854 ng/ml; Plant 22/3275-10 as containing 5,909 ng/ml; and Plant 22/3275-06 as containing 3,654 ng/ml. Reviewing these analytical results, it is clear that these plant sources are consistent with the 1.8 ng/ml plasma concentration of 20-hydroxyecdysone identified in the postrace blood sample taken from the horse following his October 13th race at Penrith.

Based on the evidence presented at the inquiry the stewards were satisfied "to the requisite standard" that "the environmental contamination emanated from plants within the registered training establishment". These being the facts presented in this matter, the stewards determined that a conviction would be recorded but the stewards chose not to impose any penalty on the trainer. The trainer was, however, cautioned that he must take all reasonable measures to ensure that his horses are not exposed to such plants and prohibited substances [28].

Specific botanical identifications of the plants associated with the harness racing new South Wales matter

More recently the plants collected at the Harness horse trainer's registered training establishment that were linked to these 20-OH identifications have to our knowledge been further identified by their full and correct scientific botanical names with an outline of their Australian distribution, as we now detail [26].

The plant numbered 02/3275-9 in Figure 2 has been identified botanically as Sida rhombifolia L., also known as Arrowleaf Sida. This plant is a drought resistant weed in Australia, with a significant distribution in New South Wales. Analytically this plant 22/3275-09 was reported as yielding 12,854 ng/ml of 20-OH, the highest 20-OH concentration recovered from these NSW plants.

The plant numbered 22/3275-10 has been identified botanically as Chenopodium Album L., also known as Lambsquarters, or colloquially in New South Wales as "fat hen" as noted in in Figure 2. The Australian distribution of this plant includes New South Wales and Victoria. Analytically this plant 22/3275–10 was reported as yielding 5,909 ng/ml of 20-OH, the intermediate concentration of 20-OH recovered from these NSW plants.

The plant numbered 22/3275-06 has been identified botanically as Amaranthus hybridus L also known as Green amaranth, or colloquially as "smooth pigweed" or "green pigweed" consistent with its colloquial name in New South Wales of "pigweed" as noted in Figure 2. The Australian distribution of this plant includes New South Wales and Victoria. Analytically this plant, 22/3275-06 was reported as yielding 3,654 ng/ml of 20-OH, the lowest concentration of 20-OH recovered from these three NSW plants.

Overall, therefore, further expert review of the plants collected by Harness Racing New South Wales from the registered training establishment/home pasture of the Harness horse in question shows them to be scientifically recognized as plants found in relevant areas of New South Wales and Victoria, the areas of concern in these current 20-OH detection matters. Additionally, as reported previously, the analytical work presented shows these plants to contain not insignificant concentrations of the plant secondary metabolite 20-OH, 20-hydroxyecdysone, fully consistent with the identification of less than 2 parts per billion plasma concentrations of this sub-

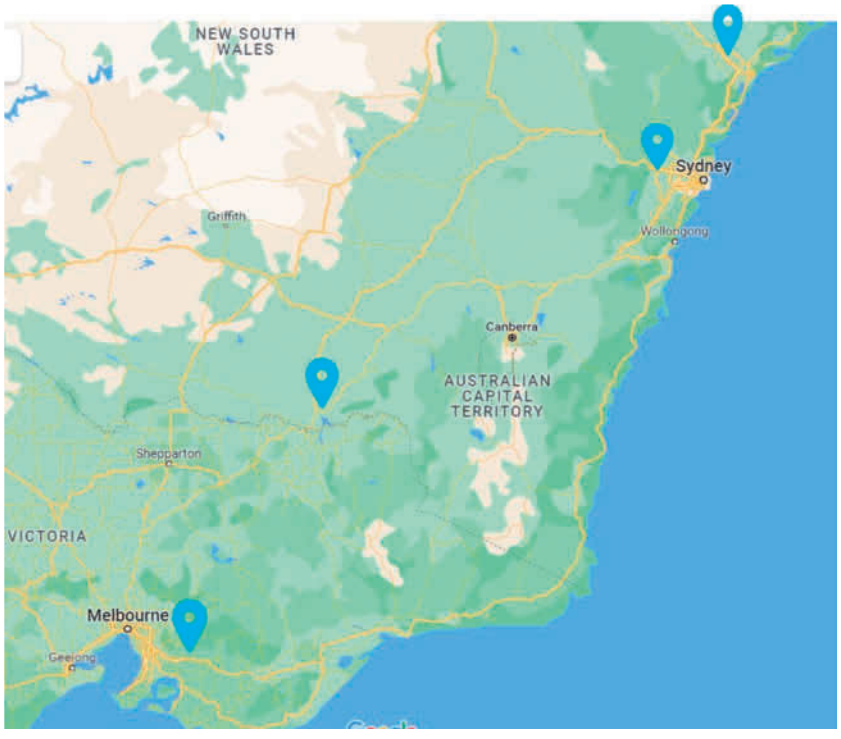

Fig. 3 The home farms and racing locations of the horses involved in these 20-hydroxyecdysone matters. The far north symbol marks the home pasture registered training establishment of the Harness Horse, while the Sydney symbol marks the location of the racetrack where the Harness Horse raced and was postrace sampled on October 13th, 2022. The next symbol, southwest of Canberra marks the home farm location of the Endurance mare, from which location she shipped to the fourth symbol close to Melbourne, location of the Tonimbuk Equestrian Centre in Victoria, Australia where the September 10th Endurance Race was held and in which the mare competed and was blood sampled. [Map image courtesy of Google Maps] | *Die Heimatfarmen und Rennorte der Pferde, die in diese 20-Hydroxyecdyson-Angelegenheiten verwickelt sind. Das Symbol im äußersten Norden markiert die auf der heimischen Weide registrierte Ausbildungsstätte des Trabers, während das Sydney-Symbol den Standort der Rennstrecke markiert, auf der dieser am 13. Oktober 2022 lief und nach dem Rennen beprobt wurde. Das nächste Symbol, südwestlich von Canberra, markiert den Heimatfarmstandort der Endurance-Stute (Distanz-Pferd), von dem aus sie zum vierten Symbol in der Nähe von Melbourne transportiert wurde, Standort des Tonimbuk Equestrian Centre in Victoria, Australien, wo am 10. September das Distanzrennen stattfand und an dem die Stute teilnahm und ihr Blut entnommen* wurde. [Kartenbild mit freundlicher Genehmigung von Google Maps]

stance, 20-OH, (20-hydroxyecdysone) in blood samples from both the Harness horse and the Endurance horse involved in these 20-hydroxyecdysone matters. Plant nomenclature and 20-OH concentrations are summarized in Table 1.

The geographic locations of the home farms and racing locations at which these events occurred are presented in Figure 3. The registered training establishment home pasture of the Harness horse is Sawyer's Gully, NSW, the northmost location identified in a map of the relevant New South Wales area, Figure 3. Next south is the location of the racetrack where the horse raced and was postrace blood sampled, close to Sydney NSW. Then further to the Southwest we have the location of OSO Arabian Farm, the home farm of the Endurance mare, with the fourth symbol further south and west being the Tonimbuk Equestrian Centre in Victoria, Australia, the location of the Endurance ride, with all of these locations being in the same general geographic and climatic region of southeastern Australia.

Discussion

In summary, in Australia, in at least the months of September/October in the geographic area between Sawyers Gully NSW and the Tonimbuk Equestrian Centre in Victoria, Australia, there is a significant likelihood of a horse being randomly exposed to plants (Figure 2) containing sufficient 20-hydroxyecdysone to give rise to a plasma/serum identification of 20-hydroxyecdysone. In the Harness racing case presented above the best estimate of the serum concentration identified in the Harness horse was in the order of 1.8 nanograms/ml plasma [26], a concentration that the stewards considered consistent with inadvertent exposure to the identified plant sources of this substance in the local New South Wales area [28]. Similarly, the blood samples taken from the Endurance mare on September 10th, 2022, also tested positive for a somewhat lower plasma concentration of 20-hydroxyecdysone, in Victoria, a not insignificant distance from the Sawyers Gully/Penrith sample events and to our knowledge associated with inadvertent exposure of the mare to similar plant species as in the Harness horse matter [26, 23].

Plants containing measurable concentrations of 20-hydroxyecdysone are distributed worldwide. Furthermore, the apparently extensive cultivation, marketing, and human consumption of some members of these plant families, the classic example being spinach, has the potential to give rise to unexpected trace level serum identifications of 20-hydroxyecdysone in humans, as was identified in the human subject control samples in the *Isenmann* study [22]. These *Isenmann* et al. (2019) Limit of Quantification or thereabouts human control sample 20-hydroxyecdysone identifications – our best estimates from review of their presented data being from 0.5 to 2.0 ng/ml – show that the detection of small plasma concentrations of 20-hydroxyecdysone in human serum samples similar to those reported in these current equine matters are not unexpected events.

The *Isenmann* et al. [22] reported low serum concentrations of 20-hydroxyecdysone are also consistent with earlier data of *Koolman* and *Simon* [24] who reported apparent concentrations of ecdysteroids in the serum samples of mammals using their best available detection technologies. These authors concluded that "ecdysteroids (or better ecdysteroid like material) occur in vertebrates at low but constant concentration". Assaying for immunoreactive ecdysteroids in mammalian serum samples these authors reported apparent ecdysterone concentrations of from 0.432 ng/ml in canine serum to 16 ng/ml in rat serum, consistent with the somewhat less than 2 ng/ml concentrations reported identified in these Australian 20-hydroxyecdysone matters. Overall, these data suggest that identification of low nanogram/ml serum concentrations of 20-hydroxyecdysone in equines should always be evaluated in the context of the environment in which the horses in question are training or competing. With regard to these current 20-hydroxyecdysone identifications, it seems clear that the environment in New South Wales and Victoria is an environment in which there is a significantly increased likelihood of environmentally related serum/plasma identifications of 20-hydroxecdysone at least in the September/October months, based on these current Endurance and Harness Racing matters.

As the sensitivity of equine drug testing has increased there have been concomitant increases in the incidence of detections of plant substances. These identifications have led the *International Federation of Horseracing Authorities* (IFHA) to recommend Residue Limits for a number of such substances [21], many of which are plant substances that directly transfer from consumed plants to horses, e.g., atropine, scopolamine, and hordenine [16, 4, 5]. One characteristic of these plant related substance identifications is that the likelihood of transfer of a pasture plant substance to a horse can depend to a significant extent on local seasonal and environmental conditions. In this regard the classic examples are hordenine [16], synephrine [5] and scopolamine [4], the inadvertent transfer to horses of each of these substances being to a significant extent driven by both regional and seasonal factors.

The likely pharmacological effect of 20-hydroxyecdysone in a horse showing less than a 2 nanogram/ml serum concentration of this substance is minimal. In human administration studies reported by *Dinan* et al. [10] a 1,400 mg dose of ecdysone yielded peak plasma concentrations of 20-hydroxyecdysone in the order of 600 ng/ml, 300-fold greater that

Table 1 Plants identified as containing 20-OH and collected from the paddock of a harness horse at the New South Wales (NSW) training establishment in Case #2. | *Pflanzen, bei denen 20-OH identifiziert wurde und die auf der Koppel eines Geschirrpferdes in der Trainingseinrichtung in New South Wales (NSW) in Fall #2 gesammelt wurden.*

Genus/species	Common name	Plant ID	20-OH, ng/mL	Australian Distribution
Sida rhombifolia L	Arrowleaf Sida	Plant 22/3275-9	12854	NSW
Chenopodium Album L	Lambsquarters	Plant 22/3275-10	5909	NSW, Victoria
Amaranthus hybridus L	Green amaranth	Plant 22/3275-06	3654	NSW, Victoria

the less than 2 ng/ml concentrations reported present in the horses in these current equine 20-hydroxyecdysone matters. This more than 300-fold difference in plasma concentration between the intentional administration and the inadvertent plant exposure in these current equine samples is fully consistent with these plasma concentrations being pharmacologically insignificant.

Review of the scientific literature shows that 20-hydroxyecdysone is a widely distributed plant substance which readily transfers to both humans and horses, usually in small but analytically detectable amounts, as shown by the *Isenmann* study [22] and earlier work by *Koolman* and *Simon* [24]. As with all plant substances, the likelihood of a significant plant to horse transfer depends to a large extent on local plant growth conditions, and there are planetary regions with an unusually high potential for growth of specific plant species and resultant inadvertent transfer of such substances, as has been seen previously with hordenine, scopolamine and synephrine. The solution to this problem is to identify an irrelevant plasma or urinary concentration of the plant substance of concern, in this case 20-hydroxyecdysone, below which concentration the reporting of an analytical identification is not required. At this point the region west of Sydney, New South Wales, and the region east of Melbourne, Victoria, appear to be geographic areas with a high September/October seasonal potential for inadvertent plant driven identifications of 20-hydroxyecdysone. Given this circumstance and based on review of the scientific literature and the HRNSW findings on 20-hydroxyecdysone in local plants and the relevant HRNSW regulatory decisions, we now suggest 2 ng/ml of 20-hydroxyecdysone as an interim Screening Limit of Detection (SLOD) for 20-hydroxyecdysone in equine blood/plasma/serum samples.

The widespread availability of plants containing 20-hydroxyecdysone takes on a greater importance considering a recent report of counterfeiting of food supplements in the European Union with extracts of Cyanotis arachnoidea, a plant native to China [20]. This plant can reach 20-OH levels on the order of 4–5 %, in contrast to spinach's relatively weak content of 0.005–0.08 % by weight.

With respect to the *Fédération Equestre Internationale* (FEI) Atypical Findings (ATFs) policy first communicated November 23, 2020, [13] we note that these two 20-hydroxyecdysone identifications meet many of the presented FEI/ATF policy criteria [14]. These criteria include a requirement that there be identifications of the same prohibited substance arising from other samples taken at relevant event (s), which criterion is approached by these two time and New South Wales location related 20-hydroxyecdysone identifications. The second criterion is that there be ATFs arising from the same prohibited substance from other samples taken in events held at the same venue and/or in the same region, which criterion is also approached. The third criterion is that samples taken from feed or bedding at the relevant event test "positive" for the substance in question, which criterion was clearly met in the Harness Racing New South Wales events. Finally, there is the matter of the concentration of the prohibited substance identified in the samples identified which, as we have detailed from the scientific literature, are entirely consistent with plant driven atypical findings. Based on this ATF policy set forth by the FEI it is clear that the interim SLOD proposed in this communication above is an appropriate interim SLOD for 20-hydroxyecdysone, and also consistent with presumably now in-place regulatory practice in Harness Racing New South Wales.

Abbreviations

AERA: Australian Endurance Riders Association Incorporated
ARCI: Association of Racing Commissioners International
NSW: New South Wales
HRNSW: Harness Racing New South Wales
ARFL: Australian Racing Forensic Laboratory
WADA: World Anti-Doping Agency
RASL: Racing Analytical Services Limited
EA: Equestrian Australia
IFHA: International Federation of Horseracing Authorities
SLOD: Screening Limit of Detection
FEI: Federation Equestre Internationale
ATFs: Atypical Findings
20-OH: 20-Hydroxyecdysone

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Authors' contributions

TT conceived and directed the project and TT, CF of the North American Association of Racetrack Veterinarians (NAARV), GAM, Director of the New York Drug Testing and Research Program and AMB of Caracas, Venezuela and Abu Dhabi, United Arab Emirates reviewed the data interpretation and analysis and approved the proposed interim SLOD from an equine practitioner, researcher, and regulatory scientist's perspective. KB and AFL performed the data searching, chemical structure evaluations and statistical analyses and TT coordinated and edited all drafts of this manuscript with ongoing contributions from all authors and all authors reviewed approved the final manuscript submitted for publication.

Availability of data and materials

The datasets used and/or analyzed during the current study are available in the public domain as referenced in the manuscript or from the corresponding author on reasonable request.

Declarations

Ethics approval and consent to participate not applicable: As a review of the relevant scientific and regulatory literature no ethics approval and consent to participate is necessary or required and all the authors consent to publication of this case report and analysis.

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Exhibit

9

Pferdeheilkunde – Equine Medicine 39 (2023) 6 (November/December) 550–556 DOI 10.21836/PEM20230606

Trace level identifications of fentanyl and eutylone in equine plasma, pharmacological significance and probable origins – a case report and analysis

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Summary: Consistent with recently increased street availability and recreational use of the potent synthetic opioid fentanyl, there has been a parallel increase in trace level plasma identifications of fentanyl in racing horses, at times in association with trace level amounts of other human recreational substances such as the synthetic cathinone eutylone, currently classified as a designer drug. Fentanyl has three basic effects in the horse, i.e., an analgesic effect, a locomotor stimulation response and a potential endurance effect. Fentanyl is considered relatively straightforward to synthesize following a four-step procedure. Eutylone is the most frequently identified cathinone-related substance identified in the US and is considered a synthetic stimulant. Eutylone is inexpensive to produce and mimics the effects of cocaine, methamphetamine and 3,4-methylenedioxymethamphetamine, commonly known as ecstasy, and is, like fentanyl, a street marketed human recreational substance. Both substances are listed in horseracing as Class 1 with Penalty Class A substances, therefore having the highest penalties for identifications in horses given their stimulant properties, according to the ARCI (*Association of Racing Commissioners International*). Given that racing authorities recognize the potential for substances of "human use and addiction" to inadvertently transfer to racing horses, we were asked to develop an Irrelevant Plasma Concentration (IPC) for fentanyl in horses. Additionally, the finding of a trace level of eutylone along with a trace level of fentanyl increases the likelihood that these paired trace level identifications were caused by inadvertent transfer from a human using a combination of recreational substances. With regard to fentanyl, review of the published pharmacology of fentanyl suggested that locomotor responses disappear below plasma concentrations of 5 ng/mL, with the locomotor response peaking at 50 ng/mL. Similarly, the antinociceptive effects of fentanyl require concentrations above 6.5 ng/mL. An effective plasma concentration (EPC) of 25 ng/mL was therefore decided on. Dividing this EPC by 500, the conservative *Toutain & Lassourd* safety factor (SF) gives a 50 pg/ml IPC for fentanyl in the horse. This value was not exceeded by any of the low fentanyl concentrations identified in 125,000 post-race samples in the 2018–2022 time range. Plasma concentrations of fentanyl in the sub-40 pg/mL range are therefore pharmacologically irrelevant with a significant likelihood of transfer from recreational users. This IPC value is consistent with a number of recent trace level plasma fentanyl identifications in equine samples and judgements in these matters by regulatory authorities that the likely source of these trace level plasma fentanyl identifications was inadvertent transfer from human recreational users to the horses in question. The first detections of fentanyl detections in racehorses occurred in the period 1978–1983 corresponding to the introduction of sensitive radioimmunoassay screening methods. This contrasts with the more recent uptick in 2018–2022, apparently due to exposure of horses to inadvertent trace level transfers from recreational users of fentanyl. Eutylone continues to be a concern, but more information on its equine pharmacokinetics is necessary before similar development of EPC and IPC can be determined.

Keywords: fentanyl, eutylone, environmental exposure, detection, pharmacological significance, racehorses

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Introduction

On September 17th, 2020, two winning horses at Charles Town, West Virginia (WV) were disqualified for post-race "positives" based on the detection of fentanyl and eutylone in their postrace samples (*King* 2020). Eutylone (*Krotulski* et al. 2021) is an illicit recreational substance/drug known colloquially as a "bath salt", chemically related to the naturally occurring plant substance cathinone (*Kind* et al. 2012), and apparently being used recreationally in combination with fentanyl. The regulatory rulings specifically noted that "the standard penalty for a first offense Class A medication violation of year suspension/$10,000.00 fine" was not imposed. As reported in BloodHorse magazine, (*King* 2020) the ruling noted both trainers' overall clean medication records and the fact that the substances were identified at "a trace level, which

lends credibility to the probability that the horse was inadvertently exposed to the drug in some manner."

These rulings also noted that an employee of one trainer who had access to both horses prerace had refused to take a drug test for fentanyl, and which employee was subsequently suspended. Overall, these events are consistent with these trace level postrace fentanyl and eutylone identifications being an outcome of their inadvertent transfer to the horses in question at trace levels from a human or humans using these substances recreationally. These likely events were considered by the regulatory authorities, who redistributed the purses but imposed no multiple medication violation points on the trainers involved, recognizing the substantial evidence supporting lack of trainer culpability with regard to these trace level "substances of human use and addiction" identification events.

In response to this specific West Virginia case and related regulatory events we were invited to review the pharmacology of fentanyl in horses and to identify an Irrelevant Plasma Concentration (IPC) for fentanyl as a guide for regulators with regard to the pharmacological significance or lack thereof of low concentration serum/plasma identifications of fentanyl, as we now report.

The substances in question, fentanyl and eutylone

Fentanyl, N-phenyl-N-[1- (2-phenylethyl)piperidin-4-yl]propenamide (Fig 1) , is a μ-opioid receptor agonist about 100 times more potent than morphine (*Tobin* 1978). Like all morphine related μ-opioid agonists fentanyl has three basic actions in the horse, an analgesic response, a clearcut locomotor stimulation response and a potential endurance effect. Regarding the latter, there are suggestions that pharmacologically effective concentrations of a μ-opioid agonist may prolong the racing endurance of a horse by suppressing the perception of exercise stress approaching the end of a race (*Tobin* et al. 1979). For these reasons horse racing has long been concerned about fentanyl and its improper use in racing horses, with the matter of fentanyl being an opiate and a DEA Schedule II substance making fentanyl identifications a particularly sensitive issue with regard to the social image of horse racing (*Tobin* 1981).

Fentanyl was first synthesized by Dr. Paul Janssen in Belgium (*Stanley* 2014) who synthesized a number of opiate related agonists including phenoperidine in 1957 and fentanyl in 1960. As well as being 100 or so times more potent than morphine, fentanyl is much more lipid soluble, giving it a remarkably rapid onset of action following IV administration and a significantly better therapeutic index (safety record) than morphine. Fentanyl was introduced into human medicine in Europe in 1963, in the US in 1968 and fentanyl is currently the opioid most often used intravenously for intraoperative analgesia in the United States and worldwide (*Stanley* 2014).

Fentanyl is marketed worldwide as an opioid analgesic. Approved formulations include transdermal patches, oral transmucosal lozenges, buccal tablets, buccal soluble film, sublingual tablets, nasal sprays and most recently a sublingual spray (*Stanley* 2014). Furthermore, given that its chemical synthesis is relatively straightforward as set forth in Figure 2, the street availability of illicitly synthesized fentanyl, hereinafter street fentanyl, has significantly increased, leading to increased recreational use of fentanyl, with the associated potential for trace level environmental transfers to horses from humans either prescribed or using fentanyl recreationally. A further point of interest is that recreational use often involves combi-

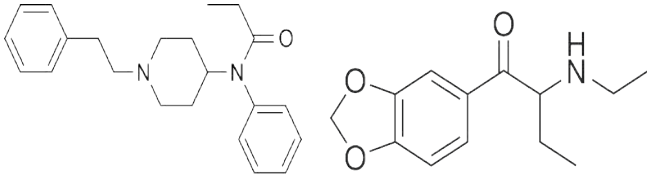

Fig. 1 a) Left, Fentanyl (N-phenyl-N-[1-(2-phenylethyl)piperidin-4-yl] propenamide); Molecular Formula $C_{22}H_{28}N_2O$, Molecular Weight, 336.471 g/mol. b) Right, Eutylone, ((±)-1-(1,3-benzodioxol-5-yl)-2-(ethylamino)butan-1-one); Molecular Formula $C_{13}H_{17}NO$, Molecular Weight, 235.283 g/mol. | *a) Links: Fentanyl (N-Phenyl-N-[1-(2-phenylethyl)piperidin-4-yl]propenamid), Summenformel $C_{22}H_{28}N_2O$, Molekulargewicht 336,471 g/mol. b) Rechts, Eutylon, ((±)-1-(1,3-benzodioxol-5-yl)-2-(ethylamino)butan-1-on), Summenformel $C_{13}H_{17}NO$, Molekulargewicht, 235,283 g/mol.*

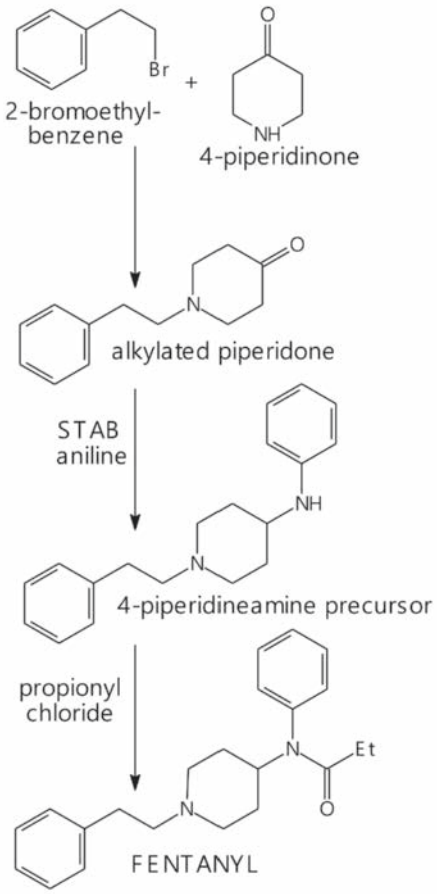

Fig. 2 Simple 4-step synthetic routes to fentanyl. The more recent *Valdez* et al. (2014) method has the advantage that all intermediate product yields exceed 90%, unlike the older *Suh* et al. (1998) method with yields in the 60–80% range. (STAB is sodium triacetoxyborohydride). | *Einfache 4-stufige Synthesewege zu Fentanyl. Das neuere Valdez et al. (2014) Methode hat den Vorteil, dass alle Zwischenproduktausbeuten über 90% liegen, im Gegensatz zur älteren Methode von Suh et al. (1998) mit Ausbeuten im Bereich von 60–80%. (STAB ist Natriumtriacetoxyborhydrid)*

nations of fentanyl with other psychoactive substances (*Wernau* 2023, *Daniulaityte* et al. 2019) such as eutylone, as in this West Virginia matter, and more recently with the veterinary sedative xylazine (US DEA, 2023).

Eutylone, Figure 1b, is a synthetic cathinone-related Novel Psychoactive Substance (NPS) and in 2021 it was the most frequently identified cathinone related substance by the US Drug Enforcement Administration (DEA) (*Krotulski* et al. 2021). Eutylone was first forensically identified in the US in September 2018 and by 2020 it was one of the most prevalent illicit synthetic stimulants reported in US forensic toxicology. *Krotulski* et al (2021) reported that by April 2020 eutylone had been confirmed in 83 human forensic cases, all but one case east of the Mississippi River, an east coast US distribution consistent with this current equine case being reported in West Virginia racing. This combined fentanyl and eutylone identification is of particular interest because the amounts identified are consistent with recent racing industry evaluations of the likely inadvertent transfer of fentanyl from human recreational users as the reason for the recent 2018 and thereafter uptick in trace level plasma identifications of fentanyl in racing horses.

Low concentration detections of fentanyl in racing horses

Concerns first arose in horse racing in the 1970s about the possible misuse of fentanyl in racing horses (*Combie* et al. 1979a). Administering fentanyl by rapid intravenous injection to horses we observed clearcut, rapid onset and highly reproducible dose related locomotor responses to fentanyl. We soon showed that this locomotor response is characteristic of opioid-related substances in horses, as we reported in the veterinary literature (*Combie* et al. 1979 a and b, *Kamerling* et al. 1985). Additionally, we developed a number of immunoassay-based screening tests for fentanyl in racing horses. Such tests had a Limit of Detection (LOD) for fentanyl of about 100 picograms/ml, which at that time provided a rapid, sensitive, and highly useful screening test for fentanyl (*Woods* et al. 1986, *McDonald* et al. 1987, *Tobin* et al. 1988).

With regard to the matter of equine drug testing, in the 40 or so years since fentanyl was first detected in horse racing the sensitivity of equine drug testing has increased at least 1,000-fold (*Fenger* et al. 2018). In a recent sequence of low plasma concentration fentanyl identifications the Limit of Detection (LOD) for fentanyl was 1 picogram/ml or less and the lowest concentration equine plasma fentanyl identifications that we are aware of were reported at 0.30 picograms/ml, and at 0.76 picograms/ml, both in Pennsylvania racing (*Mostollor* 2018). In all, eleven identifications were analyzed and detected in approximately 125,000 post-race equine samples analyzed between January 2018 and August 2022. Values ranged from 0.3–40 pg/mL. The question that has therefore arisen in the equine regulatory community is what if any is the pharmacological significance of these low picogram or sub-picogram/ml plasma concentration identifications of fentanyl.

A significant part of the reason for this question is the now widespread availability and use of street fentanyl, often in combination with other psychoactive substances and the associated increased incidence of human overdose deaths related to fentanyl abuse (*Wernau* 2023, *Daniulaityte* et al. 2019). This now widespread availability and use of street fentanyl has the potential to give rise to inadvertent transfer of trace level amounts of human use fentanyl to racing horses, as has long been the case for cocaine/benzoylecgonine (*Camargo* et al. 2006), morphine (*Camargo* et al. 2005), methamphetamine (*Brewer* et al. 2016), and gabapentin (*Brewer* et al. 2022) and other human prescription medications and recreational substances (*Washington Horse Racing Commission* 2022). As set forth in *Camargo* et al. (2006) in the case of cocaine, inadvertent transfer could arise 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 racehorses. Spread of cocaine by casual contact is consistent with the fact that it is readily absorbed through human skin. Dermal and mucosal exposures of horses may result in the presence of cocaine metabolites in urine. Similar considerations likely relate to fentanyl, a drug known to be more lipophilic than morphine, as well as eutylone and related synthetic cathinones.

An irrelevant plasma concentration (IPC) for fentanyl

Review of the published scientific literature on fentanyl in horses shows that the locomotor response to fentanyl is lost when the plasma concentration of fentanyl falls below 5 ng/mL (*Combie* 1979). Similarly, *Echelmeyer* and coworkers (2019) have shown that fentanyl concentrations of greater than 6.5 ng/mL are required for an antinociceptive effect of fentanyl in the horse. These data are in good agreement with the results of *Combie* (1979) who reported the minimal plasma concentration of fentanyl associated with a locomotor response in horses as being 5 ng/mL and the peak locomotor response occurring at plasma concentrations of about 50 ng/mL. Assuming an Effective Plasma Concentration (EPC) for fentanyl in equines of about 25 ng/mL and dividing this concentration by 500, the highly conservative Effective Plasma Concentration (EPC) value divisor suggested by *Toutain & Lassourd* (2002) gives an Irrelevant Plasma Concentration (IPC) for fentanyl of 50 picograms/ml. We note that the eleven fentanyl concentrations determined post-race during 2018–2022 come in well below this conservatively calculated fentanyl Irrelevant Plasma Concentration (IPC). As such, it is highly unlikely that any of these $\leq$ 40 picogram/ml reported plasma concentrations of fentanyl were associated with any pharmacological effect of fentanyl at the time of the races in question. Eutylone identification was important in establishing that the source of fentanyl was likely from a human recreational user; however, more research is necessary before we can develop an IPC for eutylone.

History of fentanyl detections in racing horses

These environmental transfer interpretations for recently reported trace level identifications of fentanyl are consistent with the biphasic pattern of US fentanyl identifications as recorded by the *Association of Racing Commissioners International*

(ARCI) (*Holloway* 2020). As presented in Figure 3, fentanyl detections in racing horses were first reported in 1978 consistent with the then availability of a highly sensitive radioimmunoassay screening test for fentanyl. Fentanyl "positive" calls peaked at 29 in 1979 and then declined to zero in 1984/1985, consistent with recognition by potential users of the fact that fentanyl had become readily detectable by racing chemists. The following years from 1986 to 2017, a period of 31 years, presented a total of 10 fentanyl identifications, with zero identifications between 1995 and 2008, but 2018 presented 12 fentanyl identifications, zero in 2019 and a total of five in 2020.

The most likely explanation for this recent 2018 and thereafter increase in fentanyl identifications is an increase in the number of low concentration trace level fentanyl detections. Such an uptick in trace level identifications would be consistent with the presumably increased random exposure of horses to trace amounts of environmental fentanyl associated with the increased recreational use of street fentanyl (*Wernau* 2023, *Daniulaityte* et al. 2019). Given this environmental reality and the lack of pharmacological significance of trace level fentanyl, it may be appropriate for racing authorities to identify an analytical "cut-off" or Screening Limit of Detection (SLOD) for fentanyl in equine plasma, similar in principle to the environmental "cut-offs"/screening limits in place for cocaine/benzoylecgonine, methamphetamine and gabapentin and other human medications and recreational substances in many US racing jurisdictions (*Tobin* et al. 2012).

Based on the above analysis, there is no likelihood of a significant pharmacological effect associated with an identification of fentanyl at a plasma concentration of less than 50 picograms/ml. The second consideration is that given the now widespread availability of street fentanyl, the likelihood is that detections of trace level amounts of fentanyl at concentrations < 40 pg/mL are caused by inadvertent transfer of trace amounts of fentanyl from humans to horses. As such, the sub-40 pg/ml plasma identifications of fentanyl at concentrations are pharmacologically irrelevant with a significant likelihood of being the result of inadvertent transfer of fentanyl to the horses in question from a recreational user of street fentanyl or less likely from an individual prescribed an FDA approved product containing fentanyl (*Stanley* 2014).

Regulatory recognition of fentanyl as a substance of human use inadvertently transferring to racing horses

The final question is the identification of fentanyl sources in cases of possible environmental contamination. In the matter of the 300 femtogram/ml Pennsylvania identification the Stewards decided that "due to mitigating circumstances, there will be no further action on this drug positive" (*Fenger* et al. 2018). In the 760 femtogram/ml Pennsylvania matter the fentanyl identification was linked to the horse trailer driver being a user of fentanyl (*Mostollor* 2018), presumably non-prescription fentanyl. As such, the lowest concentration plasma identification was apparently dismissed, and the second lowest was considered caused by non-trainer related environmental transfer from a human user. Similarly, two of the 1–40 pg/ml plasma samples were associated with the employee in the West Virginia fentanyl/eutylone matter who had declined three requests to present for fentanyl testing, which was considered a mitigating circumstance for the trainers involved. Based on the prior good medication regulation record of the trainers involved, the Stewards adjudicating this matter considered these circumstances as indicative of inadvertent contamination being the source of this fentanyl identification and the trainers were found "not responsible for the medication violation" and no penalties were assessed against their licenses (*King* 2020). A point of particular interest is that the Pennsylvania Rules of Racing specifically recognize "substances of human use" as set forth in 403.16. "Environmental contaminants and substances of human use", in which section (b) notes that "Substances of human use and addiction may be found in the horse due to its close association with humans". Section (c) of this rule states that "If probative and substantial evidence is presented to the Bureau Directors prior to a hearing or presented to the Judges or Stewards during a hearing which indicates that a positive test may have been a result of environmental contamination, including inadvertent exposure due to human drug use, or dietary intake, or is endogenous to the horse, those factors may be considered in mitigation of any disciplinary action against the affected trainer" (*Legal Information* Institute, 2019) fully consistent with the above referenced fentanyl rulings.

The Pennsylvania Rules of Racing are also consistent with the current *Association of Racing Commissioners International* (ARCI) Model Rules which note that " (2) Substances of hu-

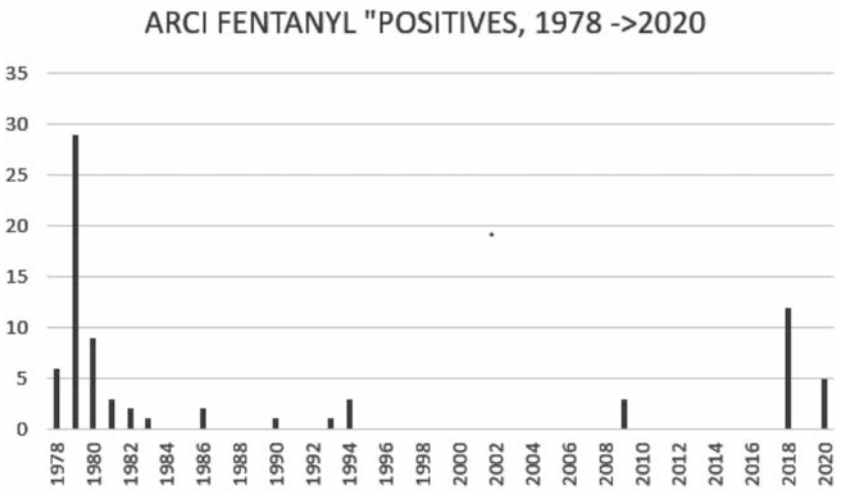

Fig. 3 Fentanyl identifications in US racing 1978–2020 as reported to the Association of Racing Commissioners International (ARCI). Shown are the number of positives versus year. | *Fentanyl-Identifizierungen im US-Rennsport 1978–2020, wie der Association of Racing Commissioners International (ARCI) gemeldet. Das Diagramm zeigt die Anzahl der positiven Ergebnisse auf der y-Achse und das Jahr auf der x-Achse.*

man use and addiction may be found in the horse due to its close association with humans. (3) If the preponderance of evidence presented in the hearing shows that a positive test is the result of environmental contamination, including inadvertent exposure due to human drug use, or dietary intake, or is endogenous to the horse, those factors should be considered in mitigation of any disciplinary action taken against the affected trainer. Disciplinary action shall only be taken if test sample results exceed the regulatory thresholds in the most recent version of the ARCI Endogenous, Dietary, or Environmental Substances Schedule." We also note, however, that the current ARCI Endogenous, Dietary, or Environmental Substances Schedule does not include fentanyl on its list of substances, so these West Virginia and Pennsylvania fentanyl rulings break new ground with respect to their recognition of fentanyl as a cause of trace level contamination of racing horse and their equine drug testing samples resulting from "inadvertent exposure due to human drug use" (ARCI, 2017, 2020).

Human drug testing "cut-offs" for fentanyl

Consistent with these moves by racing regulators away from zero tolerance testing for fentanyl, on October 24th, 2019, the Biochemical Testing Advisory Committee (BTAB) of the US Military Personnel Drug Abuse Testing Program "voted unanimously to add fentanyl and norfentanyl to the panel with a cutoff of one nanogram per milliliter." To the best of our knowledge, this "cut-off" is both an ELISA screening and a confirmatory testing "cut-off" (DOD Instruction 2020). This BTAB action is presumably a recognition of events similar to those set forth above in racing regulation, namely an increase in trace level fentanyl detections associated with inadvertent transfer from recreational users to "clean" individuals. It is also interesting that in a SAMHSA Drug Testing Advisory Board June 11–12, 2019 Minutes – Open Session, during the discussion, it was explained "that, although there is not a screening test specifically for fentanyl in the DoD panel, the presumption of a positive test will rely on an individual being positive for fentanyl and another drug – for example, cocaine and fentanyl, or an opioid and fentanyl ", formal recognition in human drug testing of a pattern of low concentration fentanyl detections occurring in association with detections of other recreational substances (*Dept Health & Human Services* 2019).

Conclusions

In summary, in the United States street fentanyl is now a widely available and used recreational substance, and is apparently being detected in racing horses at plasma concentrations of 40 picograms/ml or less, including one plasma identification at 0.30 picograms/ml. These plasma concentrations are well below the quite conservative 50 picograms/ml *Toutain & Lassourd* (2002) based Irrelevant Plasma Concentration (IPC) and at least two of these identifications, one at 0.76 picograms/mL and another at 0.30 picogram/mL, were considered by the regulatory authorities as being most likely caused by inadvertent transfer from a human user or users of presumably non-prescription fentanyl. Given these circumstances and the current widespread availability of street fentanyl, it is appropriate for equine regulatory authorities to carefully consider the lack of pharmacological relevance of plasma identifications of fentanyl at plasma concentrations of less than 50 picograms/ml and the significant probability that such irrelevant trace level identifications are associated with inadvertent transfer of fentanyl from human users, most likely recreational users of street fentanyl to racing horses.

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Conflict of interest statement

C. Fenger, K. Brewer, and T. Tobin are veterinarians and equine forensic scientists who have testified in equine forensic science matters and related areas. A. Morales-Briceño and C. Fenger are equine veterinarians who practice in the United States (C. Fenger) and South America and the Middle East (A. Morales-Briceño) in horse racing and sports-related areas. A. F. Lehner is an analytical toxicologist.

Author contributions

T. Tobin and K. Brewer performed the primary regional regulatory and forensic literature searching and analysis and assembled the forensic data, with A. Morales-Briceño focusing particularly on the European, Spanish, Middle East and South American veterinary and regulatory literature and experience. C. Fenger, Executive Director of the North American Association of Racetrack Veterinarians (NAARV), contributed to the writing and reviewed and approved the proposed Irrelevant Plasma Concentration for fentanyl. A. F. Lehner reviewed and

edited the manuscript and researched fentanyl syntheses. T. Tobin 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.

Animal welfare 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. Thomas *Tobin* https://orcid.org/0000-0001-8506-3147

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Exhibit

10

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Metformin as an environmental substance transferring to horses – a case report and analysis

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Summary: Metformin is a widely prescribed oral antihyperglycemic agent and currently a first-line medication in the treatment of human type 2 diabetes, with a total of 92 million US prescriptions in 2022. The daily dose per human can be as much as 2.5 grams/day which is excreted largely unchanged into the environment. Metformin is chemically stable and a widely distributed environmental substance. Metformin therefore has the potential to be identified at trace levels in equine blood and urine samples as a result of random exposure to environmental metformin. Given these circumstances we have reviewed the scientific literature and calculated an irrelevant blood/plasma/serum concentration of metformin of 5 nanograms/ml. We now therefore propose this plasma concentration of metformin as an interim Screening Limit of Detection (SLOD) for metformin, below which concentration a blood/plasma/serum identification of metformin should not be considered appropriate for regulatory action.

Keywords: Metformin, environmental contamination, antihyperglycemic agent, Screening Limit of Detection, racehorses

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Introduction

As the world population of humans has increased so has the environmental presence of anthropogenic substances. With respect to horse racing, the increased sensitivity of drug testing now allows racing chemists to detect pharmacologically irrelevant trace level amounts of numerous human pharmaceuticals in regulatory samples [1 1]. The human prescription medication Gabapentin is the classic example; the human dose is large, up to 3 grams/day, a dose that is largely excreted unchanged into the environment. Gabapentin is stable in the environment so it persists and may accumulate. Gabapentin is orally absorbed, so environmental Gabapentin can give rise to trace level detections in horses. Gabapentin is a classic example of a human prescription medication that is not infrequently detected at pharmacologically irrelevant concentrations in equine plasma, as set forth by Brewer and colleagues [6,7].

The regulatory solution to this circumstance is to define a Screening Limit of Detection (SLOD) below which such identifications are by definition pharmacologically irrelevant and not reported for regulatory action. For example, in October 2019 a plasma Screening Limit of Detection of 8 ng/mL was introduced for Gabapentin in Ohio racing, where regulators may be guided with regard to the significance of trace level plasma detections of Gabapentin [6,7]. We now review the current status of trace level plasma/serum identifications of a substance broadly similar in pharmacokinetic and regulatory terms to Gabapentin, namely the human prescription medication Metformin. Based on available data we propose an interim Screening Limit of Detection for Metformin of 5 ng/ml in equine plasma/serum to handle the regulatory problem of irrelevant trace level detections of Metformin in equine plasma samples.

Metformin, a widely prescribed high dose human medication

Metformin, *N*,*N*-dimethylbiguanide, $C_4H_{11}N_5$, Molar mass, 129.167 g·mol^{-1} (Figure 1), is a widely prescribed oral antihyperglycemic agent that is currently a first-line medication in human medicine in the treatment of type 2 diabetes and

other conditions associated with insulin resistance. Metformin is a biguanide molecule chemically related to phenformin and also to the plant substance galegine, a guanidine derivative found in the French lilac, botanically *Galega officianalis*[14,21]. Galegine is a substance with blood glucose-lowering properties and the foundation for the discovery of metformin[22].

At physiological pH, Metformin is a cationic (positively charged) hydrophilic molecule with low lipid solubility. Its direct diffusion through cell membranes is therefore minimal and intestinal absorption and tissue distribution of Metformin is facilitated by various Organic Cation Transporters (OCTs)[17]. The oral bioavailability of Metformin in humans is on the order of 50 %[14]. Following intravenous administration, the elimination pharmacokinetics of Metformin are multiphasic, with post IV administration blood concentrations at first declining rapidly, but followed by a much longer 17 hours or so terminal plasma half-life, reflecting the presence of a slow release "deep kinetic" Metformin compartment. Overall, it appears that the effective plasma half-life of Metformin in patients with good renal function is about 5 hours[14,21]. In older nonracing horses Metformin has been proposed as a treatment for equine metabolic syndrome despite low bioavailability and increased rate of elimination compared to humans[19]. The insulin resistance associated with equine metabolic disorder is also considered a likely predisposing factor to laminitis[9].

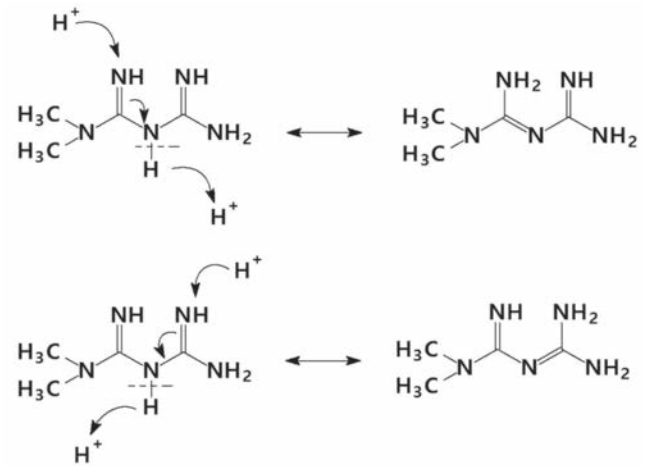

Fig. 1 Equilibrium structures of metformin, *N,N*-dimethylbiguanide, $C_4H_{11}N_5$, molar mass 129.167 g/mol. In acid, protonation of the C-2 imine induces release of a proton from the central nitrogen resulting in a 2-3 double bond (top). Protonation of the C-4 imine induces release of a proton from the central nitrogen resulting in a 3-4 double bond (bottom). | *Gleichgewichtsstrukturen von Metformin, N,N-Dimethylbiguanid, $C_4H_{11}N_5$, Molmasse 129,167 g/mol. In Säure induziert die Protonierung des C-2-Imins die Freisetzung eines Protons aus dem zentralen Stickstoff, was zu einer 2-3-Doppelbindung führt (oben). Die Protonierung des C-4-Imins induziert die Freisetzung eines Protons aus dem zentralen Stickstoff, was zu einer 3-4-Doppelbindung führt (unten).*

In human medicine, dosing with Metformin usually starts at 500 mg/day, a daily dose that may be increased to control the patient's blood sugar level. The daily dose for some patients may therefore at times be as high as 2,500 mg/day. Metformin is similar to gabapentin in that it is not significantly metabolized, and humans prescribed Metformin therefore contribute most of their daily Metformin dose to the environment. Metformin is also stable in the environment with potential to accumulate in the environment local to an individual prescribed Metformin. Metformin is therefore a classic high dose and frequently prescribed human medication with significant potential to become present in and detectable in the environment of individuals prescribed Metformin[14,21].

In the year 2022 there were more than 92 million US prescriptions for Metformin making it the third most frequently prescribed medication in the United States[20]. Given this circumstance and the above pharmacological characteristics of this human prescription medication it is not surprising that Metformin is a widely distributed anthropogenic trace level environmental substance[1].

Metformin, a widely distributed environmental substance

Consistent with these chemical and pharmacological characteristics of Metformin, in a study on the detection of pharma-

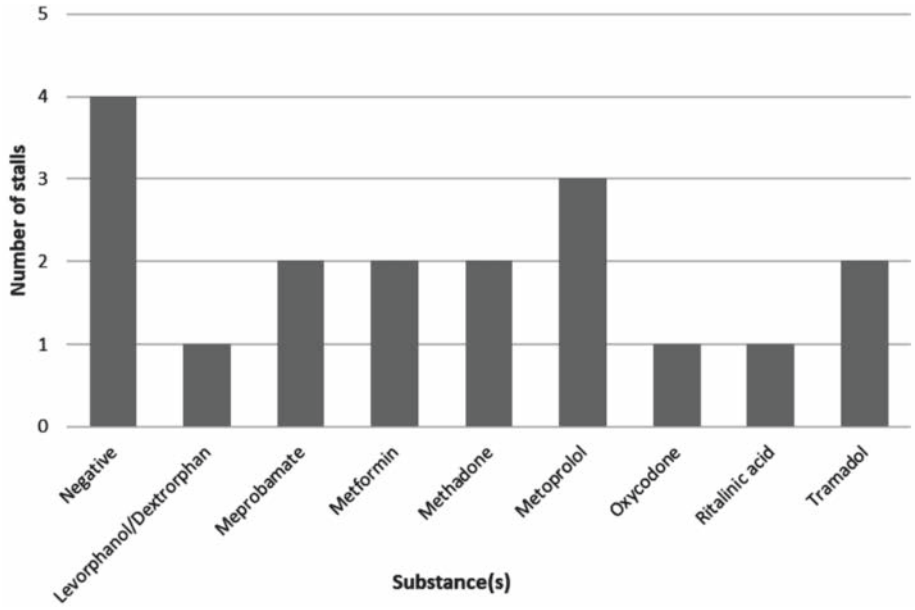

Fig. 2 Human therapeutic substances found in the "ship-in" stalls at Charles Town races. | *Humantherapeutische Substanzen, die in den „Ship-in"-Ständen bei den Rennen in Charles Town gefunden wurden.*

ceuticals in 59 "wadeable" streams of the Southeastern United States, Bradley and his U.S. Geological Survey colleagues detected Metformin at 57 of their sample sites and Metformin was confirmed in 89% of samples analyzed. Bradley and his colleagues described Metformin in these "wadeable stream" samples as a "pervasive presence" and also as "near ubiquity". These authors also noted that "metformin is reported widely in wastewater effluent, increasingly in environmental samples and even in tap water" [4,5].

Responding to concerns that Charles Town Racetrack "ship-in" stalls might be contaminated with Naproxen, the Charles Town racing authorities had 21 "Ship-in" stalls at Charles Town racetrack "*swabbed*" and the swabs tested in their racing analytical laboratory for substances of concern to racing regulators. Metformin was detected in two of these swabbed stalls as depicted in Figure 2. Overall, a total of 25 different substances of regulatory concern were detected in these stalls, 12 equine therapeutic medications, 8 human therapeutic medications and 5 human recreational substances [11].

Fully consistent with these chemical and environmental characteristics of Metformin, it was detected in two "ship-in" stalls when twenty-one "ship-in" stalls at Charles Town racetrack were swabbed for pharmacological contaminants/environmental substances. In fact, Metformin was one among a total of eight human medications identified in these "ship-in stalls", as presented in Figure 2, taken from *Fenger* et al., 2017 [11]. This presence of detectable levels of Metformin in "ship-in" stalls is fully consistent with its occasional identification at trace levels in racehorse blood and urine samples.

The current regulatory status of Metformin in humans and equines

Metformin is not listed as a prohibited substance by the World Anti-Doping Agency [25]. The Association of Racing Commissioners International (ARCI) lists Metformin as Category 2 penalty B substance [3], a classification that has at times been suggested not to be entirely consistent with the published ARCI definition of a Category 2 penalty B substance. As of 2023 the Federation Equestre Internationale (FEI) lists metformin as a "Prohibited Substance-Controlled Medication" with the notation that it may be used in the treatment of Equine Metabolic Syndrome [10].

The scientific literature is mixed on whether Metformin has an effect on athletic performance on any species at any plasma concentration. No studies have specifically evaluated the effect of Metformin on exercise in horses. However, a PubMed search of "metformin" with "exercise performance" yields 182 results, which makes Metformin one of the most researched drugs in the field of exercise physiology. The majority of scientific studies evaluating any effect of Metformin on performance conclude that there is none, or a negative (ergolytic) effect. A meta-analysis evaluating this group of scientific papers concludes that, overall, these studies failed to show any effect of Metformin on indices of athletic performance, other than an increased rating of perceived exertion [8].

Given the widespread distribution of environmental contamination with Metformin, it is not surprising that Metformin has been detected at trace levels in equine blood and urine samples in circumstances involving no known administration

Table 1 Metformin identifications reported in US racing, 2017 to date; data from Mr. Kerry Holloway, Association of Racing Commissioners International and from review of Horseracing Integrity and Welfare Unit, (HIWU) records. The table lists the date of the race in question, names of trainer and horse, racetrack, regulatory matrix in which the metformin was reported detected, the concentration where available ("amount"), the fine amount and suspension duration where known. | *Metformin-Identifikationen im US-Rennsport, 2017 bis heute; Daten von Kerry Holloway, Association of Racing Commissioners International und aus der Überprüfung der Aufzeichnungen der Horseracing Integrity and Welfare Unit (HIWU). In der Tabelle sind das Datum des betreffenden Rennens, die Namen des Trainers und des Pferdes, die Rennstrecke, die regulatorische Matrix, in der das Metformin nachgewiesen wurde, die Konzentration, sofern verfügbar („Menge/Amount"), die Höhe der Geldbuße und die Dauer der Aussetzung, sofern bekannt, aufgeführt.*

Date	Trainer	Horse	Track	Matrix	Amount	Fine	Penalty	Notes
5/8/2017	Ronald Gene Davis Jr	Story on the Street	Will Rogers Downs	Urine		$1000	Suspended	
5/9/2017	Recil L Payton	Bless Jessica R	Will Rogers Downs	Urine		$1000	Suspended	
4/28/2021	Wesley Ward	Averly Jane	Churchill Downs	Plasma/ serum	4.2 ng/mL	$5000	5 days	
7/15/2022	Wesley Ward	Insanity It Seems	Monmouth	Urine	577 ng/ml	$2000	15 days	Also, Naproxen
6/2/2023	Jonathan Wong	Heaven and Earth	Horseshoe Indianapolis	Plasma/ serum	630 pg/ml 242.5 ng/ml urine	$25000	Suspended 2 years; forfeit $21600 purse	Pay $8000 HIWU arbitration costs
6/11/2023	Guadalupe Munoz Elizondo	Quinton's Charmer	NM	Plasma/ serum	162 pg/ml Plasma			Work, not race
6/24/2023	Javier Morzan	Lady Liv	Delaware Park	Plasma/ serum	253 pg/ml; Corrected 222 pg/ml		Dismissed	Work, not race
8/3/2023	Angel J Castillo	Pylon	Delaware Park				Provisonally suspended	
8/5/2023	Michael Lauer	Mowins	Horseshoe Indianapolis	Urine	40 ng/mL; Plasma data unavailable			

and therefore presenting as inadvertent environmental transfer events. Further, given the unlikely possibility that it would either be administered to the young athletic population of racing horses, and the unlikely possibility that it may be in any way ergogenic, the establishment of a screening limit guideline for horse racing regulators is critically important.

Reported Metformin identifications

Table 1 presents a summary of reported Metformin identifications. In 2017 there were two Metformin identifications at the Will Rogers Downs Racetrack, both of which resulted in modest fines and suspensions for the trainers involved and in both of which cases mitigating circumstances were mentioned[16] In 2021, the winner of the Kentucky Filly Juvenile Stakes was disqualified following an identification of 4.2 ng/mL of Metformin in serum. The trainer was fined $5000 and suspended for 5 days. The following year, the same trainer had combined "positives" for Metformin and Naproxen in a horse racing at Monmouth Park. The trainer was fined $2,000 and suspended for 15 days, the horse disqualified, and the prize money forfeited. The Metformin in this Monmouth Park matter was identified in a urine sample, and the concentration was about 577 ng/ml. Naproxen was also identified in this sample at a trace level and the presence of both substances was considered due to inadvertent environmental contamination, with the naproxen concentration in this matter not communicated[24].

On Monday, May 22nd, 2023, the Horseracing Integrity and Safety Authority's (HISA) Anti-Doping and Medication Control (ADMC) program was activated in most US racing jurisdictions and to date there have been five reported Metformin identifications. The first of these occurred on or about June 2nd at Horseshoe Indianapolis, and the Metformin concentration was reportedly about 630 picograms/ml in plasma and 242.5 nanograms/ml in urine[12]. The next Metformin positive was reported following a workout at a Quarter Horse racetrack in New Mexico at a plasma concentration of about 162 picograms/ml. The third Metformin identification was in Delaware, with concentration data reported at 253 pg/ml in plasma, but review of the relevant data files suggests a more correct value is 222 pg/ml. The most recent HIWU metformin identification was reported on 8/5/23 at Horseshoe Indianapolis for 40 ng/ml in urine, with no information concerning the corresponding plasma concentration[12]. What is interesting about these HISA calls is the rapid increase in the call rate for the environmental substance Metformin at sub-nanogram/ml plasma concentrations very shortly after HISA assumed regulatory responsibility for medication control in the relevant states, and with all of the identifications to our knowledge being reported from the same laboratory, Industrial Laboratories. One possible explanation for this apparently sharp increase in the rate of Metformin calls is that HISA is reporting out for regulatory action trace level identifications of Metformin that previously were not considered as being of regulatory concern.

The matter of determining an irrelevant plasma concentration (IPC) of Metformin in equines

Given the various characteristics of Metformin outlined above that make clear that Metformin is a widely distributed substance in the environment, it is important to determine what would be an appropriate Irrelevant Plasma Concentration (IPC) Screening Limit of Detection (SLOD) for Metformin in equine plasma and urine samples. Review of the therapeutic plasma concentrations of Metformin at steady state in humans as presented by *Graham* et al.[14], shows that the "*concentration average at steady state*" for Metformin in human therapeutics is around 2.5 ug/ml or 2,500 ng/ml. Dividing this plasma concentration by the very conservative Toutain Irrelevant Plasma Concentration (IPC) factor of 500[23] gives an Irrelevant Plasma Concentration for Metformin of 5 ng/ml, based on these human Metformin "concentration average at steady state" data referenced above.

Reviewing published equine Metformin data and applying the above referenced Toutain approach to determining an Effective Plasma Concentration (EPC) in the horse *Dr. Richard Sams* divided the reported 30 mg/kg IV every 8 hours dose as used by *Hustace* et al..[19] by a plasma clearance value of 9 ml/kg/minute. Assuming an 8-hour interval between dosing, Dr. Sams obtained an effective plasma concentration of 6.9 ug/ml equine in plasma serum.[24] Dividing this value by the Toutain Safety Factor (SF) of 500 as referenced above, one obtains the equine/Toutain Irrelevant Plasma Concentration (IPC) for Metformin of 13.9 ng/ml, somewhat greater than the figure based on the human IPC value calculated from the human plasma "concentration average at steady state" data presented above.

Determining an interim screening limit of detection (SLOD) for Metformin in horses

A Screening Limit of Detection – hereinafter a Screening Limit – is a defined analyte concentration in plasma/serum or urine or other forensic matrix below which concentration the identification is considered of no regulatory concern, in other words an Irrelevant Concentration (IC). A screening limit must be influenced by the characteristics of the environment in which the horse is racing. Simply put, if the substance in question is a plant substance there will obviously be regional and seasonal factors affecting the incidence of random exposure of racing horses. Similarly for anthropogenic substances and particularly for substances of human use, including substances humans are using either medicinally or recreationally, it is not possible to predict exposure, so the setting of a Screening Limit is largely based on the range of values identified in routine sample analysis as long as the concentrations identified are below an appropriately calculated Irrelevant Plasma or Urinary concentration.

The screening limit of detection for Metformin based on the range of Metformin values below the IPC reported to date in US racing

As this case report goes to press the total number of Metformin identifications reported in US racing is in the order of nine identifications with all of the five most recent identifications in plasma serum being to our knowledge between 5 ng/ml and the 25-fold lower 160 picograms/ml concentration reported in the New Mexico identification and the unknown but presumably very low plasma concentration in the 8/5/2023

Horseshoe Indianapolis 40 ng/ml urinary identification. We specifically note that all these identifications are less than the conservative 5 ng/ml IPC calculated from the available human concentration average at steady state data, and even further below the IPC calculated from the best available equine data as calculated by *Dr. Richard Sams* based on the referenced equine pharmacokinetic data.

Given the fact that the Sams calculated Irrelevant Plasma Concentration for Metformin was 13.9 ng/ml and the highest of the recent Metformin identifications was 4.2 ng/ml in plasma, it is reasonable to propose an interim Screening Limit of Detection for Metformin of 5 ng/ml in blood/serum/plasma.

The Horseracing Integrity and Welfare Unit (HIWU) introduces a limit of detection for Metformin in US racing

On October 19th, 2023, as this communication was being readied for submission, numerous press reports appeared reporting that HISA had "met with all six laboratories to establish an updated uniform "Limit of Detection". On the basis of this meeting HIWU "will be withdrawing the Equine Anti-Doping Charge letters from trainers Guadalupe Munoz Elizondo and Javier Morzan due to their Covered Horses testing positive for Metformin at levels in blood that would not have been reported as Adverse Analytical Findings under the updated Limit of Detection." Furthermore, since at this time the 630 picograms/ml Equine Anti-Doping Charge is still in place for the Jonathan Wong 630 picograms/ml metformin identification, the currently undisclosed HIWU Metformin plasma serum "Limit of Detection" is apparently somewhere between the Guadalupe Munoz Elizondo value of 162 picograms/ml and the Jonathan Wong 630 picogram/ml value, since the Jonathan Wong Equine Anti-Doping Charge has not to our knowledge been withdrawn under this new HIWU regulation[15].

We must also draw attention to the fact that a "Limit of Detection" (LOD) is the lowest concentration that can be detected by an analytical method in its optimal configuration. The "Limit of Quantification" (LOQ) is the lowest concentration at which the concentration of a substance in a specified sample/matrix can be reliably quantified. The technically and scientifically correct term for the Metformin regulatory level introduced on October 19th by HIWU is "Reporting Level" which as a quantitative level is by definition above the LOD and also equal to or above the LOQ of most if not all of the involved laboratories.

The definition of a "Reporting Level" as presented by the Association Of Official Racing Chemists (AORC) is as follows. "Reporting Level. The concentration, as instructed by the authority or determined by the laboratory in consultation with the authority, of a specified PROHIBITED SUBSTANCE (usually a legitimate equine therapeutic substance or a normally occurring substance) below which a laboratory does not normally report its presence in a SAMPLE."[2]

We also note that this HIWU presented "Limit of Detection" more correctly a "Reporting Level" at an apparent concentration of less than 650 picograms/ml is in the order of eight-fold or more lower than our very conservatively calculated and now presented Irrelevant Plasma Concentration (IPC) Screening limit of Detection (SLOD) of 5 ng/ml in blood/plasma/serum for Metformin in horses.

At this time, it was unclear as to whether or not this HIWU metformin "Limit Of Detection" is defined in plasma or urine. It is therefore appropriate to draw attention to the fact that it is well understood in equine forensic science that urinary concentrations of a substance/medication can be highly variable depending on the pKa of the substance and the pH and specific gravity of the urine sample in question. The effect of pH on urinary drug concentrations has been demonstrated to be a potentially 200 fold or greater effect for acidic medications[18]. For basic medications such as lidocaine *Gerken* et al.[13] 1991 demonstrated a 1,000-fold greater concentration of lidocaine in an acidic post exercise urine. The take home message in equine forensic science is that regulatory thresholds are best defined in plasma and where a plasma threshold is defined the urinary concentration data are of extremely limited forensic significance.

Closing summary

Under the current HISA regulatory system all of the Metformin plasma values reported out as Metformin "positive" are to our knowledge less than this proposed 5 ng/ml in plasma interim Screening Limit of Detection. These HISA reported Metformin identifications are at pharmacologically irrelevant concentrations and had no possible effect on the outcome of the race in question. As such, and given the fact that Metformin is a widely prescribed high dose human therapeutic medication with significant potential to transfer indirectly at trace levels to horses either from humans prescribed Metformin or from other environmental sources, it is appropriate that blood/plasma/serum identifications of Metformin at concentrations less than 5 ng/ml in racing horses not be reported for regulatory action.

Abbreviations

ADMC	Anti-Doping and Medication Control
ARCI	Association of Racing Commissioners International
EPC	Effective Plasma Concentration
FEI	Federation Equestre Internationale
HISA	Horseracing Integrity and Safety Authority.
HIWU	Horseracing Integrity and Welfare Unit.
IC	Irrelevant Concentration
IFHA	International Federation of Horseracing Authorities
IPC	Irrelevant Plasma Concentration
IUC	Irrelevant Urinary Concentration
SF	Safety Factor
SLOD	Screening Limit of Detection.
WADA	World Anti-Doping Agency

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Author's' contributions

TT conceived and directed the project and TT, CF of the North American Association of Racetrack Veterinarians (NAARV), GAM, Director of the New York Drug Testing and Research Program, RH of Holland Management Inc., and AMB of Caracas, Venezuela and Abu Dhabi, United Arab Emirates reviewed the data interpretation and analysis and approved the proposed interim SLOD from an equine practitioner, researcher, and regulatory scientist's perspective. KB and AFL performed the data searching, chemical structure evaluations and statistical analyses and TT coordinated and edited all drafts of this manuscript with ongoing contributions from all authors and all authors reviewed and approved the final manuscript submitted for publication.

Availability of data and materials

The datasets used and/or analyzed during the current study are available in the public domain as referenced in the manuscript or from the corresponding author on reasonable request.

Declarations

Ethics approval and consent to participate are not applicable: As a review of the relevant scientific and regulatory literature no ethics approval and consent to participate is necessary or required and all the authors consent to publication of this case report and analysis.

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Exhibit

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Trace-level detections of methaphetamine in racing horses – a review and forensic analysis

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Summary: Methamphetamine is a central stimulant and an approved human therapeutic medication which is also clandestinely synthesized and marketed worldwide as a recreational substance. Users of clandestinely synthesized methamphetamine may handle and use methamphetamine in far greater amounts than medically approved dosages. Given that mucus membrane exposure of a horse to 10 milligrams of methamphetamine has produced jugular blood plasma/serum methamphetamine concentrations of 88,400 picogram/ml, inadvertent transfer of picogram/ml amounts of methamphetamine from recreational users to racing horses is a well understood process. Evaluating such picogram/ml methamphetamine identifications, the first factor to consider is that methamphetamine presents as two chemically distinct mirror image enantiomers, d-methamphetamine and l-methamphetamine. D-methamphetamine is the more pharmacologically active enantiomer, marketed in the United States (US) as Desoxyn®, a US Drug Enforcement Administration (DEA) Schedule II prescription medication. l-methamphetamine is pharmacologically less active and is marketed in the US in several Over-The-Counter (OTC) nasal decongestant inhalers. Forensically correct evaluation of picogram/ml jugular blood/plasma/serum methamphetamine identifications in racing horses requires quantitative evaluation of the blood, urinary and hair concentrations of each methamphetamine enantiomer, as well as the presence or absence of the expected amphetamine metabolite. Evaluation of the regulatory significance of a jugular blood/plasma/serum concentration of methamphetamine must also take into account the fact that following oral exposure to methamphetamine jugular blood concentrations will be much higher than systemic blood concentrations, given that the jugular vein is the direct venous connection between the local high mucus membrane concentration of methamphetamine and the systemic circulation of the horse. Based on published scientific data, mucus membrane exposure of a horse to 100 micrograms of methamphetamine, a very conservative 1/1,500 of a possibly pharmacologically effective equine dose may give rise to jugular blood/plasma/serum concentrations of methamphetamine of 884 picograms/ml, a conservative guideline value for evaluating the pharmacological and forensic significance of jugular blood/plasma/serum concentrations of methamphetamine.

Keywords: trace-level detection, racing horse, methamphetamine, forensic, analysis

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Introduction

Methamphetamine (R,S)-N-methyl-1-phenylpropan-2-amine, formula, $C_{10}H_{15}N$, molar mass 149.237 g·mol− (Figure 1) is an amphetamine related substance at times detected in post-race blood and urine samples from racing horses[1]. The methamphetamine concentrations involved in these equine blood/plasma/serum or urinary identifications are usually picogram/ml concentrations and unlikely to be pharmacologically significant. Such relatively low concentration blood/plasma/serum/urinary identifications are consistent with their being the result of inadvertent exposure of the horse to trace amounts of environmental methamphetamine from recreational methamphetamine users in contact with the horse, either directly or indirectly.

The first published scientific report detailing a case of random and indirect exposure of horses to environmental methamphetamine is that presented by *Brewer* et al. 2016[1], who reported on an October 2014 sequence of events in which horses were transported to a race meet in Ontario in a newly purchased methamphetamine contaminated horse trailer that had apparently previously been used as an illicit methamphetamine synthesis laboratory. Three horses transported in this trailer tested post-race *"positive"* for picogram/ml urinary concentrations of methamphetamine, while a fourth horse transported in another trailer tested negative. The urinary methamphetamine concentration in these horses ranged from 56 picograms/ml to 340 picograms/ml. Reviewing these identifications the Ontario Racing Commission (ORC) noted

"the very low levels of methamphetamine identified in these horse urines, levels in the opinion of the ORC with no possible impact on the performance health and safety of horses and levels consistent with inadvertent environmental contamination". The ORC also noted the need *"to set limits high enough to cut-off the environmental noise and low enough to stop performance enhancement."* Reporting these regulatory events in the scientific literature, Brewer et al 2016 wrote that *"an interim regulatory cut-off of 15 ng/mL for methamphetamine in post-race urine is proposed"*.

Consistent with these Ontario Racing Commission rulings, the year 2016 saw a sequence of six urinary methamphetamine identifications at Lone Star Park in Grand Prairie, Texas, the first reported on April 17th, rapidly followed by two more identifications on April 23rd, and 24th. The next identifications in this sequence were two identifications on May 13th, in one of which May 13th horses the urinary methamphetamine concentration was reported at 460 picograms/ml., in the same general range as the 2014 methamphetamine identifications in Ontario. The sixth and to our knowledge last horse in this sequence tested methamphetamine "positive" on July 4th, 2016. All of these six horses tested blood/plasma/serum negative for methamphetamine, leading the Texas Racing Commission (TRC) regulatory authority to consider these identifications as not being trainer related [1]. Among the factors considered by the TRC were the low concentrations of methamphetamine identified in the urine samples in question, the fact that the corresponding blood/plasma/serum samples were negative for a detectable concentration of methamphetamine, the fact that methamphetamine is recognized as a substance of human use and addiction and potentially can be found in a horse due to its close association with humans as an inadvertent contaminant. Additionally, no evidence was found indicating that the drug was intentionally or inadvertently administered by any of the trainers in question or their employees. Given these circumstances the Texas Racing Commission ruled that the presence of methamphetamine in the samples was sufficient cause to disqualify the horses in the races in question but that the mitigating circumstances warranted no further penalty for either the Owners or Trainers.

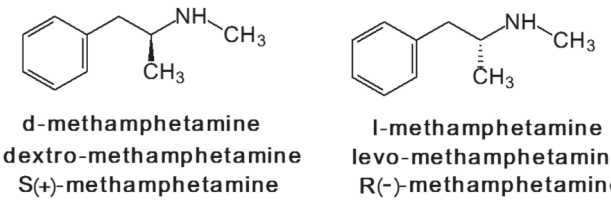

Fig. 1 Methamphetamine (N-methyl-1-phenylpropan-2-amine) exists as two mirror image enantiomers, d-methamphetamine and l-methamphetamine. d-Methamphetamine, the more pharmacologically active enantiomer, is a US DEA Schedule II controlled substance available in the US as the prescription medication Desoxyn®. l-Methamphetamine is less pharmacologically active and available in the US as a number of Over-The Counter (OTC) preparations such as Vicks Vapoinhaler® and NeilMed Sinu Inhaler®. | *Methamphetamin (N-Methyl-1-phenylpropan-2-amin) existiert als zwei spiegelbildliche Enantiomere, d-Methamphetamin und l-Methamphetamin. d-Methamphetamin, das pharmakologisch aktivere Enantiomer, ist eine kontrollierte Substanz der US-amerikanischen DEA Schedule II, die in den USA als verschreibungspflichtiges Medikament Desoxyn® erhältlich ist. l-Methamphetamin ist pharmakologisch weniger aktiv und in den USA als eine Reihe rezeptfreier Präparate (OTC) wie Vicks Vapoinhaler® und NeilMed Sinu Inhaler® erhältlich.*

These inadvertent methamphetamine transfer events are consistent with a similar sequence of events that occurred at Canterbury Park in Minnesota [3]. Two methamphetamine "positives" were reported in different horses for the same trainer, one horse in 2014 and a second in 2017. This second methamphetamine "positive" was for the pharmacologically insignificant concentration of 126 picograms/ml of blood/serum/plasma, a violation of the then-in-place Minnesota Racing Commission's "zero tolerance" policy. Other Canterbury trainers have also had horses test methamphetamine positive in 2015 and 2017 respectively, consistent with the number of US racing methamphetamine *"positives"* presented in Figure 2.

With regard to how these Canterbury Park inadvertent environmental transfer events may have occurred, one prominent regulatory veterinarian was cited in the *Lyden* Fox News report as saying that *"I think it is probably an incidental transfer from a human substance abuser likely through contact with the human hands to the horse's mucus membranes"* [3]. Consistent with this suggested mechanism of methamphetamine contamination of a racing horse, shortly after the Canterbury Park 2014 methamphetamine identification, the local Shakopee Minnesota police arrested two members of the Canterbury Park starting gate crew, both of whom were found to be in possession of methamphetamine, and the Fox 9 investigators also reported that they *"discovered at least five other horse handlers at Canterbury busted for drugs during this period"* [3]. Review of the history of methamphetamine detections in US Racing shows that while these identifications are sporadic (Figure 2), they are also ongoing [4]. This ongoing pattern of

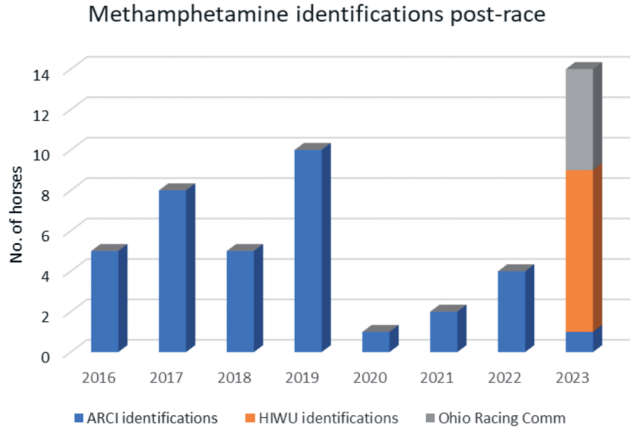

Fig. 2 Methamphetamine identifications reported in US racing, 2016 to October 2023, as per the Association of Racing Commissioners International (ARCI). Methamphetamine identifications reported since May 22nd, 2023, as per the Horse Racing Integrity and Welfare Unit (HIWU) are represented by the orange section under 2023 and detailed in Table 1. Ohio State Racing Commission identifications are identified by the gray section under 2023. | *Gemeldete Methamphetamin-Identifizierungen im US-Rennsport von 2016 bis Oktober 2023, gemäß der Association of Racing Commissioners International (ARCI). Methamphetamin-Identifizierungen, die seit dem 22. Mai 2023 von der Horse Racing Integrity and Welfare Unit (HIWU) gemeldet wurden, sind im orangefarbenen Abschnitt unter 2023 dargestellt und in Tabelle 1 aufgeführt. Identifizierungen der Ohio State Racing Commission sind im grauen Abschnitt unter 2023 gekennzeichnet.*

methamphetamine identifications is consistent with the associated horsemen being unaware of the source (s) of these identifications and therefore not being in a position to proactively prevent these identifications.

The likelihood of these methamphetamine identifications being associated with recreational use of methamphetamine by individuals working with or around these horses is supported by the number of racetrack workers identified as being linked to methamphetamine use in the Association of Racing Commissioners International (ARCI) methamphetamine records [4]. As shown in Figure 3, recording of the number of racetrack workers per year in the ARCI records with methamphetamine charges commenced in 2016 and increased in numbers to the high forties in 2019, the same year in which the number of equine methamphetamine identifications peaked prior to 2022, and after which year both the number of equine methamphetamine identifications and the number of racetrack workers with methamphetamine charges declined. These post-2019 declining numbers for equine methamphetamine identifications and racetrack workers with methamphetamine charges are presumably based on both increased industry awareness of human methamphetamine use and its potential to give rise to inadvertent transfer of trace level amounts of environmental methamphetamine to racing horses.

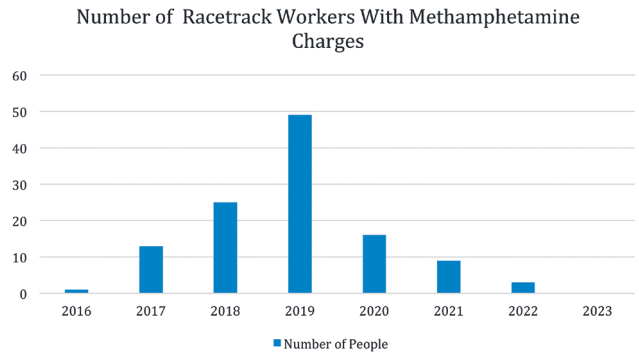

Fig. 3 Number of racetrack workers per year recorded as having methamphetamine related charges per year in Association of Racing Commissioners International (ARCI) methamphetamine records.[4] | *Anzahl der Rennstreckenarbeiter pro Jahr, bei denen in den Methamphetamin-Aufzeichnungen der Association of Racing Commissioners International (ARCI) pro Jahr Anklagen im Zusammenhang mit Methamphetamin gemeldet wurden.* [4]

Other evidence of methamphetamine being a substance of environmental concern in horse racing comes from data developed by the West Virginia Racing Commission who in or about 2017 swabbed the ship-in stalls at the Charles Town Racetrack based on concerns that the stalls might be contaminated with Naproxen and therefore causing environmental Naproxen identifications. A total of 21 ship-in stalls were swabbed yielding identifications of no fewer than 25 substances of regulatory concern, 5 being identifications of human recreational substances. Among the human recreational substances, cocaine/BenZoylEcgonine (BZE) led the list with 10 total identifications, with 1 identification of methamphetamine and 2 identifications of amphetamine, the expected human urinary metabolite of methamphetamine, as set forth in Figure 4 [5] .

Consistent with the ongoing pattern of these identifications, there has been increasing regulatory understanding of the circumstances driving these identifications and starting in about 2020 there has been an increasing tendency for regulatory authorities to identify mitigating circumstances, namely random environmental exposure, as factors in rulings on these low concentration methamphetamine identifications, and where appropriate to evaluate and treat these identifications as random and pharmacologically insignificant events occurring largely outside of the control of the horsepersons involved.

Regulation of methamphetamine under the horseracing integrity and safety authorit (HISA) and the horseracing integrity and welfare unit (HIWU)

More recently, however, since May 22nd, 2023, when medication regulation in Thoroughbred horse racing in most US states came under the control of the *Horseracing Integrity and Safety Authority* (HISA) [6] and its enforcement arm, the Horseracing Integrity and Welfare Unit (HIWU), there has been a significant increase in the frequency with which methamphetamine identifications/"positives" are being called, as set forth in Figure 2 and Tables 1 and 2. As detailed in Table 1, between May 23rd and October 7th, 2023, there have been a total of eight reported identifications of methamphetamine, starting with three identifications involving the same trainer at Prairie Meadows, Iowa. The penalties involved in these

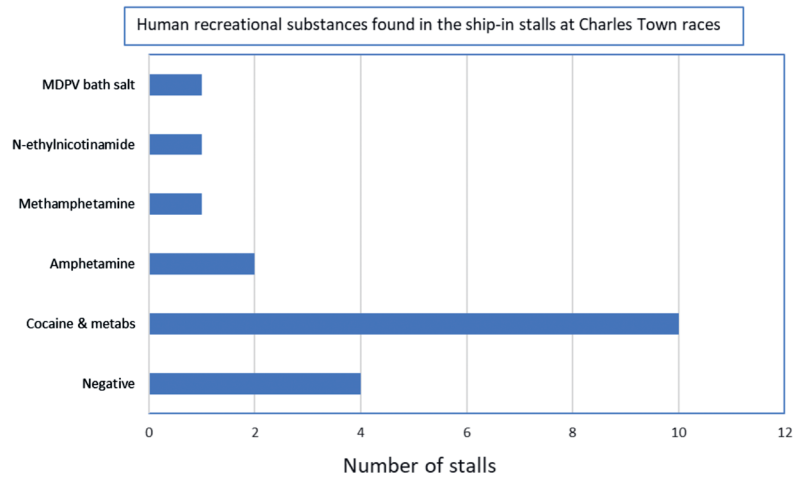

Fig. 4 Listing of the human recreational substances identified in equine ship-in stalls at the Charles Town racecourse, where 79% of the stalls were found to contain substances of regulatory interest. MDPV is 3,4-methylenedioxypyrovalerone; N-ethylnicotinamide is a metabolite of the stimulant nikethamide; cocaine & metabolites include benzoylecgonine. | *Auflistung der Substanzen für den menschlichen Freizeitgebrauch, die in Pferdeställen auf der Pferderennbahn Charles Town identifiziert wurden, wo festgestellt wurde, dass 79% der Pferdeboxen Substanzen von regulatorischem Interesse enthielten. MDPV ist 3,4-Methylendioxypyrovaleron; N-Ethylnicotinamid ist ein Metabolit des Stimulans Nikethamid; zu Kokain und seinen Metaboliten gehört Benzoylecgonin.*

Table 1 HIWU reported methamphetamine identifications in US Thoroughbred racing, May 22nd-December 15th, 2023, showing date of the race, name of the trainer, name of the horse, claimed violation, substance in question, racetrack, testing matrix, claimed concentration, type of race and, where available, resultant regulatory action and trainer response, if any. DQ = DisQualification; Meth = methamphetamine; Presence = Presence of a banned substance and/or its metabolites or markers; PS = Provisional Suspension; PS-lifted = Provisional Suspension lifted based on ADMC Program Rule submissions to FTC & case stayed pending the FTC's approval of the new Rules. | *HIWU gemeldete Methamphetamin-Identifizierungen bei Vollblutrennen in den USA, 22. Mai, 15. Dezember 2023, mit Angabe des Datums des Rennens, des Namens des Trainers, des Namens des Pferdes, des behaupteten Verstoßes, der fraglichen Substanz, der Rennstrecke, der Testmatrix und der angeblichen Konzentration, Art des Rennens und, sofern verfügbar, daraus resultierende behördliche Maßnahmen und Reaktion des Trainers, falls vorhanden. DQ = DisQualifikation; Meth = Methamphetamin; Presence = Vorhandensein einer verbotenen Substanz und/oder ihrer Metaboliten oder Marker; PS = Vorläufige Aussetzung; PS-aufgehoben = Die vorläufige Aussetzung wurde auf der Grundlage der Einreichungen der ADMC-Programmregeln bei der FTC aufgehoben und der Fall wurde bis zur Genehmigung der neuen Regeln durch die FTC ausgesetzt.*

Date	Trainer	Horse	Offense	Substance	Location	Concentration	Subsequent activity	Penalty	Trainer plea
6/19/2023	Dick Clark	Colonel Kink	Presence of a banned substance and/or its metabolites or markers	Methampheta-mine	Prairie Meadows, Altoona Iowa	n/a	Raced 3 times after that (7/3, 7/9, 7/22)	Provisional suspension 7/20/23, then 18-month suspension, DQ, Fine $12,500	Admission of EAD violation and acceptance of consequences
6/19/2023	Dick Clark	My Heart's On Fire	Presence of a banned substance and/or its metabolites or markers	Methampheta-mine	Prairie Meadows, Altoona Iowa	n/a	Won maiden special weight	18-month suspension, DQ, fine $12,500	Admission of EAD violation and acceptance of consequences
7/22/2023	Dick Clark	Kissed a Cadet	Presence of a banned substance and/or its metabolites or markers	Methampheta-mine	Prairie Meadows, Altoona Iowa	n/a	1st maiden special weight	18-month suspension, DQ, fine $12,500	Admission of ECM violation and acceptance of consequences
7/30/2023	Hector Palma	Baladi	Presence of a banned substance and/or its metabolites or markers	Methampheta-mine	Del Mar, California	n/a	Claiming	n/a	n/a
5/29/2023	John Pimental	Golovkin	Presence of a banned substance and/or its metabolites or markers	Methampheta-mine	Monmouth, New Jersey	n/a	Last in claiming race, claim voided	n/a	n/a
7/7/2023	Ramon Rechy	Night Livin	Presence of a banned substance and/or its metabolites or markers	Methampheta-mine	Horseshoe, Indianapolis	n/a	Won claiming race	n/a	n/a
7/20/2023	Randy Preston	Fly Home	Presence of a banned substance and/or its metabolites or markers	Methampheta-mine	Belterra Park, Ohio	824 pg/ml blood d-Methamphetamine	1st maiden claiming	n/a	n/a
10/7/2023	Jimmy Corrigan	Stay Lost	Presence of a banned substance and/or its metabolites or markers	Methampheta-mine	Belterra Park, Ohio	143 pg/ml plasma serum	n/a	n/a	n/a

HISA/HIWU Iowa methamphetamine identifications are also considerably more severe than those utilized prior to the new HISA/HIWU regulation, for example 18-month suspensions and a US$12,500.00 fine in each of the three Iowa identifications presented in Table 1, sharply differ from pre-HISA/HIWU regulatory approaches to trace level methamphetamine identifications.

Commercially available d- and l-methamphetamine products

Methamphetamine, Figure 1, is a member of the amphetamine group of sympathomimetic amines. Chemically, methamphetamine exists as two mirror image enantiomers, d- and l-methamphetamine [7]. D-methamphetamine is the more pharmacologically active enantiomer, being a potent central nervous system stimulant, producing euphoria, increased energy and alertness and improved self-esteem in humans. In the US, d-methamphetamine is a DEA Schedule II stimulant under the Controlled Substances Act, with just one legal methamphetamine product, the human prescription medication Desoxyn® approved in the US for use in obesity and Attention Deficit Hyperactivity Disorder (ADHD) [8]. The l-isomer, l-methamphetamine is considered to be less pharmacologically active, acting primarily as a sympathomimetic vasoconstrictor and is available in a number of OTC nasal decongestant inhalers in the US [9]. Additionally, methamphetamine is also synthesized in clandestine laboratories and available and used worldwide as a recreational substance, including in the United States [10]. The enantiomer ratios in these clandestinely synthesized products are uncertain and depend on the starting materials and synthetic methodologies used by the clandestine laboratories in question. Synthesis from ephedrine or pseudoephedrine results in a relatively pure d-methamphetamine, whereas the alternative synthesis from phenyl-2-propanone results in a racemic mixture of d- and l-methamphetamine [11].

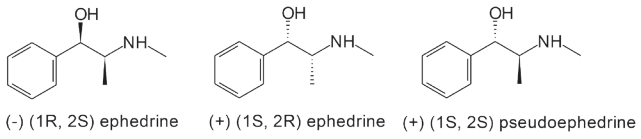

Fig. 5 Ephedrine stereoisomers that are used as methamphetamine precursors. (-)1R, 2S-ephedrine (left) is marketed as pharmaceutical grade ephedrine; (+)1S, 2R-ephedrine (middle) constitutes its mirror image as would be found in racemic ephedrine. A different ephedrine stereoisomer, (+)1S, 2S-pseudoephedrine (right), is the active component of decongestants. | *Ephedrin-Stereoisomere, die als Methamphetamin-Vorläufer verwendet werden. (-)1R,2S-Ephedrin (links) wird als Ephedrin in pharmazeutischer Qualität vermarktet; (+)1S, 2R-Ephedrin (Mitte) stellt sein Spiegelbild dar, wie es im razemischen Ephedrin zu finden wäre. Ein anderes Ephedrin-Stereoisomer, (+)1S,2S-Pseudoephedrin (rechts), ist der aktive Bestandteil von abschwellenden Mitteln.*

The pharmacological and regulatory differences between these d- and l- enantiomers of methamphetamine have been recognized in US racing where d-methamphetamine is an Association of Racing Commissioners International Drug Class 1, Penalty class A substance [12]. with the notation that "*recommended Penalty B if testing can prove the presence of only levo-methamphetamine in the sample*", reflecting the lesser pharmacological efficacy and Over The Counter availability of l-methamphetamine. However, review of the HISA banned substances list [13] shows that HISA does not specifically distinguish between d- and l-methamphetamine. Under the heading "*SUBSTANCE*" HISA lists just "*Methamphetamine*" noting that its "*ACTION*" is "*Stimulant*" and under "*COMMERCIAL/DEVELOPMENTAL NAME(S) where available*" listing "Desoxyn DEA Schedule II". The absence of l-methamphetamine and its various OTC commercial products from the HISA banned substances list is somewhat unusual.

More recently, on or about October 23rd, 2023, HIWU created a subcategory for human-abuse drugs including cocaine, methamphetamine, MDMA (3,4-methylenedioxy-methamphetamine and THC (delta-9-tetrahydrocannabinol) [14]. Under this new rule, public disclosure of a "positive" test will not result in a suspension until a second test (if this second

Table 2 Methamphetamine identifications in Harness Racing reported by the Ohio Department of Agriculture Analytical Toxicology Laboratory during 2022–2023 showing where available the date of the race, name of the trainer, name of the horse, location, claimed violation, substance in question, racetrack, testing matrix, claimed concentration, type of race and where available, resultant regulatory action and trainer response, if any. | *Vom Ohio Department of Agriculture Analytical Toxicology Laboratory im Zeitraum 2022–2023 gemeldete Methamphetamin-Identifizierungen bei Trabrennen mit Angabe des Datums des Rennens, des Namens des Trainers, des Namens des Pferdes, des Ortes, des behaupteten Verstoßes und der fraglichen Substanz, sofern verfügbar. Rennstrecke, Testmatrix, beanspruchte Konzentration, Art des Rennens und, sofern verfügbar, daraus resultierende behördliche Maßnahmen und Reaktion des Trainers, falls vorhanden.*

Date	Trainer	Horse	Breed	Location	Lab	Matrix	Concentration
3/19/23	Brewer	Ilovemywoody	Standardbred	Miami Valley Raceway	Ohio Department of Agriculture Analytical Toxicology Laboratory	Plasma	253 pg/ml
11/26/22	Hagerman	Dashintothe-beach	Standardbred	Northfield Park	Ohio Department of Agriculture Analytical Toxicology Laboratory	Blood	130 pg/ml
	McGinnis	HP Maestro	Standardbred		Ohio Department of Agriculture Analytical Toxicology Laboratory	Blood	113 pg/ml
11/8/22	Rhoades	Sheswildnfree		Northfield Park	Ohio Department of Agriculture Analytical Toxicology Laboratory	Blood	645 pg/ml
	Sharp				Kenneth L. Maddy Analytical Chemistry Laboratory/split sample	Blood	30 pg/ml

test is requested within seven days of the original identification) confirms the presence of the detected substance. HIWU states that this grace period, usually about three weeks, will (in the opinion of HIWU) allow the trainer to investigate the source of the prohibited substance and provide an explanation to HIWU. HIWU, however, apparently will NOT assist the trainer in his or her investigations (our emphasis on NOT). In this regard we specifically note the critical role of the Ontario Racing Commission (ORC) drug testing personnel in identifying the unusual origins of the "cluster" of methamphetamine identifications described by *Brewer* et al. 2106[1], and the central role that their high sensitivity analysis of samples taken from the suspected horse trailer played in the identification of the horse trailer source of the trace level urinary methamphetamine identifications involved in this Ontario Racing Commission matter.

Clandestine (street) synthesis of methamphetamine

Clandestine laboratories synthesizing methamphetamine are reported as largely using the synthetic method of Akira Ogata, who in 1919 first synthesized methamphetamine by combining ephedrine, iodine and red phosphorus[15] .

The starting material for this methamphetamine synthesis is ephedrine, typically l-1R,2S-ephedrine (Fig. 5) from cold medications and by the early 1990s[16] was able to report that the "*hydroidic acid-red phosphorus*" method was the most common clandestine synthesis route to methamphetamine, used in clandestine laboratories since the early 1980s. The reaction scheme is shown in Fig. 6 as the "Red Phosphorus" method, and *Skinner*[16] reported that reduction of l-ephedrine or d-pseudoephedrine resulted in formation of d-methamphetamine.

Optimal reaction yields in established laboratory settings are as high as 92%, while clandestine synthetic yields are likely to be in the 50–75% range. Hypophosphorus acid apparently also works well in place of red phosphorus, at least in smaller scale syntheses[17] .

Regulatory responses to clandestine synthesis of methamphetamine

Responding to these clandestine synthesis of methamphetamine events, the US Congress passed the "Combat Methamphetamine Epidemic Act in 2005", wherein the most important provision involved restrictions on the availability of pseudo-

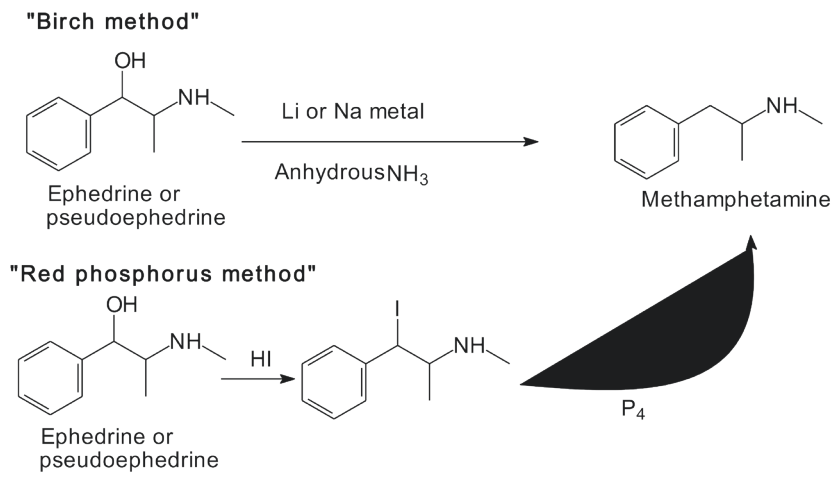

Fig. 6 Common routes to methamphetamine starting from ephedrine or pseudoephedrine. The "Birch method" is a chemical reduction method relying on reaction with ammonia in the presence of alkali metal catalysts. The "Red phosphorus method" requires hydrogen iodide and the phosphorus allotrope known as red phosphorus. The Birch method has been common throughout the American Midwest, whereas the red phosphorus method has been more frequently used in Mexican laboratories for mass production. | *Gängige Wege zu Methamphetamin ausgehend von Ephedrin oder Pseudoephedrin. Die „Birch-Methode" ist eine chemische Reduktionsmethode, die auf der Reaktion mit Ammoniak in Gegenwart von Alkalimetallkatalysatoren beruht. Die „Rote-Phosphor-Methode" erfordert Jodwasserstoff und das als roter Phosphor bekannte Phosphorallotrop. Die Birch-Methode ist im gesamten Mittleren Westen der USA verbreitet, wohingegen die Methode mit rotem Phosphor in mexikanischen Labors häufiger für die Massenproduktion eingesetzt wird.*

Table 3 Synthetic methods and their methamphetamine stereoisomer production. The first three methods have been used in clandestine laboratories, whereas the bottom two methods are considered too challenging for significant clandestine laboratory synthesis. | *Synthesemethoden und ihre Herstellung von Methamphetamin-Stereoisomeren. Die ersten drei Methoden wurden in geheimen Laboren verwendet, während die beiden unteren Methoden als zu anspruchsvoll für eine umfangreiche Synthese im geheimen Labor gelten.*

Starting compound	Available in	Reaction type	Product	Reference
1-phenyl-2-propanone	Readily synthesized from phenylacetic acid	Leuckart method or Reductive amination	Racemic methamphetamine	[36] *Kunalan* et al., 2009 [37] *Cunningham*, et al., 2010
(+)-pseudoephedrine	OTC decongestants	Birch reduction	S (+)-methamphetamine	[20] *Abbruscato & Trippier*, 2018
(-)-ephedrine	OTC decongestants	Birch reduction	S (+)-methamphetamine	[20] *Abbruscato & Trippier*, 2018
(-)-norephedrine	OTC decongestants and appetite suppressants	Hydrogenation of carbodiimide product	R (-)-methamphetamine	[38] *Hazama* et al., 2008
D-phenylalanine	Dietary supplements	LiAlH4 reduction & benzyl chloroformate reaction	S (+)-methamphetamine	[39] *Repke* et al., 1978

ephedrine and other key synthetic components [18]. The Mexican authorities followed suit, but Mexican laboratories have largely turned to alternative synthetic routes starting from phenyl-2-propanone. Figure 7 shows two routes from phenyl-2-propanone (phenylacetone) to methamphetamine by either reductive amination with methylamine or the Leuckart reaction. These latter approaches are capable of high production yields but produce racemic methamphetamine (dl) [19, 20].

These synthetic procedures and the methamphetamine enantiomer status of the synthetic product are summarized in Table 3. Simply put, the Leuckart method starting with 1-phenyl-2-propanone yields racemic methamphetamine, while the Birch reduction method starting with d-pseudoephedrine or l-ephedrine both yield l-methamphetamine.

Pharmacokinetics and pharmacodynamics of d- and l-methamphetamine

With regard to the comparative pharmacokinetics of the d- and l-forms of methamphetamine, *Mendelson* [19] studied the methamphetamine stereoisomer pharmacokinetics (PK) and found they showed similar PK parameters, and at high doses, l-methamphetamine intoxication is similar to that of d-methamphetamine, but the psychodynamic effects of l-methamphetamine were shorter-lived and less desired by recreational users. The authors concluded that racemic and d-methamphetamine have similar effects and would be expected to have comparable abuse liabilities. More recently [21] reviewed methamphetamine stereoisomeric effects focusing on l-methamphetamine and confirmed that cardiovascular and subjective effects from d-methamphetamine (0.5 mg/kg) were much longer-lasting than those from l-methamphetamine (0.5 mg/kg).

The National Institute of Drug Abuse (NIDA) [18] in 2019 claimed significant decreases of up to 80% of lab incidents owing to successful reduction in the availability of methamphetamine precursors, a claim supported prospectively in the earlier review by *McKetin* et al. [22]. Given that illicit methamphetamine synthesis involves many hazards beyond physiological addiction, with hazards including explosive chemicals such as anhydrous ammonia, drain cleaners, paint thinner, metallic lithium, hydrochloric or sulfuric acids, starter fluid, camping fuel, and others that can damage the respiratory tract, mucous membranes, eyes, and skin [23], legal restrictions designed to reduce the ability to synthesize methamphetamine continue. One approach involves development of new pseudoephedrine compositions that make extraction of the active component difficult [24]. However, *Presley* et al. [25] raise doubts about the efficacy of such formulations. Meanwhile, illicit labs have developed masking agents to make detection of precursors and products more difficult for analytical labs; five commonly employed protecting groups – acetyl, p-tosyl, methoxycarbonyl, Fmoc, and t-Boc – were recently studied by *Mayer* et al. [26] in efforts to assist labs in the recognition of masked compounds.

Regulatory significance of the d- and l-isomers of methamphetamine

The regulatory distinction between d-methamphetamine and l-methamphetamine raises an interesting regulatory/forensic point, namely that identifying only the l-enantiomer is consistent with exposure of the horse to the less active l-enantiomer isomer, as has happened in Kentucky [27]. On the other hand, identification of predominantly d-methamphetamine may be evidence of exposure to either commercially approved synthesized, purified and marketed d-methamphetamine product, or illegal synthesis from ephedrine or pseudoephedrine. Identification of a mixture of approximately equal concentrations of the d- and l-methamphetamine enantiomers may suggest exposure to an illicitly synthesized and not enantiomerically purified mixture of d- and l-methamphetamines, i.e., racemic methamphetamine.

The pharmacokinetics and pharmacodynamics of methamphetamine in horses

To our knowledge there are only two published studies addressing the pharmacokinetics and pharmacodynamics of methamphetamine in horses. In the early seventies, *Ray* and colleagues [28] administered 150 mg of Desoxyn®, namely d-methamphetamine to six horses, one of which was a Thoroughbred. As shown in Figure 8 replotted from *Ray* et al. 1972 [28], plasma concentrations of d-methamphetamine in this Thoroughbred horse peaked rapidly at 1 hour post-administration and then declined to be present at less than

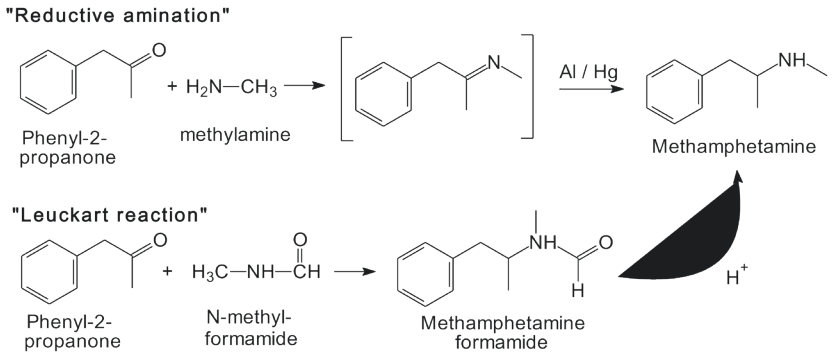

Fig. 7 Synthetic routes to methamphetamine from phenyl-2-propanone, a.k.a. phenylacetone. The "Reductive amination" route combines phenyl-2-propanone and methylamine to create an N-methylimine that undergoes reduction catalyzed by aluminum amalgam. The "Leuckart reaction" relies on nucleophilic attack of the phenyl-2-propanone carbonyl carbon by N-methylformamide to produce a methamphetamine formamide product that is converted by treatment with acid to methamphetamin. | *Synthesewege zu Methamphetamin aus Phenyl-2-propanon, auch bekannt als Phenylaceton. Der Weg der „reduktiven Aminierung" kombiniert Phenyl-2-propanon und Methylamin, um ein N-Methylimin zu erzeugen, das einer durch Aluminiumamalgam katalysierten Reduktion unterliegt. Die „Leuckart-Reaktion" beruht auf einem nukleophilen Angriff des Phenyl-2-propanon-Carbonylkohlenstoffs durch N-Methylformamid, um ein Methamphetamin-Formamid-Produkt zu erzeugen, das durch Behandlung mit Säure in Methamphetamin umgewandelt wird.*

5.7 ng/ml at 8 hours post-administration. We specifically note that this 150 mg/horse d-methamphetamine dose used by *Ray* et al. is an approximately six-fold greater dose than the suggested human daily dose of Desoxyn® and *Ray* et al. [28] did not report any behavioral changes in any of their six horses administered this I/M dose of d-methamphetamine.

Clandestinely synthesized methamphetamine and trace level identifications in horses

Given its relative ease of synthesis and worldwide use as a recreational substance, methamphetamine is a widely illicitly synthesized and marketed substance, as exemplified by the Canadian horse trailer methamphetamine events [1]. In the United States during 2015–2018 an estimated 1.6 million US adults aged 19 and over reported past year methamphetamine use. Of these, 52.9% had a methamphetamine misuse disorder, i.e., US individuals misusing methamphetamine, essentially all of which was the product of illicit synthesis, hereinafter "street" methamphetamine.

This pattern of illicit synthesis and presumably variable chemical presentations and packaging of street methamphetamine adds to the variables influencing the likelihood of inadvertent transfer of trace amounts to horses. In the first place, street methamphetamine is unlikely to be presented to street users in a chemically and mechanically stable pill format. A further consideration is that as an abused substance methamphetamine is likely to be used recreationally by individuals at far higher doses than medically approved doses. While the medically approved human dose of Desoxyn® is 2.5–10 mg daily and not to exceed 60 mg/day, common recreationally abused doses are 100–1,000 mg/day and up to 5,000 mg/day in chronic binge use. These product format and use variables that apply principally to street methamphetamine greatly increase the variability in the amounts of a recreational substance such as methamphetamine that a horse is likely to be inadvertently exposed to, as compared to exposure to amounts of prescribed or over the counter human medications.

High jugular blood concentrations following mucus membrane exposure of horses to methamphetamine

The second and most recent study of the pharmacokinetics and pharmacodynamics of methamphetamine is that of *Knych* et al. 2019 [29]. In this study *Knych* et al. administered d-methamphetamine from Sigma-Aldrich to six exercised Thoroughbred horses. Intravenous administration of 10 mg of methamphetamine produced mean peak post-injection plasma concentrations of 9.90 ng/ml, which plasma concentrations declined following a two-compartment model, rapidly at first and then more slowly to fall below the Limit of Detection (LOD) of 5 picograms/ml between 12- and 18-hours post-administration. This relatively small equine dose of methamphetamine was deliberately selected for this study based on the *"high likelihood that inadvertent exposure (to methamphetamine) would be to lower amounts of the drug"*, in other words an important goal of this *Knych* study was to address the matter of random oral exposure of racing horses to relatively small amounts of methamphetamine.

Addressing this random mucus membrane exposure matter *Knych* et al. [29] administered this same 10 mg dose of methamphetamine *"transmucosally"* meaning that the *"Methamphetamine powder was applied directly to/rubbed onto the oral mucosa by an individual wearing a glove"*. As set forth in Figure 9, peak jugular blood plasma/serum concentrations following transmucosal administration occurred rapidly, between zero to 15 or 30 minutes post-administration and were widely variable, ranging from about 4 ng/ml to an unexpected 88.4 ng/ml, presumably reflecting both the skill of the individual performing the "rubbing" and the cooperativeness of the equine involved. By far the most important take home message from these data is that mucous membrane application of a 10 mg dose of methamphetamine produced jugular vein blood concentrations on average four-fold greater than the peak plasma concentration following intravenous administration and in one horse at least as high as 88,400 picograms/ml, dashed line in figure 9. The words "at least as high" allude to the fact that the 88,400 picograms/ml plasma concentration in Figure 9 replotted from *Knych* et al. [29] presents as an apparently declining plasma concentration of methamphetamine, with the true peak plasma concentration of methamphetamine in this horse likely being higher than the presented 15-minute time point, with the actual peak jugular blood/plasma/serum concentration occurring at some time between 0 and 15 minutes following the oral transmucosal administration procedure.

The reason for these fourfold and higher jugular vein blood concentrations observed after transmucosal oral administration of methamphetamine is that the jugular vein drains the oral cavity and as such is actually delivering the high local

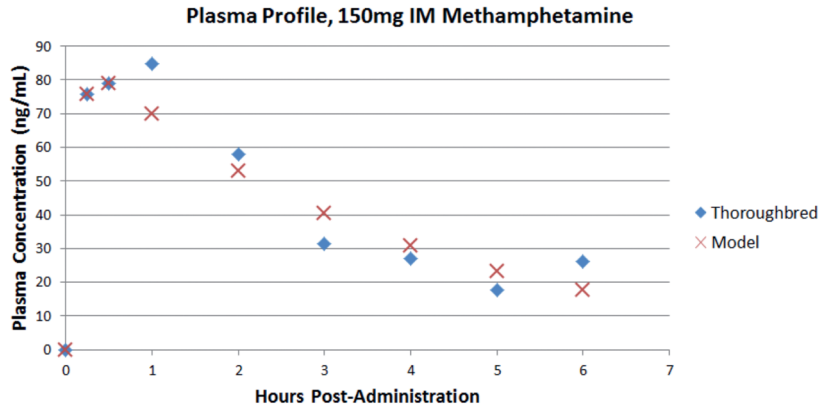

Fig. 8 The blue diamonds show the plasma concentrations of methamphetamine in a Thoroughbred horse administered 150 mg of methamphetamine IM replotted from Ray et al. [28]. The crosses show a first pass best fit kinetic model of these data obtained by back stripping. | *Die blauen Rauten zeigen die Plasmakonzentrationen von Methamphetamin bei einem Vollblutpferd, dem 150 mg Methamphetamin IM verabreicht wurden, neu aufgetragen von Ray et al. [28]. Die Kreuze zeigen ein kinetisches First-Pass-Best-Fit-Modell dieser Daten, das durch Back-Stripping erhalten wurde.*

oral tissue concentration of methamphetamine to the systemic circulation of the horse. This is a factor that must be kept in mind when evaluating the pharmacological or regulatory significance of jugular blood concentrations of a substance that may have entered the horse by oral transmucosal absorption, such as in this case methamphetamine. Review of the *Knych* data [29] from horse #8 shows a declining plasma concentration of 88.4 nanograms/ml at 15 minutes post-oral 10 mg transmucosal administration, suggesting that a 100 microgram oral transcutaneous exposure to methamphetamine could be expected to give rise to a 884 picogram/ml jugular blood plasma identification of methamphetamine, a dose 1,500-fold less than the dose administered by *Ray* and his colleagues and well below any dose expected to produce a pharmacological response in the horse in question and above the range of all of the more recent serum/plasma methamphetamine concentrations as presented in Tables 1 and 2 .

Previously in place or proposed screening limits for methamphetamine

Reviewing the urinary methamphetamine identifications reported in Canadian racing in 2016, *Brewer* et al. [1] proposed an interim 15 nanogram/ml Screening Limit in urine based on the concentrations reported in these Canadian urinary identifications. At about the same time the Oklahoma Horse Racing Commission (OHRC) published a urinary Screening Limit of 100 ng/ml, based on their regulatory experience in Oklahoma [30]. This higher Screening Limit presented by the OHRC is consistent with the fact that methamphetamine has a pKa of 9.8, meaning that it will carry a positive charge and may be expected to trap at high concentrations in acidic pH urines. These chemical characteristics mean that post-administration urinary concentrations of methamphetamine are highly variable as shown by *Ray* et al. [28] where the peak urinary concentrations of methamphetamine ranged from 1,145 ng/ml in one horse to 17,930 in another, approaching a 16-fold range in peak urinary concentrations following administration of the same dose of methamphetamine to non-exercised horses. Even more compelling is the highly variable relationship between the peak plasma and peak urinary concentrations, the urinary concentrations of methamphetamine in one horse being, at 17,930 ng/ml, a 996-fold greater concentration amount than the 18 ng/ml peak plasma concentration observed in that particular horse. Simply put, urinary concentrations of methamphetamine are highly variable, presumably largely driven by the ability of methamphetamine, as a basic medication, to concentrate in acidic urine [31], similar to the 1,000-fold concentrating effect of acidic post-exercise urinary pH on urinary lidocaine concentrations [32] and also consistent with the well-recognized inherent variability of post-race urinary pH values and resultant equine urinary drug concentrations [33,24]. In short, regulatory thresholds and regulatory evaluations involving methamphetamine are best based on plasma data, given the extreme variability in urinary methamphetamine concentrations as presented in the paper of *Ray* et al. [28].

Suggested evaluation process for an equine methamphetamine identification

To correctly evaluate the pharmacological and regulatory significance of a claimed methamphetamine identification in a jugular blood sample from a racing horse, we suggest the following approaches. First, given that the currently in place HISA/HIWU penalties for a trace level methamphetamine identification can be career terminating for a horseperson, it is incumbent on the parties involved to rigorously evaluate all available chemical and other evidence. The evidence evaluated should therefore include quantitative blood and urinary analysis for both methamphetamine isomers. Quantitative analysis of a suitably timed post-event hair sample from the horse in question should be included in a full investigation of any methamphetamine positive to distinguish between in-

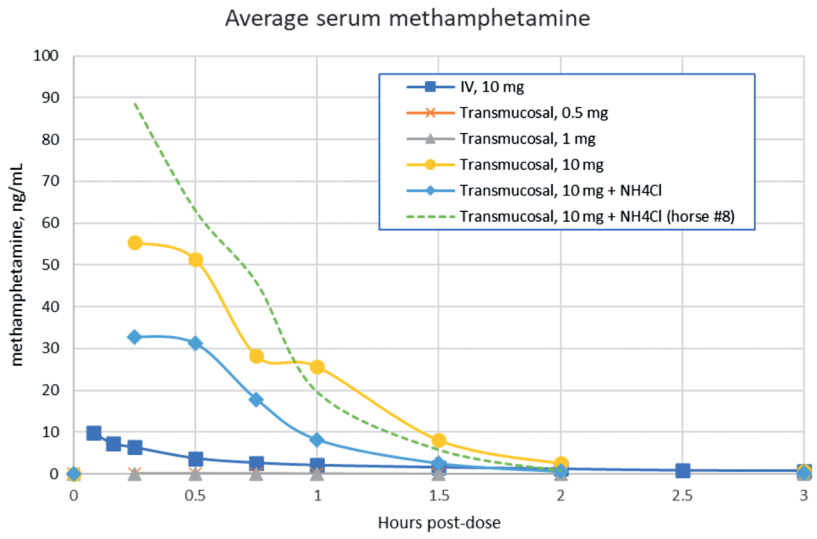

Fig. 9 The blue squares (■) starting at 0.08-hr present mean serum concentrations of methamphetamine following IV administration (10 mg/horse to six horses). Traces for the remaining symbols (×,Δ,●,⧫) present mean serum concentrations following oral transmucosal administration of 0.5, 1, 10 and 10 (NH_4Cl) mg/horse, respectively. The last of these included 165 g ammonium chloride administered via nasogastric tube 10 hr prior to methamphetamine administration. Transmucosal administration results represent the average of two horses, except for those given NH_4Cl, three horses. The one horse in this six-horse experiment with the highest serum methamphetamine values following oral transmucosal administration is shown with the dashed line. Data replotted from Table 2 of *Knych* et al. 2019.[30] | *Die blauen Quadrate (■), beginnend bei 0,08 Stunden, zeigen die mittleren Serumkonzentrationen von Methamphetamin nach intravenöser Verabreichung (10 mg/Pferd bis sechs Pferde). Spuren für die übrigen Symbole (×,Δ,●,⧫) zeigen mittlere Serumkonzentrationen nach oraler transmukosaler Verabreichung von 0,5, 1, 10 bzw. 10 (NH_4Cl) mg/Pferd. Die letzte davon umfasste 165 g Ammoniumchlorid, die 10 Stunden vor der Methamphetaminverabreichung über eine Magensonde verabreicht wurden. Die Ergebnisse der transmukosalen Verabreichung stellen den Durchschnitt von zwei Pferden dar, mit Ausnahme von drei Pferden, denen NH_4Cl verabreicht wurde. Das eine Pferd in diesem Sechs-Pferde-Experiment mit den höchsten Methamphetamin-Serumwerten nach oraler transmukosaler Verabreichung ist mit der gestrichelten Linie dargestellt. Daten aus Tabelle 2 von Knych et al. 2019.*

cidental environmental exposure and intentional administration. [35]

Review of the *Knych* et al. data [29] presented in Figure #9 shows that oral transmucosal exposures to 10 mg of methamphetamine gave rise to a jugular vein blood/plasma/serum methamphetamine reading of 88,400 picograms/ml in one of the six horses used in these experiments. On this basis, exposure to 0.1 mg of methamphetamine may reasonably be expected to give rise to jugular vein methamphetamine identifications of 884 picograms/ml or thereabouts. Given that 0.1 mg of methamphetamine is in the order of 1,500-times less than the 150 mg IM dose used by *Ray* and his colleagues [28] and which dose produced no reported behavioral effects, it is reasonable to assume that transmucosal exposure to sub-milligram amounts of methamphetamine can give rise to readily detectable picogram/ml jugular blood/plasma/serum concentrations of methamphetamine. Based on these data, a simple low nanogram/ml or less identification of methamphetamine in a jugular blood/plasma/serum sample is most likely evidence of nothing more than inadvertent environmental exposure of the horse to a pharmacologically insignificant amount of environmental methamphetamine.

Absence of, or a very low concentration of, methamphetamine in the corresponding urine sample would be evidence that the exposure resulting in the jugular blood sample identification was of relatively recent occurrence, namely within 60 minutes or so of the urine sample collection time, which time frame may be of assistance in identifying the circumstances under which the exposure event occurred. Finally, absence of a detectable concentration of the correct enantiometic forms of methamphetamine in an appropriately timed hair sample from the horse would be evidence that the jugular blood/plasma/serum identification was a transient trace level detection associated with environmental exposure of the horse to methamphetamine and not in any way associated with a deliberate horseperson-related administration to the horse of a pharmacologically significant amount of methamphetamine.

In closing, given the clandestine synthesis and widespread street availability and use of methamphetamine including recreational use by racetrack personnel and its detection in ship-in stalls, simple identification of a jugular blood/plasma/serum sub-nanogram amount of methamphetamine is most likely evidence of innocent and inadvertent exposure of the horse to environmental methamphetamine. If the horse person is at risk of significant penalty for such an unpredictable occurrence, the horseperson should be allowed to pursue all of the above presented scientific approaches to establish that a simple trace level detection in jugular blood/plasma/serum is not *per se* evidence of a knowing and deliberate administration of a pharmacologically significant amount of methamphetamine to the horse or horses in question. Based on the data available to date, identification of less than 1 nanogram/ml in jugular vein plasma serum can be associated with oral exposure of the horse to amounts of methamphetamine in the order of 1,500-fold less than a pharmacologically effective dose. A jugular blood plasma/serum concentration of 1 nanogram/ml of methamphetamine is thus a highly conservative regulatory "cut-off" or Screening Limit of Detection" concentration and, based on the data presented in Figure 9, a jugular blood plasma serum concentration of up 3 nanograms/ml would not be inconsistent with "*incidental transfer from a human substance abuser or a similar inadvertent environmental source.*"

Abbreviations

ADHD	Attention Deficit Hyperactivity Disorder
ARCI	Association of Racing Commissioners International
DEA	Drug Enforcement Administration
DQ	DisQualification.
FEI	Federation Equestre Internationale
HISA	Horseracing Integrity and Safety Authority.
HIWU	Horseracing Integrity and Welfare Unit.
MDMA	3,4-MethyleneDioxyMethAmphetamine
NIDA	National Institute on Drug Abuse
OHRC	Oklahoma Horse Racing Commission
ORC	Ontario Racing Commission
OTC	Over-The-Counter
PK	PharmacoKinetics
TRC	Texas Racing Commission
US	United States

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Author's contributions

TT conceived and directed the project and TT, CKF of the North American Association of Racetrack Veterinarians (NAARV), GAM, Director of the New York Drug Testing and Research Program, RLH of Holland Management Inc., and AMB of Caracas, Venezuela and Dubai, United Arab Emirates reviewed the data interpretation and analysis and approved the proposed regulatory guidelines from an equine practitioner, researcher, and regulatory scientist's perspective. KB and AFL performed the data searching, chemical structure evaluations and statistical analyses and TT coordinated and

edited all drafts of this manuscript with ongoing contributions from all authors and all authors reviewed approved the final manuscript submitted for publication.

Availability of data and materials

The datasets used and/or analyzed during the current study are available in the public domain as referenced in the manuscript or from the corresponding author on reasonable request.

Declarations

Ethics approval and consent to participate are not applicable: As a review of the relevant scientific and regulatory literature, no ethics approval or consent to participate was necessary or required and all the authors have consented to publication of this case report and analysis.

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Erweiterte Zusammenfassung

Spurennachweise von Methamphetamin bei Rennpferden – eine Überprüfung und forensische Analyse

Methamphetamin ist ein zentrales Stimulans und ein zugelassenes Humantherapeutikum, das auch heimlich synthetisiert und weltweit als Freizeitsubstanz vermarktet wird. Konsumenten von heimlich synthetisiertem Methamphetamin können Methamphetamin in weitaus größeren Mengen als den medizinisch zugelassenen Dosierungen handhaben und konsumieren. Angesichts der Tatsache, dass die Schleimhautexposition eines Pferdes gegenüber 10 Milligramm Methamphetamin zu Methamphetaminkonzentrationen im Halsblutplasma/Serum von 88400 Pikogramm/ml geführt hat, ist die unbeabsichtigte Übertragung von Pikogramm/ml Methamphetaminmengen von Freizeitkonsumenten auf Rennpferde ein gut verstandener Prozess. Bei der Auswertung solcher Picogramm/ml-Methamphetamin-Identifikationen ist zunächst zu berücksichtigen, dass Methamphetamin als zwei chemisch unterschiedliche spiegelbildliche Enantiomere vorliegt, nämlich D-Methamphetamin und L-Methamphetamin. D-Methamphetamin ist das pharmakologisch aktivere Enantiomer und wird in den Vereinigten Staaten (USA) als Desoxyn® vermarktet, ein verschreibungspflichtiges Medikament gemäß Schedule II der US Drug Enforcement Administration (DEA). L-Methamphetamin ist pharmakologisch weniger aktiv und wird in den USA in mehreren rezeptfreien (OTC) abschwellenden Inhalatoren für die Nase vermarktet. Eine forensisch korrekte Auswertung der Methamphetamin-Identifikationen in Picogramm/ml Halsblut/Plasma/Serum bei Rennpferden erfordert eine quantitative Auswertung der Blut-, Urin- und Haarkonzentrationen jedes Methamphetamin-Enantiomers sowie der Anwesenheit oder Abwesenheit des erwarteten Amphetamin-Metaboliten. Bei der Beurteilung der regulatorischen Bedeutung einer Konzentration von Methamphetamin im Halsschlagaderblut/-plasma/-serum muss auch die Tatsache berücksichtigt werden, dass die Konzentrationen im Halsschlagaderblut nach oraler Exposition gegenüber Methamphetamin viel höher sein werden als die systemischen Blutkonzentrationen, da die Halsvene die direkte Vene ist Zusammenhang zwischen der lokal hohen Schleimhautkonzentration von Methamphetamin und dem systemischen Kreislauf des Pferdes. Basierend auf veröffentlichten wissenschaftlichen Daten kann die Schleimhautexposition eines Pferdes gegenüber 100 Mikrogramm Methamphetamin, einem sehr konservativen 1/1500 einer möglicherweise pharmakologisch wirksamen Pferdedosis, zu Methamphetamin-Konzentrationen im Halsblut/Plasma/Serum von 884 Pikogramm/ml führen, ein konservativer Richtwert zur Beurteilung der pharmakologischen und forensischen Bedeutung

Schlüsselwörter: Spurennachweis, Methamphetamin, Rennpferd, Doping, Forensik, Analyse

Exhibit

12

Pferdeheilkunde – Equine Medicine 40 (2024) 2 (March/April) 1–6 DOI 10.21836/PEMTobin

Regulatory thresholds for xylazine – review and analysis based on recent pharmacokinetic data

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Summary: Xylazine is an α2-adrenergic receptor agonist and a short acting sedative/analgesic widely used in equine practice since its original approval by the FDA in 1988. Closely related agents include Detomidine, Metdetomidine, Romifidene, Amitraz and Guanabenz. Xylazine is the shortest acting member of this group and is a Racing Medication and Testing Consortium (RMTC) "Controlled Therapeutic Medication" (version 2.2). In 2013 the RMTC interim threshold for xylazine was set at 10 pg/ml plasma with a 48-hour withdrawal and no defined dose. Application of this regulatory threshold in Washington State led rapidly to an apparent therapeutic average of about 75 pg/ml following a 200 mg dose IV at 54 hours prior to post. Based on Toutain's reported Irrelevant Plasma Concentration (IPC) for xylazine [2013] and the very short duration of action of xylazine, an interim 300 pg/ml regulatory threshold for xylazine was proposed. Soon thereafter published pharmacokinetic data for xylazine up to 12 hours post-administration showed that the terminal elimination of xylazine slows markedly from 6 hours post-administration, leading to a flat terminal half-life. The regulatory outcome of this slow terminal elimination curve for xylazine is that it can be detected in plasma for hours to days beyond any pharmacologic effect of the drug. Based on these considerations, the regulatory threshold for xylazine in Washington State was adjusted upwards to 200 pg/ml on an interim basis. Following this adjustment, review of reported plasma concentrations of xylazine in Washington State post-race samples suggests that this 200 pg/ml in plasma adjusted interim regulatory threshold is likely a more appropriate and clinically relevant 48-hour post-administration regulatory threshold for xylazine. This 200 pg/ml plasma regulatory threshold was soon adopted by the RMTC and is currently well supported by published research and practical regulatory experience.

Keywords: Xylazine threshold, RMTC, α2-adrenergic receptor agonist, horse racing regulation

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Introduction

Xylazine, (N-2,6-Dimethylphenyl)-5,6-dihydro-4H-1,3-thiazin-2-amine, $C_{12}H_{16}N_2S$, MW 220.33) is an α2-adrenergic receptor agonist, originally approved by the FDA for use in horses in 1969.[1] It is widely used as a rapid onset and short acting sedative and analgesic in equine practice.[2,3] Xylazine is one of the original members of the α2-adrenergic receptor agonist family used in equine medicine; other members are Detomidine, Metdetomidine, Romifidene, Amitraz and Guanabenz.[4,5] Xylazine has long been used in equine practice for short term sedation and analgesia, alone or in combination with other agents.[3] The manufacturers recommended dose of xylazine is 1 mg/kg intravenously (IV), although it is commonly used at lower doses, particularly when used in combination with other agents. It is generally administered by rapid intravenous injection minutes before the analgesic/sedation response is required.

The rapid onset and brief duration of action of xylazine

Xylazine is a classic rapid onset short acting agent in horses.[6] The head droop response, an easily quantified measurement of sedation/tranquilization, peaks within ten minutes after an intravenous administration of xylazine and the animal returns to clinically normal within 90 minutes.[1,7] The duration of this head droop response and the analgesic hoof withdrawal

response has been evaluated following intravenous administration of xylazine, (1 mg/kg IV), metdetomidine, (0.01 mg/kg IV), romifidene, (0.1 mg/kg IV), detomidine, (0.04 mg/kg IV), clonidine, (0.02 mg/kg IV) and guanabenz, (0.12 mg/kg IV), and revealed that xylazine has the shortest duration of pharmacological action for head droop tranquilization, hoof withdrawal analgesia, and heart rate depression despite being administered at by far the largest dose on a mg/kg basis [Figs 1–3].[4,5] Similar findings on the very short duration of analgesic effect of xylazine have been reported when either electrical stimulation or mechanical pressure were used as the nociceptive stimulus, with a return to baseline by 30 minutes after xylazine administration.[8]

The variable interval responding technique (VIR) is a method of quantifying the effect of a sedative. The VIR measures the blunting of the response to a known stimulus and has been applied to this class of drugs for the purpose of identifying subtle drug effects. Using VIR, detomidine administration was shown to be associated with a residual blunting of the variable interval response at 24 hours after administration, while xylazine administration was associated with no such residual blunting of the variable interval response at 24 hours after administration.[7] These observations confirm the very short duration of the pharmacological response to xylazine, particularly considering the markedly higher dose of xylazine (up to 1 mg/kg, or 450 mg to a typical racehorse), required for pharmacological effect.

The α2-adrenergic receptor agonist class of sedatives also possess other physiologic effects. The administration of xylazine, detomidine or romifidine is associated with a decrease in packed cell volume (PCV, median-20.9%), decrease in total protein (TP, median-5.8%), and an increase of glucose (median +28.8%).[9] In accordance with its very short duration of action, these changes all returned to baseline faster for xylazine that the other α2-adrenergic receptor agonists, within 60 min.

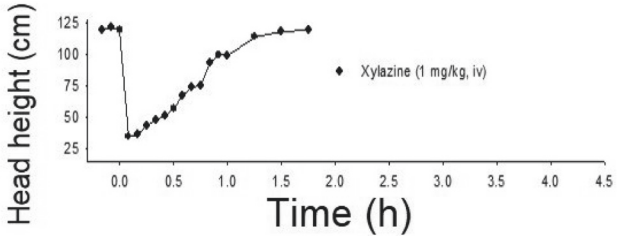

Fig. 1 Head droop following IV injection of xylazine. | *Herabhängen des Kopfes nach intravenöser Injektion von Xylazin.*

The RMTC interim regulatory threshold for xylazine

As a widely used equine therapeutic medication, xylazine has been identified by the American Association of Equine Practitioners (AAEP), the Racing Medication and Testing Consortium (RMTC) and the Association of Racing Commissioners International (ARCI) as a therapeutic medication appropriate for use in horses in training and which the RMTC has included in its list of Controlled Therapeutic Medications with defined regulatory thresholds, associated dosage schedules and withdrawal time guidelines.

In April of 2013, the RMTC interim regulatory threshold for xylazine was 10 pg/mL in plasma with the withdrawal time recommendation of 48 hours. No dose was defined for xylazine in the RMTC guideline, suggesting that the RMTC may not/did not have a well-defined database for this medication.[10] This threshold was presented to the American racing industry as a regulatory threshold and withdrawal time guideline for the clinical use of xylazine in competition horses, and this threshold and the associated withdrawal time guideline were soon thereafter adopted by the Washington State Horse Racing Commission.

Implementation of the xylazine regulatory threshold in Washington State

In Washington State horse racing, the first xylazine positive under this new medication rule was called on July 29th, 2014, when Truesdail Laboratories (Pasadena, CA) reported a plasma/serum xylazine finding in excess of 0.01 ng/ml serum, (estimated as 0.075 ng/mL) and the split sample was quantified at 0.093 ng/mL. Given that these concentrations exceeded the RMTC threshold of 10 pg/mL by more than 7 to 9 times, there was consideration that this violation was likely to have resulted from xylazine administration within the 48-hour RMTC guideline.

On the other hand, the principals responsible for the care of the horse indicated that the horse in question was tranquilized by the veterinarian for routine dental work with 200 mg of xylazine by rapid IV injection at 11:30 AM on Friday, July 18th, 2014 [11] over 52 hours before the horse's scheduled race. The horse was entered in the 4th race the following Sunday, so the administration was outside of the 48-hour withdrawal time suggested by RMTC/ARCI, and the dose administered, 200 mg) less than half the manufacturer's recommended

Table 1 Post-race xylazine identifications in Washington State Horseracing [11] | *Xylazin-Identifizierungen nach dem Rennen bei Pferderennen im US-Bundesstaat Washington* [14]

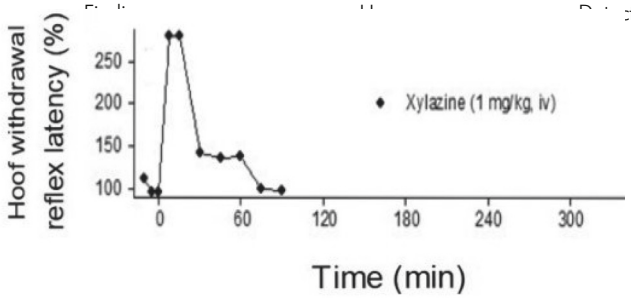

Fig. 2 Hoof withdrawal reflex latency (HWRL) following IV injection of xylazine. | *Latenz des Hufrückzugsreflexes (HWRL) nach intravenöser Injektion von Xylazin.*

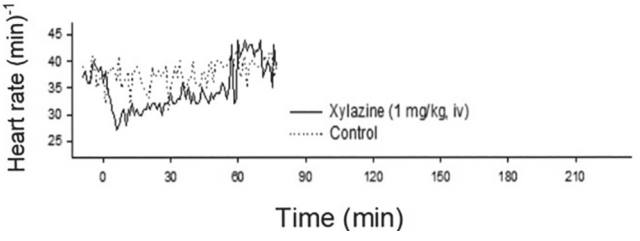

Fig. 3 Heart rates following IV administration of xylazine, showing experimental results as a solid line and control results as dotted line responses. | *Herzfrequenzen nach intravenöser Verabreichung von Xylazin, wobei die experimentellen Ergebnisse als durchgezogene Linie und die Kontrollergebnisse als gepunktete Linien dargestellt sind.*

dose of xylazine. At 3:59 pm on Sunday July 20th the mare ran and won the 4th race at Emerald Downs, and soon thereafter was reported "positive" for xylazine by Truesdail Laboratories.

Review of the RMTC interim regulatory threshold for xylazine

The Irrelevant Plasma Concentration (IPC) can be calculated for therapeutic substances.[12] The IPC is defined as "plasma concentrations that guarantee the absence of any relevant drug effect and for which there will be no regulatory action." Utilizing these calculations, taking into account a conservative 6-hour duration of action for xylazine as can be identified in Figures 1–3, the calculated IPC for xylazine is 304 pg/mL, which was provided to the Washington State Horse Racing Commission as a recommended adjustment to the RMTC xylazine regulatory threshold.

Further, only a few months after Washington State adopted the RMTC threshold for xylazine, pharmacokinetic data beyond the previously published clinical papers were presented at the 19th International Conference of Racing Analysts and Veterinarians (ICRAV) [Figure 4].[13,14] This research investigated the plasma pharmacokinetics of xylazine. Following rapid IV administration of a 400 mg/horse dose of xylazine, Dr. *Noble*'s data show that xylazine plasma concentrations decline following an initial short α phase half-life of less than an hour, reflected by a rapid post-administration decline in plasma concentrations in good agreement with previous publications which limited their investigation of xylazine pharmacokinetics to 4–6 hours post-administration.[2,3] In retrospect, it appears likely that the initial RMTC regulatory threshold for xylazine was based on mathematical extrapolation of the α-phase half-life, assuming initial xylazine plasma concentrations of less than 1,000 ng/ml. If the α-phase half-life, which is observed in the first few hours after administration, is extrapolated to 48 hours, the approximate threshold would be the April 13, 2013 RMTC proposed regulatory threshold of 10 pg/ml.

The actual plasma pharmacokinetics of xylazine are more complicated than this purported RMTC analysis. Similar to other drugs, xylazine follows a three-compartment pharmacokinetic model, wherein it moves into several different compartments and is eliminated from those compartments at different rates, resulting in a sequence of different elimination half-lives, corresponding to its tissue distribution and metabolism. The α, or initial half-life, is the parameter of most interest to the clinician, being most relevant to the duration of the clinically important effect of sedation in the case of xylazine. The terminal half-life is the parameter of most interest to the regulator, being most relevant to the duration of detectable sub-clinical and pharmacologically irrelevant plasma concentrations. Xylazine is administered as a bolus injection, and as a highly lipid soluble medication rapidly accesses and diffuses into well perfused tissues (compartments), such as the Central Nervous System (CNS), producing the rapid onset sedative/head droop, analgesic responses and heart rate depression demonstrated in Figs 1–3, respectively. As xylazine subsequently redistributes out of the CNS and other highly perfused tissues and equilibrates across less well perfused tissues, the plasma concentrations of xylazine decrease. This is consistent with the initial 1–5 hour post-administration rapid decrease of plasma concentrations of xylazine reported in most of the early pharmacokinetic studies. Given the very short duration of the pharmacological actions of xylazine, and the clinical nature of the earlier xylazine pharmacokinetic studies there was little need among those clinical researchers to follow the pharmacokinetics of xylazine for any time beyond the less than 4-hour duration of the well-defined pharmacological and physiological responses to an IV administration of xylazine.

As clinical pharmacology papers detailing the nature and duration of the pharmacological responses of the horse to xylazine, these earlier studies were not designed for determining regulatory thresholds. In order to further elucidate xylazine pharmacokinetics, Dr. *Noble* followed xylazine blood levels of horses out to 12 hours post-administration. Her data showed that after 5 hours post-administration the plasma half-life of xylazine slows markedly, as shown in Fig 4,[13] (reproduced with permission from *Noble* et al., 2016). These data suggest that the RMTC 10 pg/mL regulatory threshold, as extrapolated from the initial α half-life is inappropriate, and that an adjustment to accommodate the longer terminal plasma half-life for xylazine is necessary.

The simplest interpretation of Dr. *Noble*'s data is that by about five hours post-administration xylazine has distributed and equilibrated throughout the body of the horse and that a much slower terminal phase of metabolic clearance then commences. These findings are significantly different than the basic assumption of a one-compartment rapid elimination model and are consistent with the apparently correctly reported xylazine administration history of the first 2014 xylazine positive reported in Washington racing, as presented above.

Of considerable clinical and regulatory significance, Dr. *Noble*'s data[13] also showed that when xylazine was administered in conjunction with Butorphanol, the post five-hour terminal plasma half-life of xylazine dropped to nearly zero. This

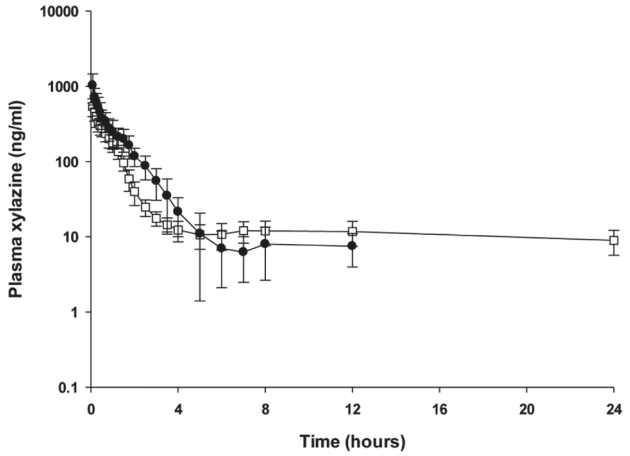

Fig. 4 Mean (± SD) plasma xylazine concentrations for 12 horses administered either 400 mg/horse xylazine I.V. alone (●) or 250 mg/horse xylazine I.V. in combination with 10 mg/horse butorphanol I.V. (□). Reproduced with permission from Noble et al, [2016].[13] | *Mittlere (± SD) Plasma-Xylazin-Konzentrationen für 12 Pferde, denen entweder 400 mg/Pferd Xylazin i.v. verabreicht wurde allein (●) oder 250 mg/Pferd Xylazin i.v. in Kombination mit 10 mg/Pferd Butorphanol I.V. (□). Wiedergabe mit Genehmigung von Noble et al., [2016].*[13]

suggests that the common practice among veterinarians of using combinations of xylazine and Butorphanol will significantly extend detection times for xylazine and possibly also for Butorphanol, if and when these two RMTC Controlled Therapeutic Medications are administered in combination. Further, no data are available on any similar effect when the α2 adrenergic medications are combined, such as the common clinical practice of combining detomidine and xylazine.

Knych et al.[15] published a study designed to enhance understanding of xylazine pharmacokinetics (PK) and pharmacodynamics (PD) in horses post-intravenous administration. Serum concentrations were best described by a 3-compartment PK model, differing from previous 2-compartment models, with a prolonged elimination half-life of 2.79 ± 0.105 hours, in contrast to 49.5 and 47 minutes in earlier studies. The study suggested discrepancies arose from varied sample collection protocols and assay sensitivity. Assessing xylazine's applicability under ARCI regulatory guidelines, the study challenged the 0.01 ng/mL threshold at 48 hours, proposing an extension to the withdrawal time or revising the threshold. Urine analysis proved ineffective but detecting the metabolite 4-OH xylazine in urine for up to 96 hours suggested an alternative regulatory approach. Additionally, the study explored xylazine's pharmacokinetics at a low dose, noting comparable sedative effects to higher doses and short-lived physiological impacts. The findings indicated a need for cautious xylazine use in racehorses, recommending extended withdrawal times.

More recently, *Habershon-Butcher* et al.[16] completed a study aimed at establishing pharmacokinetics (PK) of Xylazine in equine in urine, vital for regulatory control in racing Thoroughbreds, and complementing existing plasma PK research. These authors proposed a screening limit for xylazine in plasma at 50 pg/ml, determined by a calculated *in vivo* IPC, offering a detection time (DT) of 71 hours. Urine concentrations of parent xylazine were deemed unsuitable for regulatory monitoring, but its major urinary metabolite/regulatory analyte, 4-OH-xylazine, was considered a useful regulatory analyte. These authors suggested a screening limit for 4-OH-xylazine in urine of 10 ng/ml, further supporting regulatory control of xylazine administration. Considering the agreed DT of 72 hours by European Horserace Scientific Liaison Committee (scheduled for implementation in June 2019), a conservative withdrawal time (WT) estimate of 144 hours was recommended. This WT estimate significantly surpassed the current suggested 48-hour WT in the United States, suggesting conservative use of xylazine in European racing Thoroughbreds.

In 2020 the RMTC published a monograph on Xylazine in which they reported their own study of the administration of Xylazine hydrochloride as AnaSed™ in a single 200 mg intravenous dose to 16 exercise-conditioned Thoroughbred mares and geldings. Results in serum were determined to best fit a three-compartment model of elimination. Xylazine concentrations were found to remain above the Limit of Quantitation (10 pg/mL) in the majority of horses at 48 hours and were still quantifiable in one horse at 96 hours. Mean concentrations were 30 pg/mL at 48 hours and 10 pg/mL 72 hours post-administration. As a result, the RMTC Scientific Advisory Committee rounded up the 95/95 Tolerance Interval calculated value of 115 pg/mL and recommended a regulatory threshold of 200 pg/mL of serum or plasma of xylazine and withdrawal guidance of 48 hours for a single 200 mg intravenous dose.

Review of these findings by the Washington State Horse Racing Commission and adjustment of the regulatory threshold for xylazine

The analyses of the published Toutain IPC data and the critically important Noble data on the unusual 5-hour post-administration plasma pharmacokinetics of xylazine were communicated to the Washington Horse Racing Commission through Mrs. MaryAnn O'Connell of the Washington Horsemen's Benevolent and Protective Association. Soon thereafter, following careful review of these data by Commissioner Dr. Macomber and his fellow Washington Horse Racing Commissioners, the regulatory threshold for xylazine in Washington State was raised from 10 pg/ml to 200 pg/ml, a 20-fold upward adjustment of the plasma regulatory threshold for xylazine.[10]

This adjusted regulatory threshold was a clinically and forensically more appropriate threshold than the original RMTC 10 pg/mL threshold, an interpretation which has been confirmed by subsequent field experience and published research. Since the 200 pg/mL threshold was introduced in Washington

Table 1 Post-race xylazine identifications in Washington State Horseracing.[14] | *Xylazin-Identifizierungen nach dem Rennen bei Pferderennen im US-Bundesstaat Washington.*[14]

Finding	Horse	Detected amount	Washington State threshold	ARCI recommended regulatory threshold
xylazine	Double Shuffle	.099 ng/ml	0.2 ng/ml	.01 ng/ml
xylazine	Rhythm In May	.150 ng/ml	0.2 ng/ml	.01 ng/ml
xylazine	Alexa Alexa	0.05 ng/ml	0.2 ng/ml	.01 ng/ml
xylazine	Alota Action	0.05 ng/ml	0.2 ng/ml	.01 ng/ml
xylazine	Rainer Ice	.045 ng/ml	0.2 ng/ml	.01 ng/ml
xylazine	Among the Stars	0.14 ng/ml	0.2 ng/ml	.01 ng/ml
xylazine	Coastal Diva	0.14 ng/ml	0.2 ng/ml	.01 ng/ml
xylazine	Coolington	.045 ng/ml	0.2 ng/ml	.01 ng/ml

State there have been a significant number of reports from Truesdail Laboratories of xylazine identifications above 10 pg/mL but below 200 pg/mL, i.e., in the same range as the original xylazine identification (Table 1) fully consistent with the original xylazine identification reported in Washington State being the result of the reported administration at 54 hours prior to post.[11]

"Phase in" period for new regulatory thresholds

This sequence of events and the field data of Table 1 referenced above underscore the need to either perform population studies, as recommended by *Toutain & Lassourd*[12] or to carefully "phase-in" any newly introduced regulatory threshold, regardless of the perceived validity of the science supporting the threshold. Prior to development of the RMTC Controlled Therapeutic Medication program, regulatory thresholds and withdrawal time guidelines in the United States were worked out largely on a jurisdiction-by-jurisdiction basis by a dialogue between the analysts/regulators and the regulated, in other words by essentially the process described in this communication. No matter how well a 6–20 horse withdrawal time guideline study is designed and implemented, it is simply a research based proposed regulatory threshold. The real test of any proposed regulatory threshold is its application to the entire population of those regulated. Without actual real world population studies encompassing hundreds of horses, the details associated with the extrapolation of data generated in a research laboratory must be determined by test application in actual racing. In this case, the initial RMTC calculated 2013 regulatory threshold for xylazine failed to identify and characterize its longer terminal half-life or the unexpected interaction between butorphanol and xylazine reported in 2016 by *Noble*.[13] These issues which would have been elucidated during phase in or by a population study are examples of unanticipated medication elimination patterns. Such interactions are most likely to become apparent soon after the introduction of a regulatory threshold based on individual pharmacokinetic/elimination studies of these agents, emphasizing the need for a careful phase-in period for any new regulatory threshold.[17] Similarly, studies carried out in Thoroughbred horses may not apply to Standardbred horses, possibly based on subtle physiological differences between Thoroughbred and Standardbred horses, as has previously been demonstrated.[18]

The revised RMTC threshold for xylazine

The original April 2013 xylazine threshold was proposed after a meeting in January 2012 of the members of the RMTC Scientific Advisory Committee and other members of the scientific community. No publication was provided or referenced to serve as the basis for the threshold, other than its previous adoption in Pennsylvania. However, it was listed on the RMTC website from April 2013 until February 2016, when the threshold was revised to 200 pg/mL. The April 13, 2013 revised threshold was proposed after a February 23, 2016 meeting of the RMTC, although no additional scientific data have been produced in peer-reviewed or equivalent formats.

Conclusions

In closing, review of recently available pharmacokinetic data on xylazine shows that the terminal plasma half-life of xylazine is considerably longer than was understood prior to 2014. Given this circumstance, this pharmacokinetic reality strongly suggests that the original 2013 RMTC threshold for xylazine was inappropriate. Reviewing these pharmacokinetic realities, the Washington State Horse Racing Commission elected to adjust its plasma threshold for xylazine upwards to 200 pg/mL in plasma. Additionally, field experience to date in Washington State suggests that this 200 pg/mL in plasma is a more scientifically and forensically appropriate xylazine plasma threshold than the previously in-place 10 pg/mL threshold, fully consistent with the subsequent adoption of this 200 pg/ml plasma threshold for xylazine by the RMTC.

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Disclosure of interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Animal ethics statement

The authors confirm that the ethical policies of this journal have been adhered to, and the appropriate ethical review committee approval has been received. Animals used in these experiments were managed in accordance with the rules and regulations of the University of Kentucky Institutional Animal Care Use Committee (IACUC) which also approved the experimental protocol, assigned IACUC number 00137A2000 under the title "Drug Test Development and Validation". The

authors confirm that they adhered to the IACUC-approved protocol.

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Erweiterte Zusammenfassung

Regulatorische Grenzwerte für Xylazin; Überprüfung und Analyse auf der Grundlage aktueller pharmakokinetischer Daten

Xylazin ist ein α2-adrenerger Rezeptoragonist und ein kurzwirksames Sedativum/Analgetikum, das seit seiner ursprünglichen Zulassung durch die FDA im Jahr 1988 in der Pferdepraxis weit verbreitet ist. Zu den eng verwandten Wirkstoffen gehören Detomidin, Metdetomidin, Romifidin, Amitraz und Guanabenz. Xylazin ist das am kürzesten amtierende Mitglied dieser Gruppe und ist ein „Controlled Therapeutic Medication" (Version 2.2) des Racing Medication and Testing Consortium (RMTC). Im Jahr 2013 wurde der vorläufige RMTC-Grenzwert für Xylazin auf 10 pg/ml Plasma mit einer Entzugsdauer von 48 Stunden und ohne definierte Dosis festgelegt. Die Anwendung dieses regulatorischen Grenzwerts im US-Bundesstaat Washington führte schnell zu einer offensichtlichen therapeutischen Überschreitung von etwa 75 pg/ml nach einer 200-mg-Dosis i.v. 54 Stunden Vorlaufzeit bis zum Startpunkt eines Rennens. Basierend auf der von Toutain berichteten Irrelevant Plasma Concentration (IPC) für Xylazin [2013] und der sehr kurzen Wirkungsdauer von Xylazin wurde ein vorläufiger regulatorischer Schwellenwert von 300 pg/ml für Xylazin vorgeschlagen. Bald darauf veröffentlichte pharmakokinetische Daten für Xylazin bis zu 12 Stunden nach der Verabreichung zeigten, dass sich die terminale Elimination von Xylazin ab 6 Stunden nach der Verabreichung deutlich verlangsamt, was zu einer flachen terminalen Halbwertszeit führt. Das regulatorische Ergebnis dieser langsamen terminalen Eliminationskurve für Xylazin besteht darin, dass es über Stunden bis Tage hinaus im Plasma nachgewiesen werden kann, ohne dass eine pharmakologische Wirkung des Arzneimittels auftritt. Aufgrund dieser Überlegungen wurde der gesetzliche Grenzwert für Xylazin im US-Bundesstaat Washington vorübergehend auf 200 pg/ml angehoben. Nach dieser Anpassung legt die Überprüfung der gemeldeten Plasmakonzentrationen von Xylazin in Proben nach dem Rennen im US-Bundesstaat Washington nahe, dass dieser angepasste vorläufige Regulierungsgrenzwert von 200 pg/ml im Plasma wahrscheinlich ein angemessenerer und klinisch relevanterer Regulierungsgrenzwert für Xylazin für 48 Stunden nach der Verabreichung ist. Dieser regulatorische Schwellenwert von 200 pg/ml Plasma wurde bald vom RMTC übernommen und wird derzeit durch veröffentlichte Forschungsergebnisse und praktische regulatorische Erfahrungen gut gestützt.

Schlüsselwörter: Xylazin-Schwelle, RMTC (Racing Medication and Testing Consortium, d.h. Konsortium für Rennmedikamente und Tests), α2-adrenerger Rezeptor-Agonist, Regulierung von Pferderennen

Table 1 Post-race xylazine identifications in Washington State Horseracing.[14] | *Xylazin-Identifizierungen nach dem Rennen bei Pferderennen im US-Bundesstaat Washington.*[14]

Finding	Horse	Detected amount	Washington State threshold	ARCI recommended

Exhibit

13

RAPID COMMUNICATIONS IN MASS SPECTROMETRY
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Biomarkers of alcohol abuse in racehorses by liquid chromatography/tandem mass spectrometry

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A rapid and sensitive method was developed for the screening, quantification and confirmation of ethyl glucuronide (EG) and ethyl sulfate (ES) as biomarkers for alcohol administration to racehorses using liquid chromatography coupled on-line with triple quadrupole tandem mass spectrometry. Urine sample aliquots (0.1 mL) were pre-treated by protein precipitation. Separation of EG and ES was achieved on an Ultra PFP column. Isocratic elution with a flush step was performed using 0.1% formic acid in water (A) and 0.1% formic acid in acetonitrile (B). Analysis was performed by negative electrospray ionization in multiple reaction monitoring mode. The retention times for EG and ES were 1.7 ± 0.30 and 3.4 ± 0.30 min, respectively. The internal standard used was d_5-ethyl glucuronide with a retention time of 1.7 ± 0.30 min. The entire separation was completed in <5 min. The limit of detection (LOD) and of quantification (LOQ) for both analytes were 100 ng/mL (S/N ≥3) and 500 ng/mL, respectively. The limit of confirmations (LOC) for EG and ES were 500 ng/mL and 1.0 μg/mL, respectively. The assay was linear over a concentration range of 0.5–100 μg/mL ($r^2 > 0.995$). Intra- and inter-day accuracy and precision were less than 15%. The analytes were stable in urine for 24 h at room temperature, 10 days at 4°C and 21 days at −20°C and −70°C. Ion suppression or enhancement due to matrix effect was negligible. The measurement uncertainty was <14% for EG and <25% for ES. This method was successfully used for the quantification of EG and ES in urine samples following alcohol administration to research horses and for screening and confirmation of EG and ES in urine samples obtained from racehorses post-competition. The method is simple, rapid, inexpensive, and reliably reproducible. Copyright © 2007 John Wiley & Sons, Ltd.

Alcohol is illegally used in racehorses to manage unruly horses and control performance. It functions as a tranquilizer to calm racehorses and render them manageable, especially during movement to and loading into the starting gate. The use of alcohol in racehorses has increased because it is easy to obtain and draws less attention than prescribed tranquilizers. It is a violation to administer alcohol to a racehorse in Pennsylvania, USA. To regulate the use of alcohol in racehorses, a reliable analytical method capable of detecting the presence of alcohol and/or its metabolites as biomarkers in equine urine and plasma is necessary. Direct measurement of ethanol in equine urine and plasma is difficult as the amount administered relative to body weight is low and, following alcohol consumption, it is rapidly cleared from the body. For this reason, we have developed a method with high sensitivity and specificity for detecting biomarkers of alcohol in racehorses to prevent pre-race administration of alcohol.

The metabolites β-D-ethyl glucuronide (EG) and ethyl sulfate (ES) (Fig. 1) have been used as biomarkers to monitor alcohol consumption in humans.[1–6] Ethyl glucuronide is a phase II metabolite formed following alcohol consumption and is eventually excreted in urine. Results of studies performed in human subjects showed that the elimination of EG took much longer than that of the parent compound; ethanol. EG was detected in urine up to 80 h after alcohol consumption and long after ethanol concentration was no longer detectable.[7–9] Ethyl sulfate is a product of ethanol undergoing sulfate conjugation through sulfotransferase activity. Results from an animal study showed that ES was apparently excreted in urine mainly during the first 24 h and could persist for up to 30 h.[10,11] The longer elimination periods of EG and ES than of ethanol have both clinical and forensic significance as sensitive and selective biomarkers for alcohol consumption. Thus, developing a sensitive analytical method for detecting EG and ES as biomarkers of alcohol consumption is an effective way of regulating alcohol abuse in racehorses.

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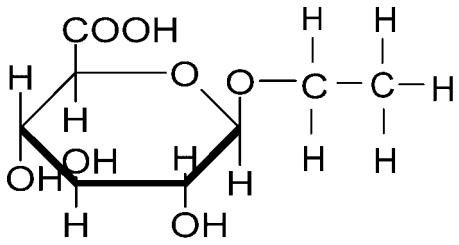
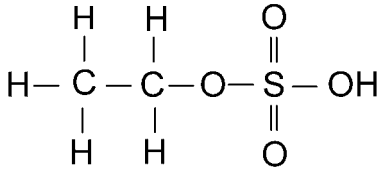


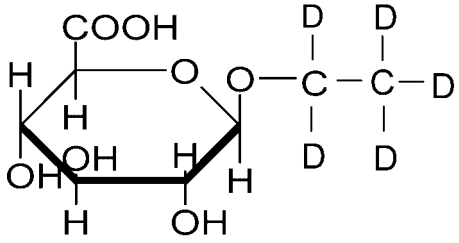
$C_8H_{14}O_7$, MW: 222

Ethyl glucuronide



$C_2H_6O_4S$, MW: 126

Ethyl sulfate



$C_8H_9D_5O_7$, MW: 227

D_5 - Ethyl glucuronide

Figure 1. Chemical structures and molecular weights of ethyl glucuronide, ethyl sulfate, and d_5-ethyl glucuronide.

Determination of EG by gas chromatography/mass spectrometry (GC/MS) after converting the highly polar glucuronide into a highly volatile substance through acetylation or silylation has been demonstrated.[7,8,12] High-performance liquid chromatography (HPLC) with pulsed electrochemical detection has also been reported for EG quantitation.[13] Since EG is non-volatile and water-soluble, liquid chromatography/mass spectrometry (LC/MS) is considered a better technique than GC/MS for the determination of EG in body fluids. Analysis of EG in urine by LC/MS following either direct sample injection or ion-exchange solid-phase extraction has been shown.[8,14] For large-scale analysis, LC/MS outperforms GC/MS in term of shorter analysis time, no need for derivatization, and robust chromatographic performance.[6] In 2004, Helander and Beck extended an existing LC/MS method for the analysis of EG to measure ES and showed that ES could be quantitatively analyzed by single quadrupole LC/MS.[5] A confirmatory method for ES was developed using a precursor and two product ions by triple quadrupole mass spectrometry.[11]

Most of the available LC/MS methods only measure EG or ES. Since both EG and ES are formed shortly after alcohol consumption, simultaneous determination of EG and ES is significant in providing defensible forensic results and meaningful evidence for alcohol consumption by humans or its administration to racehorses. Simultaneous determination of EG and ES in urine was reported using direct dilution injection of urine and pentadeuterated EG (d_5-EG) as internal standard (IS).[15] Due to the high protein and salt contents in equine urine, direct dilution injection may lead to peak broadening, shifting of retention time and shortening of column lifespan. The available LC/MS methods for EG and ES determination use human urine samples, but a method applicable to equine urine, which has far more matrix complexity than human urine, is not available. To meet this challenge, a rapid, selective and sensitive method for screening, quantification and confirmation of EG and ES as biomarkers for the use of alcohol in racehorses was developed.

EXPERIMENTAL

Materials

All HPLC grade solvents used were purchased from J&T Baker (Mallinckrodt Baker Inc., Phillipsburg, NJ, USA). EG and d_5-EG (internal standard, IS) standards were purchased from Medichem (Steinenbronn, Germany). ES was purchased from Tokyo Kasei Kogyo Co., Ltd (Tokyo, Japan). Formic acid (certified) was purchased from Merck (Darmstadt, Germany).

Preparation of stock and working solutions

Each stock solution (2.0 mg/mL) of EG, ES and the IS was prepared from dry chemical powder by dissolution in acetonitrile/water/formic acid (25:75:0.1, v/v/v), and was stored at 4°C. Working solutions containing both EG and ES at concentrations of 5.0, 10, 25, 50, 100, 250, 500 and 1000 μg/mL were prepared by diluting each stock solution with acetonitrile/water/formic acid (25:75:0.1, v/v/v), and were stored at 4°C. The working solution of the IS comprised 50 μg/mL in acetonitrile/water/formic acid (25:75:0.1, v/v/v).

Preparation of analyte standards in equine urine

The control equine urine sample used in this study was previously shown to be free of EG, ES and d_5-EG by the same LC/MS method described in this study. Calibrators ranging from 0.5 to 100 μg/mL (0.5, 1.0, 2.5, 5.0, 10, 25, 50 and 100 μg/mL) of EG and ES were prepared by spiking blank equine urine sample with appropriate concentrations of EG and ES from working solutions. Quality control (QC) samples were similarly prepared at three concentrations: 1.0, 10, and 50 μg/mL.

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Preparation of sample for analysis

An aliquot of equine urine sample (1.0 mL) was added to a 1.5 mL micro-centrifuge tube (Fisher Scientific, Pittsburg, PA, USA) and centrifuged at full speed (13 600 *g*) for 5 min in a micro-centrifuge (model 235C, Fisher Scientific). An aliquot of 0.1 mL of the supernatant was transferred into a fresh 1.5 mL micro-centrifuge tube, and 10 μL of IS (50 μg/mL) was added to each sample and mixed. The mixture was vortexed (VWR mini vortexer, Henry Troemner LLC, Thorofare, NJ, USA) prior to adding 0.5 mL acetonitrile and mixing. The sample was then re-centrifuged at full speed (13 600 *g*) for 5 min. The supernatant was transferred to a labeled culture tube (16 100 mm) and evaporated to dryness at 60°C (TechniDri-Block DB-3, Burlington, NJ, USA) under a steady stream of nitrogen. The dried extract was reconstituted in 1.0 mL of acetonitrile/water/formic acid (25:75:0.1, v/v/v). An aliquot (200 μL) was transferred into a 250 μL insert (Target PP Polyspring, National Scientific Company, Rockwood, TN, USA) and analyzed by LC/MS/MS. The injection volume was 5 μL.

Instrumentation and operating parameters

Sample analysis was performed by an LC/MS system, consisting of a Surveyor MS pump with an on-line degasser, a Surveyor autosampler, and a Finnigan Quantum triple stage quadrupole mass spectrometer equipped with an electrospray ionization (ESI) probe (ThermoFisher Scientific, San Jose, CA, USA).

LC separation was performed on a PFP column (50 3.2 mm, 5 μm particle size, Restek Corp., Bellefonte, PA, USA), with a PFP 5 μm guard column (10 4.0 mm) and a 0.25 μm pre-column filter (MAC-MOD Analytical, Chadds Ford, PA, USA). Mobile phase A consisted of 0.1% formic acid in water, while mobile phase B comprised 0.1% formic acid in acetonitrile. The mobile phase was delivered by isocratic elution program with a flush step as follows: 0 min: 75:25 (A/B) at 200 μL/min; 2.50 min: 75:25 (A/B) at 300 μL/min; 4.50 min: 75:25 (A/B) at 300 μL/min; 4.51 min: 20:80 (A/B) at 300 μL/min; 6.00 min: 20:80 (A/B) at 300 μL/min; 6.01 min: 75:25 (A/B) at 300 μL/min; 8.00 min: 75:25 (A/B) at 300 μL/min. The total analysis time was <5 min.

The mass spectrometer was operated in negative ion mode. The ESI spray was at a 45° angle to the ion transfer capillary tube that guides the ion beam into the mass spectrometer. Mass calibration of the mass spectrometer was performed with a polytyrosine-1,3,6 solution according to the user manual. The ESI source parameters were tuned by syringe infusion of each analyte into the ESI source under LC flow condition. The spray voltage was 3500 V. The ion transfer capillary temperature was 300°C. Nitrogen was the desolvation gas, at flow rates of 40 arbitrary units as the sheath gas and 14 arbitrary units as the auxiliary gas. MS and MS/MS spectra were acquired by infusion of the analyte standards in acetonitrile/water/formic acid (25:75:0.1, v/v/v) at 10 μL/min. For sample analysis, the mass spectrometer was operated in multiple reaction monitoring (MRM) mode. The source collision-induced dissociation (CID) voltage and the collision energy for MRM data acquisition had been previously optimized (Table 1). Other parameters used were: scan width 0.1 *m/z* units, scan time 0.1 s and peak width (FWHM) 0.4 *m/z* units for Q1 and 0.7 *m/z* units for Q3. Data acquisition and analysis were accomplished with Xcaliber software v.1.3 (ThermoFisher Scientific).

Table 1. ESI(–)MS/MS parameters for obtaining product ions of EG, ES and d_5-EG

Analyte	Precursor ion (*m/z*)	Source CID (V)	Collision energy (V)	Product ion (*m/z*)
Ethyl glucuronide (EG)	221	20	10	113
		15	10	85
		15	10	75
Ethyl sulfate (ES)	125	15	10	125
		10	21	97
		10	39	80
d_5-ethyl glucuronide (d_5-EG, IS)	226	10	21	85

Validation of method

The method was validated for linearity, selectivity, accuracy, precision, matrix effect and analyte stability.[16] The linearity of the method was determined by plotting peak area ratios of the analytes to IS against concentrations of the analytes. The specificity of the method was determined by analyzing six different lots of blank equine urine, urine spiked with IS and urine samples spiked with IS and the analytes. Accuracy and precision were addressed by analyzing QC samples at three different concentrations (1.0, 10 and 50 μg/mL). The matrix effect was evaluated by analyzing two batches of samples: neat standard solution versus urine extracts spiked with the analytes. Sample stability during analysis, sample freeze/thaw stability and stability during long-term storage under different temperature conditions were evaluated.

Animal administration study

The study protocol was approved by the University of Pennsylvania Institutional Animal Care and Use Committee. A 7-year-old female horse weighing 504.4 kg was used in the study. Two days before the experiment, the horse was brought from pasture into a stall and remained housed for the duration of the study. The horse was fed grass hay and water *ad libitum*. The study horse was no longer actively racing but otherwise in good health. The study started at 7 AM. The intravenous (IV) alcohol was administered via a 14-F catheter (Angiocath, Becton Dickinson, Sandy, UT, USA) placed in the jugular vein. Prior to placement of the catheter, the area over the jugular vein was clipped and washed with surgical soap (Chlorhexidine gluconate 4%, Purdue Fredrick Co., Stamford, CT, USA) and rinsed with a viricide (chlorhexidine diacetate, Fort Dodge Health, Fort Dodge, IA, USA) and 70% isopropyl alcohol. A sterile indwelling 24-F self-retaining urinary catheter (Foley Catheters, CR Bard Inc., Covington, GA, USA) was placed in the bladder and attached to a drainage bag (Bard Center Entry Urinary Drainage Bag, CR Bard Inc.) for continuous collection of urine, following washing the vulva with surgical soap and rinsing with a viricide. The horse was administered

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60 mL of absolute ethanol (200 proof) diluted in 150 mL isotonic sodium chloride. Urine samples were collected prior to IV administration (0 h) and 1, 2, 4, 8, 24 h post-administration and stored at −20°C until analysis.

RESULTS AND DISCUSSION

Method development

In the negative ion ESI mode, EG, ES and d_5-EG produced deprotonated molecules at *m/z* 221, 125 and 226, respectively, and these ions were subjected to CID. Figure 2 shows the full scan product ion mass spectra of *m/z* 221, 125 and 226 for EG, ES and d5-EG, respectively. Transitions of *m/z* 221 → 85, 125 → 97 and 226 → 85 were the major ones for EG, ES and d_5-EG, respectively. These transitions produced the highest signal-to-noise (S/N) ratio in the analysis of spiked samples and, thus, were chosen for quantitative analysis. For confirmation, transitions of *m/z* 221 → 85, 221 → 75 and 221 → 113 were selected for EG. The transitions for ES confirmation were *m/z* 125 → 97, 125 → 80 and 125 → 125. For ES, since only two product ions (*m/z* 97 and 80) were generated by CID, the precursor ion *m/z* 125 was also used for confirmation.

Chromatographic condition optimization was focused on peak shapes, matrix effects, resolution and short retention times. Due to the hydrophilicity of EG and ES, both analytes rapidly eluted from reversed-phase C_8 or C_{18} columns so these columns were not suitable for separation of EG and ES. Of the different columns with various stationary phases evaluated for the retention and resolution of EG and ES, the PFP column with a pentafluorophenyl phase was chosen for this study. Figure 3 shows the chromatograms of EG, ES and IS on a PFP column. Isocratic elution was performed for analyte separation, followed by a flush step with a high percentage of organic solvent to flush the column. The flush step was necessary to keep the column clean. The retention time was 1.7 min for EG and the IS, and 3.4 min for ES (Fig. 3). Direct dilution and one-step protein precipitation were evaluated for sample preparation. For accuracy, precision and linearity, both sample preparation procedures lead to similarly acceptable results. However, during routine sample screening it was noted that impurities in the samples prepared by direct dilution accumulated in the analytical column causing high backpressure. As the result of this problem, sample preparation was only by protein precipitation. Although protein precipitation was more labor-intensive than direct dilution-and-injection, it generated cleaner samples than those from direct dilution-and-injection. This is especially important in preparing samples for routine analysis on a large scale.

Recovery efficiency of EG and ES from equine urine

The recovery efficiency of the one-step protein precipitation method for EG and ES was determined by analyzing six replicate samples of three different concentrations (1.0, 10,

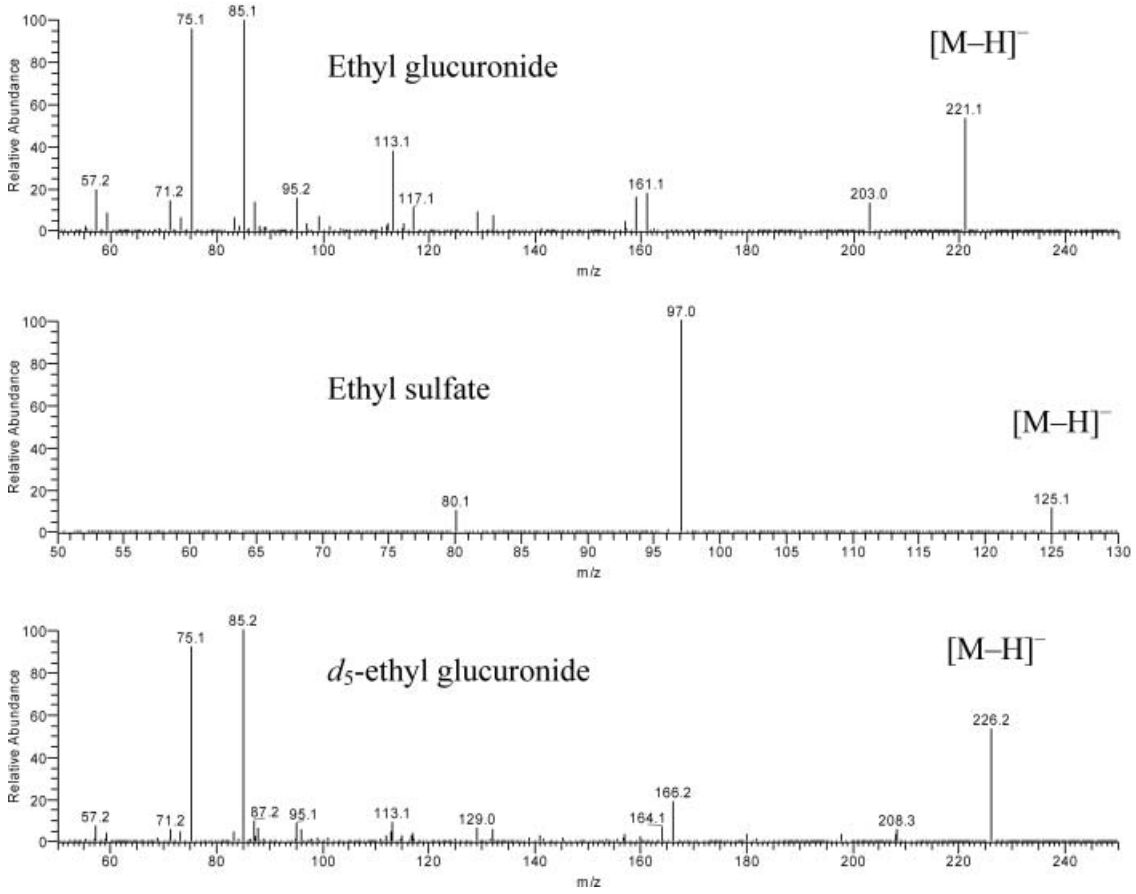

Figure 2. ESI(−)-MS/MS spectra of $[M–H]^-$ ions of ethyl glucuronide, ethyl sulfate and d_5-ethyl glucuronide.

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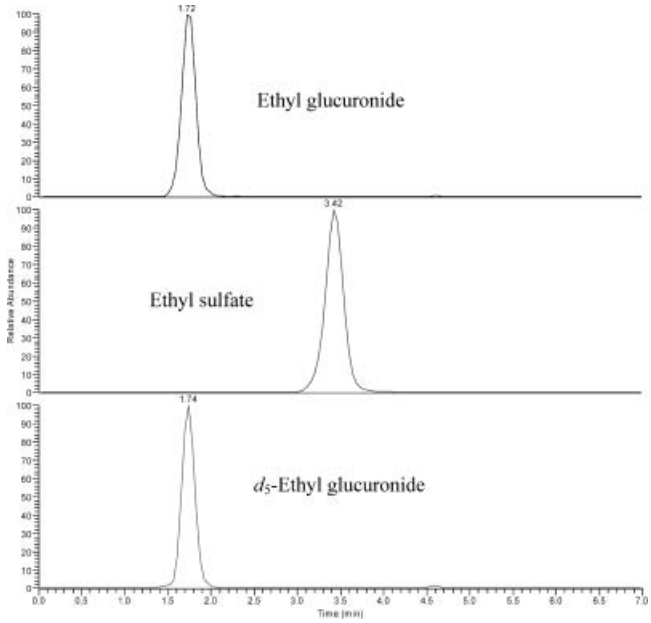

Figure 3. LC/ESI(−)MS-MRM chromatograms of ethyl glucuronide (*m/z* 221 → 85), ethyl sulfate (*m/z* 125 → 97), and d_5-ethyl glucuronide (*m/z* 226 → 85) in equine urine at 5.0 μg/mL.

and 50 μg/mL). Standard samples were prepared by spiking EG and ES into urine extract to avoid matrix effects. The recovery efficiency was calculated by comparison of the integrated peak area for the processed samples with the response for the standard samples. Average recoveries of EG were 93.5, 92.4 and 95.8% at concentrations of 1.0, 10, and 50 μg/mL, respectively. The recoveries for ES at the same concentrations were 96.0, 94.3 and 96.3%. Results obtained indicated that the one-step protein precipitation procedure produced highly consistent recovery efficiency for the analytes.

Matrix effects

Endogenous compounds from the sample matrix can suppress or enhance ionization of an analyte recovered from the matrix. This results in limitation of method precision, accuracy and reproducibility. Ion suppression/enhancement is very common in atmospheric pressure ionization sources, especially when using ESI. The matrix effect on EG and ES was evaluated at three concentrations of 1.0, 10 and 50 μg/mL in six replicates. It was calculated by comparing the chromatographic peak areas of each analyte standard in the reconstitution solvent (S) with those of the analyte added to negative equine urine extract (U), according to the following equation:

$$\text{Ion suppression or enhancement } (\%) = (A_{extract} - A_{solvent})/A_{solvent} \quad 100$$

where $A_{solvent}$ is the peak area of an analyte standard in S, and $A_{extract}$ is the peak area of an analyte standard in U. As shown in Table 2, negative values in the ion suppression/enhancement column indicated that urine induced minor ion suppression effects on both EG and ES. Ion suppression by equine urine was less than 21% for EG and less than 7% for ES, suggesting that matrix effect was not a major problem when using protein precipitation for sample preparation since the precision and accuracy of the proposed method were within acceptable experimental error.

Table 2. Matrix effect on ionization of EG and ES

Analyte	Conc. spiked (μg/mL)	Ion suppression or enhancement (%)[a]
Ethyl glucuronide (EG)	1.0	−15.6
	10	−18.9
	50	−20.7
Ethyl sulfate (ES)	1.0	−6.1
	10	−4.3
	50	−3.3

[a] Ion suppression or enhancement (%) = $(A_{extract} - A_{solvent})/A_{solvent}$ 100, where $A_{solvent}$ is the peak area of an analyte spiked in reconstitution solvent, and $A_{extract}$ is the peak area of an analyte spiked in blank equine urine extract. Negative value indicates the presence of ion suppression.

Confirmation of EG and ES

Confirmation of the presence of an analyte in a test sample is achieved by demonstrating that the 'fingerprints' of the unknown are the same as those of an authentic drug standard. Criteria for confirmation of EG and ES in equine urine included relative intensity ratios of product ions and LC retention times. The product ions employed for intensity ratio comparison were *m/z* 85, 75 and 113 at the same retention time of 1.7 min for EG and *m/z* 97, 80 and 125 at 3.4 min for ES. Figure 4 shows product ion intensity comparison and the relative intensity ratio used as criteria in the confirmation of the presence of EG or ES in equine urine. The product ion intensity ratios of an analyte may vary from batch to batch due to difference in instrument parameter settings and matrix effects, but they are unique and remain constant over the range of concentrations of calibrators in each batch. Criteria for confirmation of EG and ES in equine urine were defined as the intensity ratio difference between unknown samples and the calibrators must be less than 20%, and the retention times of EG and ES must be within 0.3 min of that of the calibrators in each batch, and a minimum of three MRM ion transitions must be demonstrated.

Method validation

Specificity

Six different lots of blank equine urine, urine spiked with IS and urine spiked with 0.1 μg/mL (limit of detection) EG and ES were analyzed to determine the specificity of the method. Figure 5 shows chromatograms of blank urine, blank urine plus IS and blank urine spiked with 0.1 μg/mL EG and ES. Direct interference caused by endogenous substances from the blank urine at the retention time of EG, ES or d_5-EG was not significant, suggesting that the protein precipitation procedure used yielded sufficiently clean samples for the analysis of the analytes and, thus, this method has high specificity for the analysis of equine urine for EG and ES.

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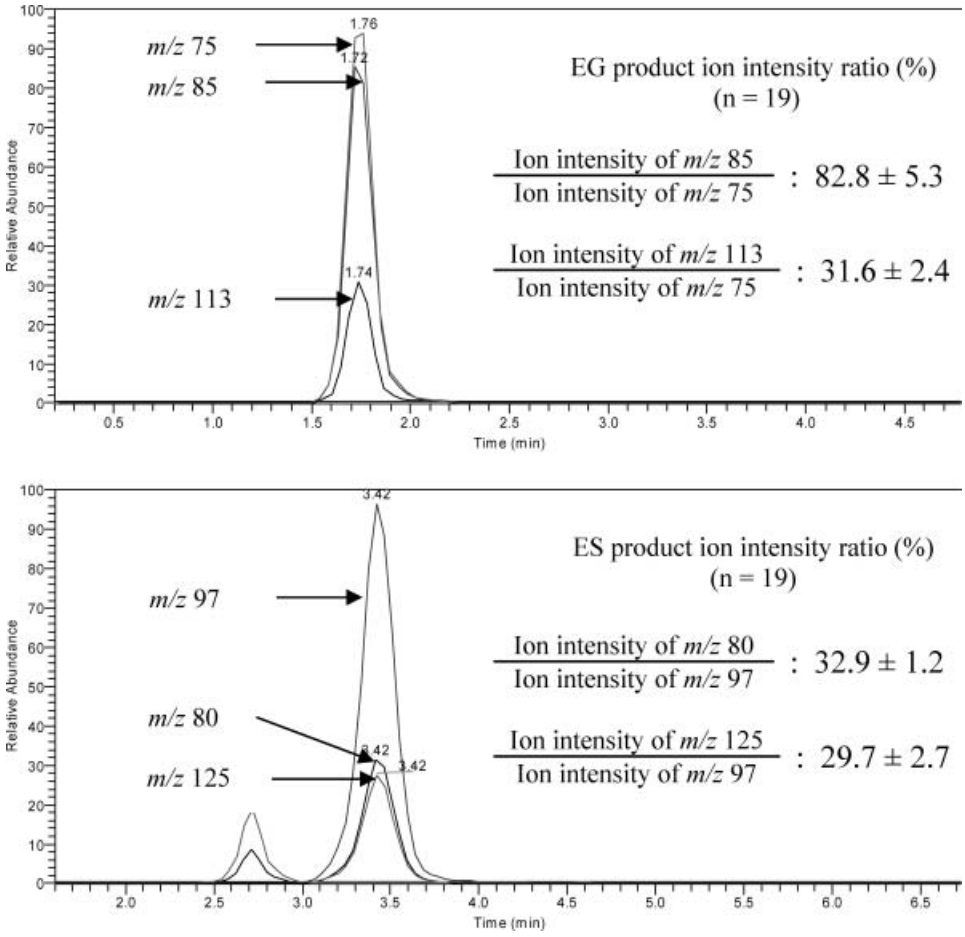

Figure 4. Product ion intensity comparison for ethyl glucuronide (upper panel) and ethyl sulfate (bottom panel) in equine urine (50 μg/mL). Product ion with the largest intensity was used as the denominator to calculate ion intensity ratios. Ion intensity ratio difference between unknown samples and the calibrators must be lower than 20% in each batch for confirmation of analytes to be acceptable within the established criteria.

Sensitivity and linearity

The method was evaluated for lowest limits of detection (LODs), quantitation (LOQs) and confirmation (LOCs). LOD (signal-to-noise (S/N) ratio 3) was 100 ng/mL. The LOQ was defined as the lowest concentration in the calibration curve that was measured with acceptable accuracy and precision. The LOQ was 500 ng/mL for both analytes in equine urine. The LOC was the lowest concentration at which the product ion intensities were sufficiently strong to produce stable product ion intensity ratio for the confirmation of each analyte, and the LOCs were 500 ng/mL for EG and 1.0 μg/mL for ES.

The ratio of the peak area of the analyte to that of the IS was proportional to concentration of the analyte from 0.5 to 100 μg/mL. A linear regression model was used in describing the regression relationship. Different weighting factors ($1/x$, $1/x^2$, and none) were examined for the best linear fit of the calibration curve, and the $1/x$ weighting factor was applied. The coefficient of determination (r^2) for both analytes was 0.995.

Accuracy and precision

Intra-day accuracy and precision were determined by analyzing eighteen validation samples at three concentrations (1.0, 10 and 50 μg/mL; n = 6 each) in one batch in a day. Inter-day accuracy and precision were measured in three consecutive batches on three separate days. The concentrations of the analytes used to determine accuracy and precision corresponded to low, medium and high concentrations used in the calibration curves. Accuracy (bias %) was determined as the agreement between the concentrations of EG and ES measured and those spiked into blank urine. The precision (RSD %) of the assay was calculated as the standard deviation expressed as a percent of the standard deviation divided by the mean of observed concentrations. Results obtained indicated that the method was accurate with an acceptance limit of 15% of the theoretical values, and the RSD around the mean value did not exceed 15% at the three concentrations tested, suggesting that the accuracy and precision of the method were acceptable (Table 3).

Stability of EG and ES in equine urine

Stability of analytes during analysis

Following sample preparation, the analytes in the reconstitution solvent may remain in an autosample tray for a few hours before injection into the LC/MS system for analysis,

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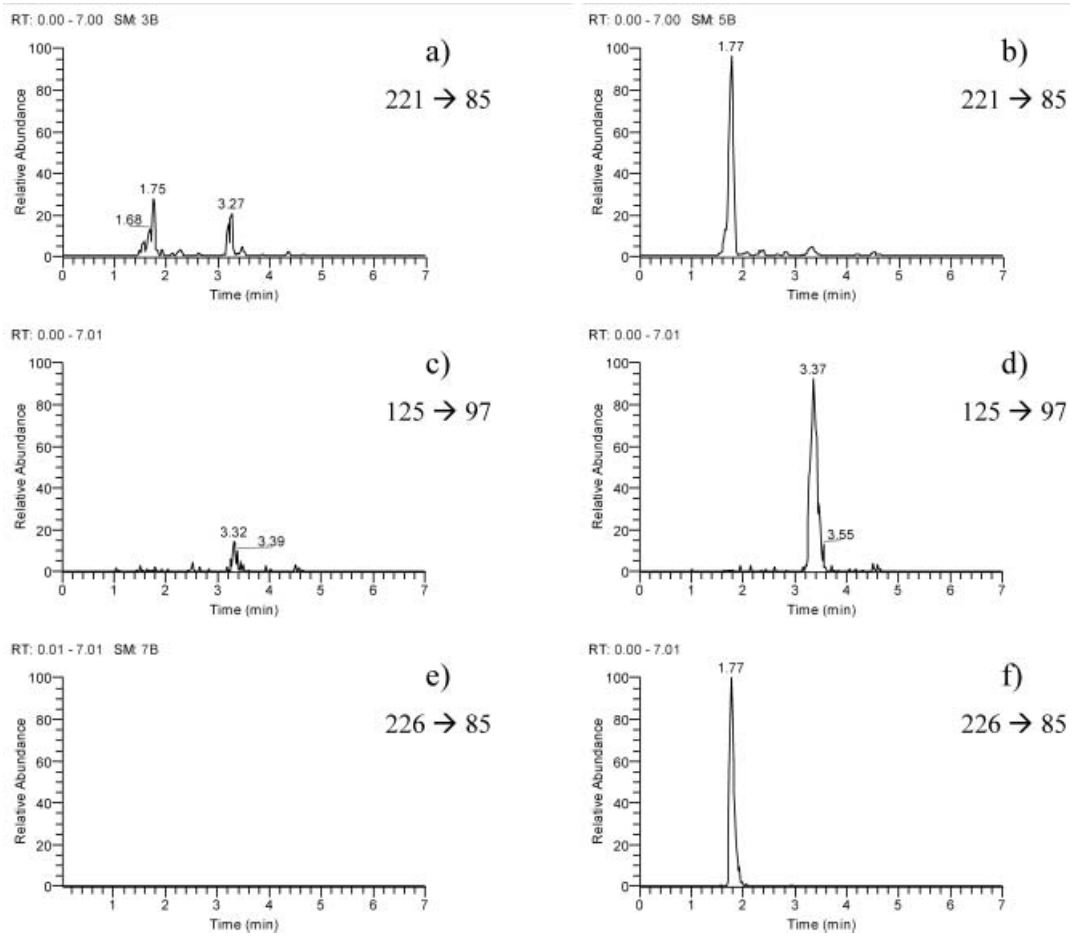

Figure 5. LC/MS-MRM chromatograms of ethyl glucuronide, ethyl sulfate, and d_5-ethyl glucuronide (IS) indicating method specificity. (a) Blank urine (scan for EG *m/z* 221 → 85) showing the absence of EG from the sample; (b) 0.1 μg/mL EG spiked in blank urine (scan for EG *m/z* 221 → 85) showing the presence of EG at 1.77 min; (c) blank urine (scan for ES *m/z* 125 → 97) showing the absence of ES from the sample; (d) 0.1 μg/mL ES spiked in blank urine (scan for ES *m/z* 125 → 97) showing the presence of ES in the sample at 3.37 min; (e) blank urine (scan for d_5-EG *m/z* 226 → 85) showing the absence of both EG and ES from the sample; and (f) 5.0 μg/mL IS spiked in blank urine (scan for d_5-EG *m/z* 226 → 85) showing IS peak at 1.77 min.

and they may degrade during this period. To determine their stability during analysis (laboratory bench-top stability), the analytes at three concentrations (1.0, 10, 50μg/mL; n = 5 each) were extracted using the sample preparation procedure previously described. The resulting extracts at each concentration were pooled together and aliquots of these pooled extracts at each concentration were transferred to autosampler vials (n = 5) and analyzed every 2 h for a 24 h period. The peak area was plotted against time to determine if the concentration of the analytes in the autosampler tray at

Table 3. Intra-day and inter-day accuracy and precision for quantification of EG and ES in equine urine (n = 6)

	Ethyl glucuronide (EG)			Ethyl sulfate (ES)		
Conc. spiked (μg/mL)	Conc. determined (μg/mL)	Accuracy[a] (bias) (%)	Precision[b] (RSD) (%)	Conc. determined (μg/mL)	Accuracy (bias) (%)	Precision (RSD) (%)
			Intra-day			
1.0	1.1	7.3	4.9	1.1	14.2	10.9
10	10.5	5.4	5.8	10.5	4.9	9.2
50	48.4	−3.2	4.3	46.6	−6.8	4.1
			Inter-day			
1.0	1.1	6.0	10.7	1.0	3.8	7.3
10	10.7	6.8	6.3	10.2	2.2	8.8
50	50.0	0.1	4.4	49.3	−1.5	6.9

[a] Accuracy (bias %) = (Conc. determined – conc. spiked)/conc. spiked 100.
[b] Precision (RSD %) = Standard deviation of conc. determined/conc. determined 100.

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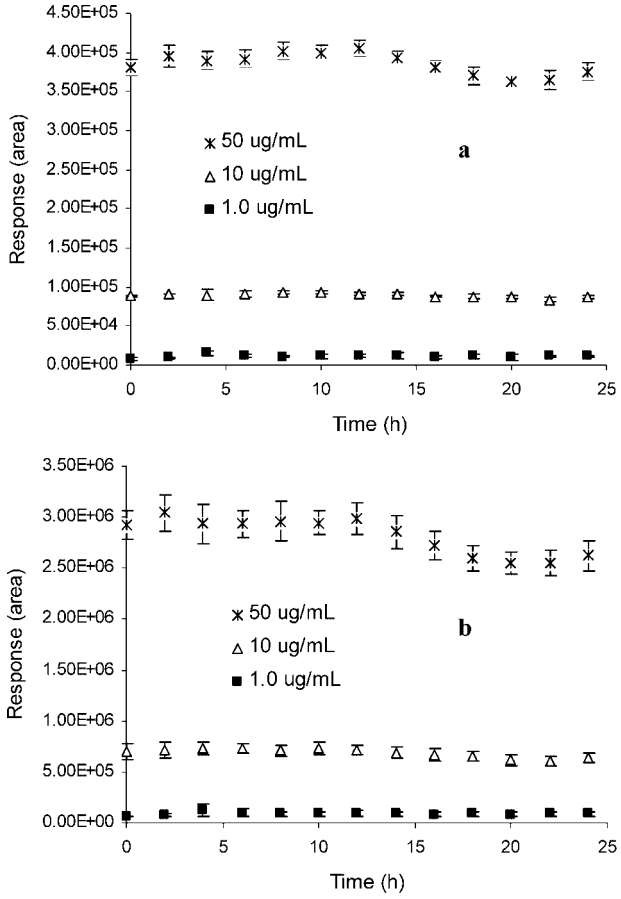

Figure 6. Stability of EG (a) and ES (b) loaded in the autosampler tray for analysis under ambient temperature conditions. Stability was determined using three different concentrations of each analyte (1.0, 10, and 50 μg/mL). Results were obtained at various time points for 24 h period. There was no significant drop in the slope suggesting that the samples were stable during the 24 h test period at ambient temperature.

room temperature decreased. The average response at each time interval was not significantly affected during the 24 h test period, suggesting that the analytes were stable for 24 h in an autosampler tray at room temperature (Fig. 6).

Effect of freeze/thaw cycles on stability of analytes

The effect of freeze/thaw cycles on the stability of the analytes was evaluated at three concentrations (1.0, 10, 50 μg/mL; n = 6 each) through one and four freeze/thaw cycles. For each freeze/thaw cycle, the samples were frozen at −20°C for 21 h, thawed and kept at ambient temperature for 3 h. The concentrations of the freeze/thaw samples were determined using daily calibration curves. The stability of EG or ES was higher than 89% after one and four freeze/thaw cycles, suggesting that EG and ES did not deteriorate after exposure to one and four freeze/thaw cycles (Table 4).

Effect of storage on stability of analytes

Racehorse samples are rarely delivered to the laboratory for analysis on the same day as they are collected. Samples are usually stored at either −20°C or 4°C for a few days before shipment to the laboratory for analysis. During shipment, the samples are not refrigerated and are subjected to variations in temperature. Thus, the effect of storage on stability of the analytes is an important factor in maintaining the integrity of the sample, and therefore, needs to be evaluated. This evaluation was conducted at three concentrations (0.5, 5.0, 50 μg/mL; n = 3 each) under different storage conditions (25°C, 4°C, −20°C and −70°C). The percentage change of the concentration of the analytes from that at 0 h (control) was calculated. The stability of EG and ES was greater than 80%, suggesting that there was no significant degradation of the analytes during sample storage at the different storage temperature conditions to which the samples were exposed (Table 5).

Table 4. Stability of EG and ES in equine urine after one and four freeze-thaw cycles (n = 6)

		One cycle		Four cycles	
Analyte	Spiked conc. (μg/mL)	Conc. determined (μg/mL)	Stability[a] (%)	Conc. determined (μg/mL)	Stability (%)
Ethyl glucuronide (EG)	1.0	1.0 0.1	101.0	1.0 0.1	97.0
	10	9.1 0.4	90.9	8.9 0.7	89.4
	50	46.4 0.9	92.7	46.2 4.0	92.5
Ethyl sulfate (ES)	1.0	1.0 0.1	99.0	1.0 0.1	95.0
	10	9.2 0.8	91.8	9.3 1.3	92.9
	50	45.5 1.2	91.1	47.0 1.0	94.1

[a] Stability (%) = Conc. determined/conc. spiked 100.

Table 5. Stability of EG and ES in equine urine at various temperature conditions (n = 3)

		Stability[a] (%)				
Analyte	Spiked conc. (μg/mL)	0 h	25°C 24 h	4°C 10 days	20°C 21 days	−70°C 21 days
Ethyl glucuronide (EG)	0.5	100	92.8	92.3	87.2	89.6
	5	100	94.0	101.1	93.2	93.7
	50	100	99.3	103.6	92.6	93.1
Ethyl sulfate (ES)	0.5	100	98.2	95.1	84.9	88.0
	5	100	97.2	93.6	82.7	86.7
	50	100	103.0	107.1	81.0	83.5

[a] Stability (%) = Conc. determined/conc. spiked 100.

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Table 6. Estimation of measurement uncertainty of EG and ES in equine urine

Symbol	Source of uncertainty	Value units (%)	Distribution	Advisor	Standard uncertainty	Degree of freedom ($n-1$)	Other
U_1	Intermediate precision	5.7	N	1	5.7	17	EG 10 μg/mL
U_2	Intermediate precision	10.6	N	1	10.6	17	ES 10 μg/mL
Combined uncertainty				1. $\sqrt{U_1^2}=5.7$; 2. $\sqrt{U_2^2}=10.6$			
Expanded uncertainty (k = 2.3)				1. (5.7 2.3) = 13.1%; 2. (10.6 2.3) = 24.4%			

Estimation of measurement uncertainty

We demonstrated previously the use of laboratory quality control samples (LQCS) as a simple, fast and acceptable method for estimating measurement uncertainty.[17] In this study, measurement uncertainty was estimated from 18 LQCS data.[18–20] The standard uncertainty values were 5.7 and 10.6 for EG and ES, respectively. The standard uncertainty is defined as the standard deviation of the QC measurements divided by 'n', the square root of the number of measurements. In this case, n is taken as 1 since a single measurement is assumed. The estimated measurement uncertainty values for EG and ES at 95% confidence interval were 13.1% and 24.4%, respectively (Table 6). Thus, an estimated quantitative value of 50 μg/mL for EG, as an example, would be reported as 50 50 13.1% μg/mL. The true value of EG in the example used, which is unknown, lies between 56.6 and 43.5 μg/mL.

Analysis of racehorse urine samples – verification of the method

The present method was applied to 'real world' post-race urine samples collected from racehorses in Pennsylvania in order to regulate alcohol abuse. Figure 7 shows the chromatographic comparison of EG in a post-race equine

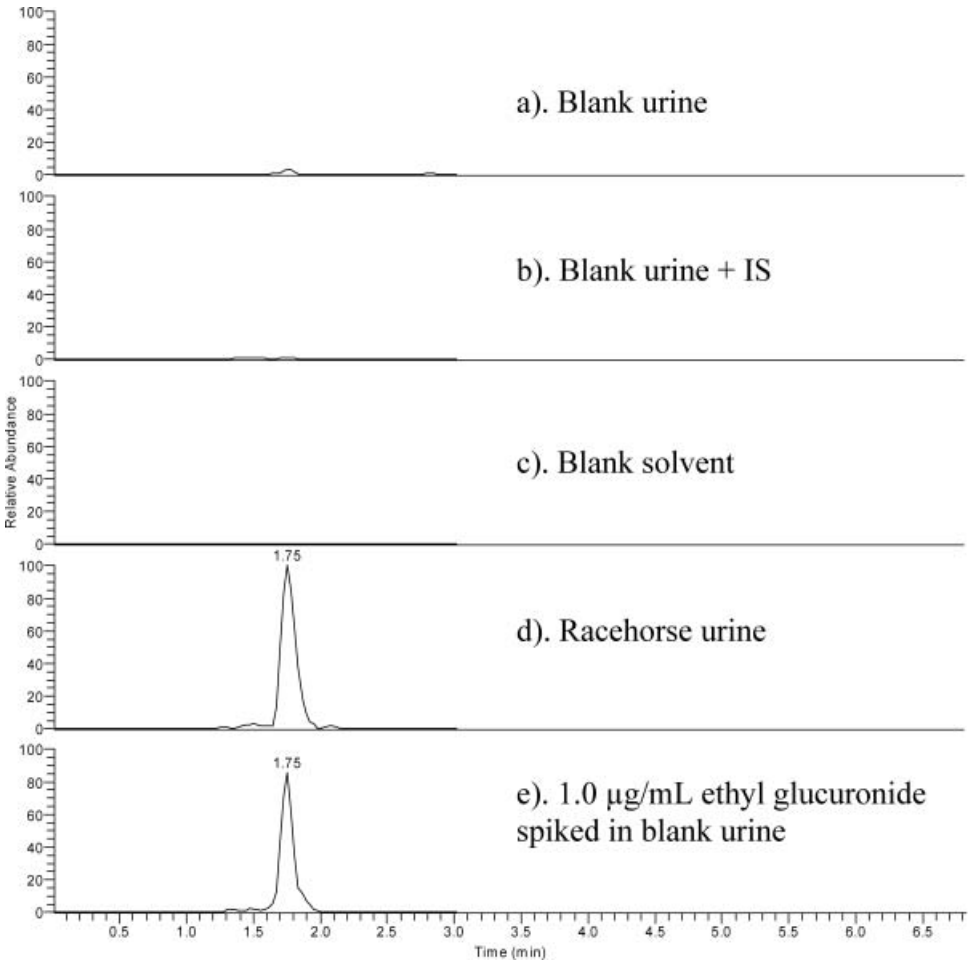

Figure 7. ESI(−)-LC/MRM chromatograms in racehorse urine sample. (a) Blank urine was scanned for EG which was not detected; (b) blank urine + IS was scanned for EG, which was absent from the sample; (c) blank solvent (mobile phase) was scanned for the presence of EG, which was absent from the sample; (d) post-race urine sample positive for EG was scanned for EG and the peak for EG at 1.75 min was detected and confirmed; and (e) EG 1.0 μg/mL spiked into bank urine as QC sample was detected at 1.75 min.

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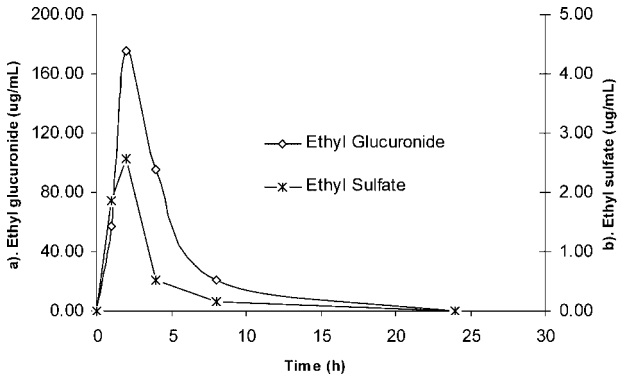

Figure 8. Concentration-time profiles of EG and ES following alcohol administration (60 mL, IV) to a research horse. Both analytes attained peak concentrations in urine within 2 h post-administration. The concentration of both analytes at 24 h post-administration was negligible.

urine sample and EG spiked in blank urine. This method was also used to quantify and confirm EG and ES in urine samples collected from research horses following alcohol administration. The results obtained indicated that EG and ES were detected in urine collected 1 h after intravenous administration of 60 mL ethanol (200 proof) to horses (Fig. 8). The concentrations of EG and ES that peaked at approximately 2 h post alcohol administration were 175.5 and 2.56 μg/mL, respectively (Fig. 8).

CONCLUSIONS

A method for detection, quantification and confirmation of EG and ES in equine urine samples by LC/MS/MS was developed, validated and verified. The method consisted of sample preparation by protein precipitation, LC isocratic separation and mass spectrometric detection, quantification and confirmation of the analytes. Since this method was developed, it has been successfully applied to a large number of urine samples obtained from racehorses post-competition and to pharmacokinetic studies. The method is simple, rapid, cost-effective and reliably reproducible.

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Exhibit

14

Pemoline and Tetramisole 'Positives' in English racehorses following Levamisole administration.

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ABSTRACT

Pemoline is a central nervous system stimulant that has been used to treat attention-deficit hyperactivity disorder and narcolepsy in humans; its identification in horses could be considered evidence of attempts to influence performance. Two recent pemoline 'positives' in English racehorses led us to review the chemical relationships between tetramisole, levamisole, aminorex and pemoline. Pemoline is a simple oxidation product of aminorex, which has been shown in the United States and elsewhere to be an equine metabolite of levamisole. Based on the clear structural relationships between aminorex and pemoline, we conclude that levamisole can metabolise to pemoline in horses and that pemoline identifications in horses post levamisole administration are likely to be associated with levamisole administration. Levamisole should not be administered to horses about to compete because of its ability to metabolise to two central nervous system stimulants, aminorex and pemoline.

KEY WORDS: Horses, tetramisole, levamisole, aminorex, pemoline, metabolism, drug 'positives'.

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Pemoline, [(*RS*)-2-amino-5-phenyl-1,3-oxazol-4(5*H*)-one] is a central nervous system stimulant used to treat attention-deficit hyperactivity disorder (ADHD) and narcolepsy in humans (Molina and others 1981). In horse racing, pemoline is classified as an Association of Racing Commissioners International (ARCI) 'Class 1' foreign substance, i.e., an agent with high potential to influence performance (Tobin 1981 and ARCI website). Pemoline is unusually identified in a post-race sample, and its identification would usually be interpreted as an attempt to improperly influence performance. However, an entirely innocent explanation is possible for such pemoline identifications, as we will now set forth.

The explanation is based on the fact that in the horse, and possibly other species, pemoline is a metabolite of levamisole, an anthelmintic and immune stimulant not uncommonly administered to horses (Mohri and others 2005). The recommended dose in the horse is 2/mg/kg orally, once a day for three-to-five days, as an immune modulator (Britt and Byars, 1997). Furthermore, in the horse levamisole, metabolism has recently been shown to produce a closely related substance, aminorex (Barker 2008, Ho and others 2009, Pellegrini, 2010). Aminorex, which is structurally related to levamisole and pemoline (**Figure 1**), is a central nervous system stimulant which was marketed as a prescription anorectic in Europe, but withdrawn when it became associated with pulmonary hypertension (Gurtner 1985). In the United States, Aminorex is a DEA Schedule 1 substance and an ARCI 'Class 1' substance, like pemoline.

In the United States, the link between levamisole administration and aminorex identifications has been associated with a series of no fewer than 82 aminorex 'positives' in post-race urines. These aminorex 'positives' started with a single identification in Belgium, then, in the United States, first in October 2004 in Ohio, and later in both Pennsylvania and Ontario (Weber 2010) as set forth in **Table 1**. In Pennsylvania, beginning

in October of 2005, testing indicated detections of aminorex in both blood and urine samples and by November 2006 aminorex 'positives' had been reported in all four Pennsylvania racetracks (Mushalko, 2010, Uboh, 2010). Each of these jurisdictions is a major North American racing jurisdiction, testing in the order of 15,000 samples per year or more at multiple racing venues, both Thoroughbred and Harness, in each jurisdiction.

Table 1: Time Sequence of Aminorex "positives" in North America, 2004-2009

State/Province	2004	2005	2006	2007	2008	2009	Total
Ohio	8	2	9	4	1	4	28
Pennsylvania		4	32				36
Ontario				18			18
Totals	8	6	41	22	1	4	82

As shown in **Table 1**, 'positive' reports for aminorex in the US and Canada peaked in 2006 with 41 total, followed by 18 in 2007, principally in Ontario. It seemed unusual that these aminorex 'positives' continued to occur in the face of significant penalties against the horsemen involved: the penalty in Ohio usually being a fine of about $500, loss of purse and a six-month suspension.

The first direct association between these aminorex 'positives' and levamisole administration was made by Dr Frank Pellegrini of Freedom Health, LLC, whose companies' products had been linked to the aminorex identifications. Dr Pellegrini made the aminorex/levamisole association in about September 2006 when at least one of a group of horses that he had treated therapeutically with levamisole gave rise to an Ohio aminorex 'positive' (Tobin 2010). This finding led Dr Pellegrini and Freedom Health LLC to perform a carefully designed series of experimental administrations, using a number of different commercial levamisole sources, a number of selected Freedom Health products and two other non-levamisole wormers, Panacur and Safe-Guard. The post administration urine samples were then analyzed under contract by Industrial Laboratories in Denver, CO, and this work confirmed Dr Pellegrini's proposed levamisole/aminorex relationship (Pellegrini 2010). The formal reporting of these findings on or about March 17th 2008 by Freedom Health, LLC, led to an abrupt decline in the number of aminorex 'positive' calls (**Table 1**). This levamisole to aminorex transformation was soon confirmed by the work of Dr Steve Barker of Louisiana State University (Barker 2008) and later by Ho and others (2009).

About this time our group in Kentucky was researching the aminorex situation on behalf of Pennsylvania horsemen, and a proposed chemical levamisole/aminorex transformation mechanism was communicated by Dr Rodney Eisenberg of Frontier Biopharm to Dr Mike Weber in Ontario (**Figure 1**). In summary, work in 2006-2007 clearly established that aminorex is found in equine urine post levamisole administration and established a proposed chemical mechanism for these transformations. These findings led to forensic and regulatory re-evaluation of the significance of the aminorex 'positives' reported in a number of jurisdictions.

Review of the data presented in **Table 1** shows differing time courses of aminorex "positives" in each jurisdiction. Pennsylvania reported no aminorex "positives" after 2006, and eventually negotiated settlements with their horsemen. In Canada, there were no aminorex "positives" tested after 2007. On the other hand, Ohio continued testing aminorex 'positives' until at least 2009, apparently largely in Standardbred racing. Since drug testing continues at about the same rate in each of these North American racing jurisdictions, these changed positive call rates are presumably due to horsemen recognising levamisole as the source of many or most of these aminorex positives, and possibly also to regulatory re-evaluation of the forensic significance of very low concentration aminorex identifications.

More recently, in October of 2009, we received a communication from a colleague of an English horse trainer who recently had two pemoline 'positives' and one apparently related tetramisole 'positive' called (Tobin 2009). Responding to this request, we revisited the then well-established link between levamisole and aminorex, and reviewed the chemistry of levamisole, aminorex and pemoline. The structure of pemoline is closely related to that of aminorex, being a simple oxidation product of aminorex. It is therefore easy to extend the levamisole/aminorex connection from levamisole, (the levo isomer of tetramisole, ergo the name levamisole) to aminorex and then, by simple oxidation, to pemoline. Im summary, any horse administered either levamisole or possibly tetramisole is at risk of producing either aminorex or pemoline positives, or, conceivably both, as set forth in this abbreviated chemical reaction scheme in **Figure 1**.

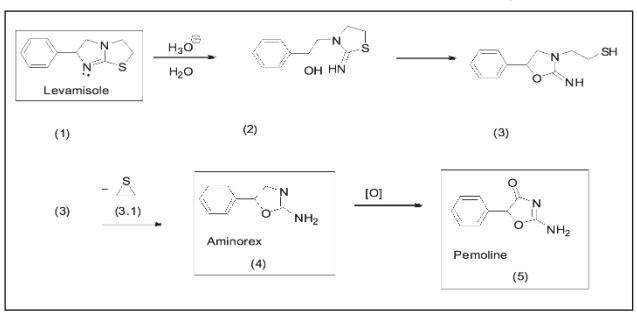

Figure 1: Proposed conversion of Levamisole to Aminorex to Pemoline. Levamisole (1) can protonate to form intermediate 2, which can undergo alkylation of the double bond to form 3, with loss of thiirane, 3.1, to yield Aminorex 4. Aminorex can then undergo biological oxidation [O] to form Pemoline 5 (Eisenberg 2008; Ho, 2009).

Furthermore, pemoline has an unusually long (150 hour) apparent terminal half-life in horse urine and is thus likely to yield prolonged 'detection times' in post-race urine testing in horses, as shown by members of our research group 30 years ago (Tobin 1981). As such, it seems probable that one of the last metabolites to be detected after levamisole administration would be pemoline, as has apparently happened in the case of our English colleague,

and as has also apparently occurred previously in English racing (Tobin 2009).

Consistent with this proposed mechanism, the horses involved in the recent English pemoline 'positive' had been administered levamisole on the recommendation of the trainer's veterinarian. Levamisole was administered as a clear yellow solution containing 3% w/v Levamisole Hydrochloride Ph.Eur. Levacide 3% Drench, which is described as a broad spectrum anthelmintic for use in the treatment and control of nematode infections in cattle and sheep. at 2.5ml per 10kg of bodyweight (.http://www.norbrook.co.uk/products/ProductPrintable.cfm/product_Key/425/CatKey/1/Section/Veterinary_Products/).

Consistent with the above chemistry, two of these horses yielded positives for pemoline and a third horse was reported positive for Tetramisole, entirely consistent with the close structural relationship between levamisole and tetramisole. (Tobin 2009).

With respect to the matter of the regulatory outcome of this very unusual circumstance, we immediately communicated the results of our researches as outlined in this report to the trainer and veterinary surgeon involved. Additionally, we can report that these findings were of relevance to an enquiry held by the British Horseracing Authority Disciplinary Department, in that "the Panel was satisfied that the source of both pemoline and tetramisole was a five-day course of 120ml of Levacide for [horse #1] and [horse #2] and [horse #3] and a four-day course for [horse #4], which commenced on August 18, 2009 and was given under veterinary advice." (http://www.britishhorseracing.com/resources/about/whatwedo/disciplinary/disciplinaryDetail.asp?item=091148)

In conclusion, administration of levamisole and presumably also tetramisole to horses which are likely to be medication tested should be avoided. This is because in the horse levamisole, and possibly also tetramisole can metabolise to two central nervous system stimulants, aminorex and pemoline, and as such have been associated with 'positive' calls for each of these substances in post-race urines.

In point of fact, in the US, the link between levamisole administrations and aminorex identifications has led to the reclassification of levamisole, previously a substance of essentially no regulatory interest, as an ARCI class two agent (ARCI website). This is an explicit regulatory recognition of the ability of levamisole administration to produce aminorex 'positives' and now, based on these English reports, pemoline 'positives' in post-race urines.

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Exhibit

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RESEARCH Open Access

Aminorex identified in horse urine following consumption of *Barbarea vulgaris*; a preliminary report

George Maylin[1], Clara Fenger[2], Jacob Machin[3], Sucheta Kudrimoti[3], Rodney Eisenberg[4], Jonathan Green[5] and Thomas Tobin[3*]

Abstract

Background: Aminorex, (RS)-5- Phenyl-4,5-dihydro-1,3-oxazol-2-amine, is an amphetamine-like anorectic and in the United States a Drug Enforcement Administration [DEA] Schedule 1 controlled substance. Aminorex in horse urine is usually present as a metabolite of Levamisole, an equine anthelmintic and immune stimulant. Recently, Aminorex identifications have been reported in horse urine with no history or evidence of Levamisole administration. Analysis of the urine samples suggested a botanical source, directing attention to the Brassicaceae plant family, with their contained GlucoBarbarin and Barbarin as possible sources of Aminorex. Since horsepersons face up to a 1 year suspension and a $10,000.00 fine for an Aminorex identification, the existence of natural sources of Aminorex precursors in equine feedstuffs is of importance to both individual horsepersons and the industry worldwide.

Results: Testing the hypothesis that Brassicaceae plants could give rise to Aminorex identifications in equine urine we botanically identified and harvested flowering Kentucky *Barbarea vulgaris*, ("Yellow Rocket") in May 2018 in Kentucky and administered the plant orally to two horses. Analysis of post-administration urine samples yielded Aminorex, showing that consumption of Kentucky *Barbarea vulgaris* can give rise to Aminorex identifications in equine urine.

Conclusions: Aminorex has been identified in post administration urine samples from horses fed freshly harvested flowering Kentucky *Barbarea vulgaris*, colloquially "Yellow Rocket". These identifications are consistent with occasional low concentration identifications of Aminorex in equine samples submitted for drug testing. The source of these Aminorex identifications is believed to be the chemically related Barbarin, found as its precursor GlucoBarbarin in Kentucky *Barbarea vulgaris* and related Brassicaceae plants worldwide.

Keywords: Horse, Urine, Brassicaceae, *Barbarea vulgaris*, Aminorex, Drug testing

Background

Aminorex, (*RS*)-5-Phenyl-4,5-dihydro-1,3-oxazol-2-amine ($C_9H_{10}N_2O$, MW 162.19, Fig. 1) is an amphetamine-like anorectic and central stimulant medication and a United States Drug Enforcement Administration [DEA] Schedule 1 controlled substance. In the nineteen sixties, Aminorex was marketed as a prescription appetite suppressant in Europe but was withdrawn when it was found to be associated with a significant incidence of cases of fatal pulmonary hypertension [1].

In the United States Aminorex began to be identified and reported as "positives" in equine samples in 2004, starting in Ohio and later in Pennsylvania and Ontario, totaling 80-plus identifications [2]. Aminorex is an Association of Racing Commissioners International [ARCI] Class 1, Penalty class A foreign substance, associated with a high potential for influencing a horse's racing performance owing to its stimulant properties. These identifications led to significant penalties against horsemen [3] but the Aminorex identifications continued, indicating that horsemen were unaware of the source of these

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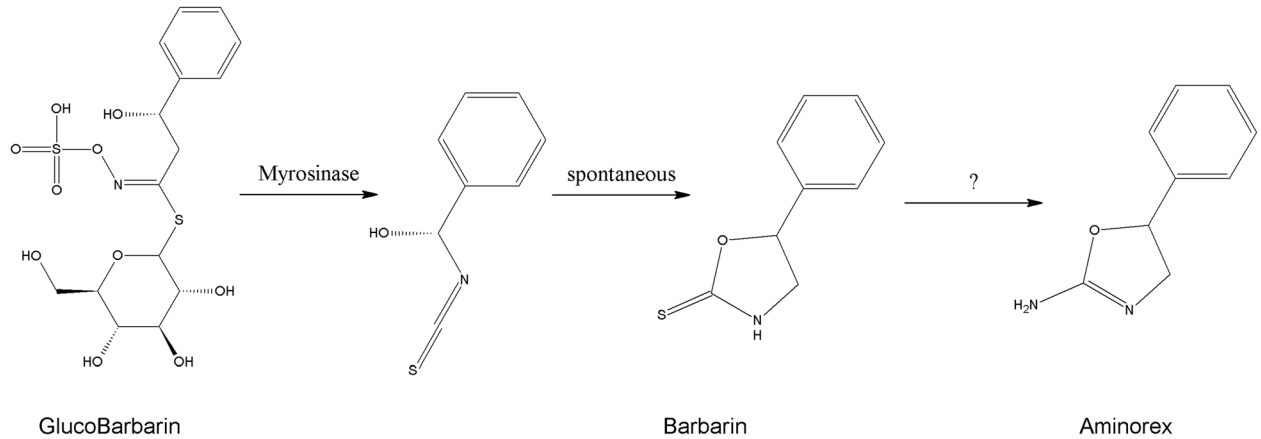

Fig. 1 Conversion of GlucoBarbarin (via Myrosinase) to Barbarin and Aminorex structure

identifications [4]. Penalty A violations include a minimum one-year suspension absent mitigating circumstances, a minimum fine of $10,000 (or 10% of the total purse) for the trainer as well as disqualification of the purse and placing of the horse on the Veterinarian's List for 180 days for the owner. (ARCI 2017), not insignificant penalties for a professional horseman.

Levamisole, an anthelmintic and immune stimulant used in horses and livestock, was implicated as a source of Aminorex identifications in 2007 [2]. Levamisole was originally identified as an immune modulator for horses in 1997 [5], and gained popularity as an adjunctive treatment for Equine Protozoal Myeloencephalitis over the following decade. In 2009 Ho et al. identified Levamisole metabolites, Rexamino and Compound II, and concluded that these metabolites could be co-identified with Aminorex in cases where the Aminorex identification resulted from treatment with Levamisole [6]. The association of Levamisole with Aminorex led to the classification of Levamisole by ARCI as a Class 2 Penalty Class B foreign substance.

While the identification of Levamisole as a source of Aminorex led to a sharp reduction in the number of Aminorex identifications, it has not eliminated such findings, and in North America sporadic unexplained Aminorex identifications have occasionally been reported [2]. Additionally, a number of recent Aminorex identifications have been reported in English sport horses where there was no history of Levamisole administration and no co-identifications of the expected metabolic markers of Levamisole administration [7].

These findings suggested an Aminorex source other than Levamisole and careful review of substances co-identified in these samples showed a number of plant-related, low molecular weight nitrogenous substances. These findings and review of the botanical literature led to the suggestion that GlucoBarbarin (Fig. 1) or its hydrolysis product Barbarin contained in certain plants and originally identified in *Barbarea vulgaris*, the plant from which the compound Barbarin was named, might be the source of these Aminorex identifications [8]. Additionally, our English colleagues reported identification of Aminorex in at least one presumably European member of the genus Barbarea [7] in the Brassicaceae family. Based on these reports, we identified a well-known member of the Brassicaceae family which flowers from late April to early May in Kentucky pasture, Yellow Rocket (*Barbarea vulgaris*, Fig. 2) and harvested this plant in flower for administration to horses, as we now report. Further, we have also synthesized and chemically purified Barbarin for use as a Certified Reference Analytical Standard and in enough quantities for equine administrations if required [9]. This reference standard was made available for use in the Aminorex related plasma and urine analyses reported in this communication.

Results

Flowering Yellow Rocket plants were harvested, cleaned, and administered to horses with pre- and post-administration blood and urine test samples collected as described in the Experimental section, below. Analyses of serum and urine samples for Aminorex and Barbarin were performed in the New York Drug Testing and Research Program Laboratory using their International Association of Standardization [ISO]-17,025 validated analytical methods. Mass Spectral data obtained for Aminorex in urine after Yellow Rocket administrations are presented in Fig. 3. The chromatogram of an 8-h urine extract scanned from 100 to 500 amu is shown in Fig. 3a. Figure 3b shows the extracted protonated ions, masses 163 and 120 which were used to identify Aminorex in the complex urine matrix of Fig. 3a. The Liquid Chromatography-

Fig. 2 Yellow Rocket, *Barbarea vulgaris* growing in Ithaca, NY, May 21th 2018

Mass Spectroscopy [LC/MS] data for the determination of Aminorex in Yellow Rocket administrations are shown in Fig. 4. The transition for Selected Ion Monitoring of Aminorex was 163.1 > 103.1. Panel A shows the chromatogram of the pre-administration urine, Panel B shows a 4-h urine extract, Panel C shows the chromatogram of a 12-h urine extract, Panel D shows the chromatogram of an 8 h urine extract, Panel E shows a pre-administration urine extract and Panel F shows the chromatogram of the Aminorex standard. All blood samples tested negative for Aminorex,

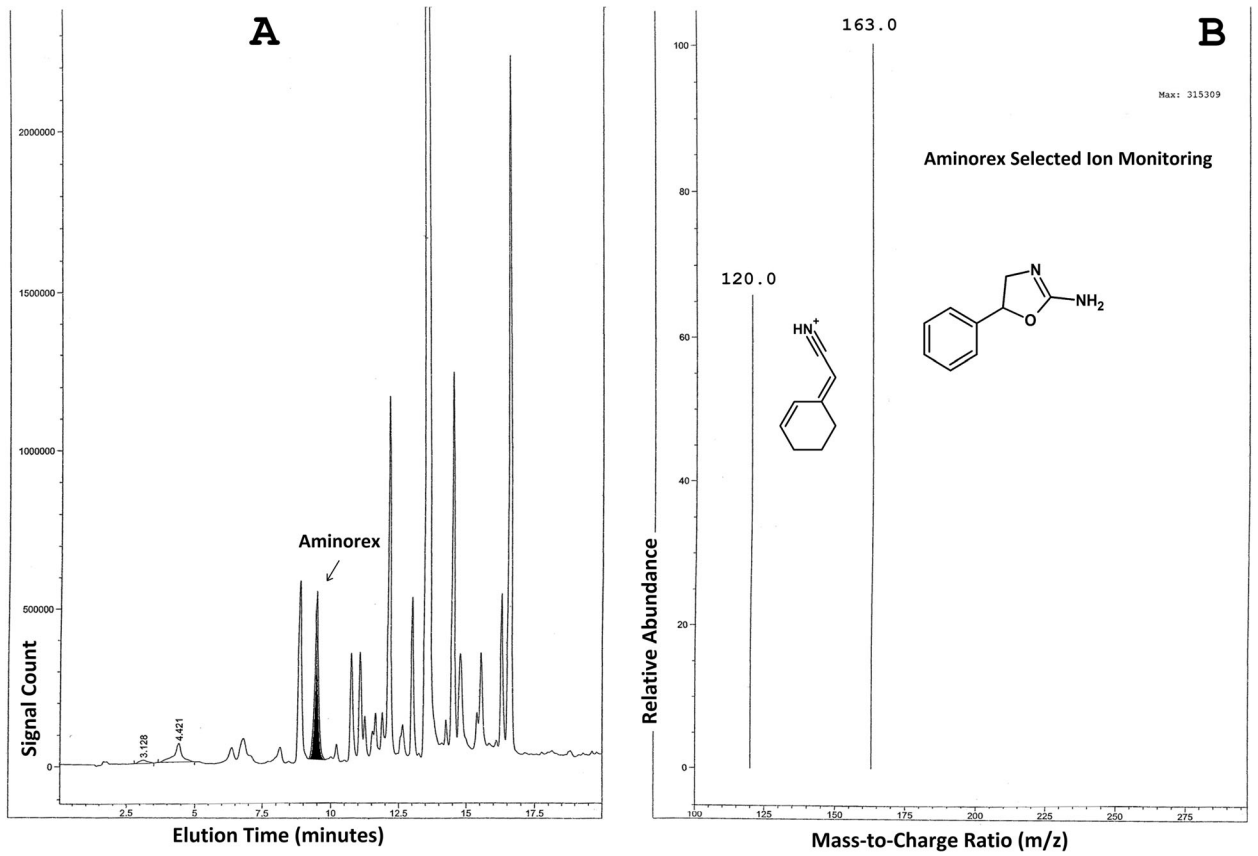

Fig. 3 a: Chromatogram of a post-8-h Yellow Rocket administration urine extract scanned from 100 to 500 am with the Aminorex peak identified. Aminorex peak determined as such by the presence of the protonated ion of Aminorex at 163 am and the major daughter ion at 120 amu, as shown in Fig. 3b. **b**: The extracted protonated ions, mass 163 (Aminorex parent ion) and mass 120 (major Aminorex daughter ion) which were used to identify Aminorex in the complex urine matrix of Fig. 3a

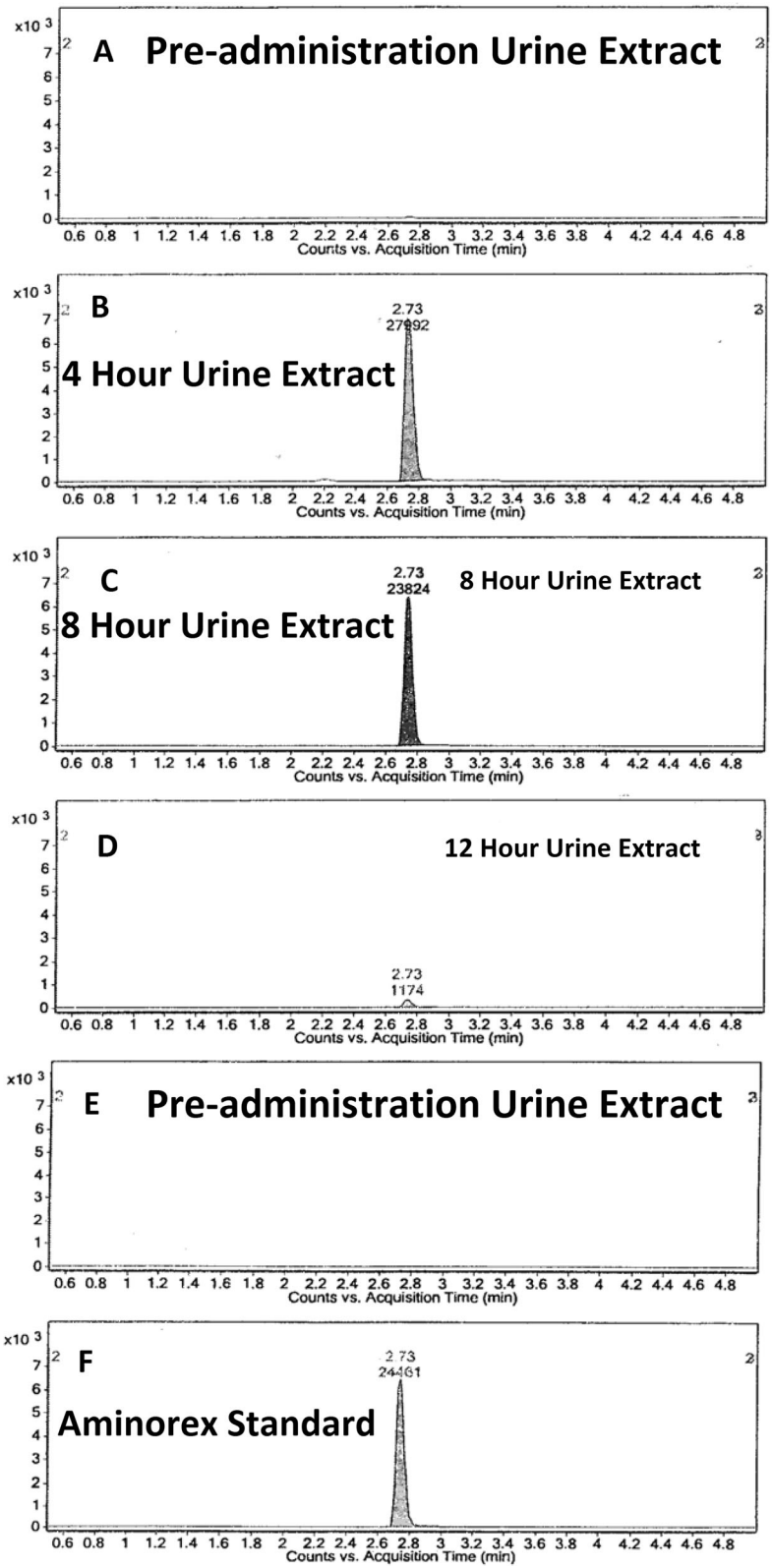

Fig. 4 (See legend on next page.)

(See figure on previous page.)
Fig. 4 The 6470 Triple Quadrupole LC/MS data for the determination of Aminorex in pre- and post- Yellow Rocket administration urines. The transition for Selected Ion Monitoring of Aminorex was 163.1 > 103.1. **a** shows the chromatogram of the pre-administration urine extract, **b** shows the chromatogram of a 4 h post–administration urine extract, **c** shows the chromatogram of a 8 h post–administration urine extract, **d** shows the chromatogram of a 12 h post–administration urine extract, **e** shows the chromatogram of a pre-administration urine extract demonstrating no carry-over, and **f** shows the chromatogram of the Aminorex reference standard. No evidence for the presence of Barbarin in these post *B. vulgaris* administration blood or urine samples has been identified to date

and both blood and urine samples tested negative for Barbarin. Pre-administration urine samples were all shown to be negative for both Aminorex and Barbarin.

Discussion

The identification of Aminorex in urine samples from horses administered *Barbarea vulgaris* is both consistent with and supports the findings and interpretations of our English colleagues [7]. Multiple members of the Brassicaceae family, including *Barbarea vulgaris,* produce GlucoBarbarin, which may be responsible for ongoing sporadic Aminorex identifications reported in post-event equine urine samples in the absence of evidence of Levamisole administration. At this time, the precise mechanism for the conversion of Barbarin to Aminorex is unclear, and it is possible that some other compound produced by *Barbarea vulgaris* is responsible. However, based on the substantial structural similarities between Aminorex and Barbarin, Barbarin is at this time the most likely candidate for the primary source of Aminorex in the Brassicaceae family.

The role of GlucoBarbarin in Brassicaceae is to serve as a Barbarin precursor. When the plant is damaged, the co-located enzyme, myrosinase, hydrolytically removes the glucose molecule from GlucoBarbarin yielding an intermediate which spontaneously cyclizes to Barbarin. GlucoBarbarin then may act like other glucosinolates in protecting the plant from potential predators [10]. Consistent with this protective function of Barbarin, the Yellow Rocket plant material was refused by the experimental horses until mixed with grass and sweet feed.

These findings are of practical significance for horse racing regulation [11], in that there are now two innocent, inadvertent, and completely unrelated sources of Aminorex identifications in post-race urine samples. The first innocent source is Levamisole, as a result of inadvertent transfer of Levamisole from its use as an anthelmintic or as an immune modulator, commonly recommended as a component of treatment for Equine Protozoal Myeloencephalitis. The second innocent source would be, as reported here, exposure to Aminorex and/or Aminorex precursors associated with inadvertent inclusion of plant fragments of the Brassicaceae family in equine feedstuffs and their subsequent ingestion by horses about to race.

Aminorex identifications in post-race urines following Levamisole administration may be confidently identified as Levamisole related if other post-Levamisole administration metabolites, Rexamino and Compound II, described by Ho et al. [12] are identified in the sample. The inability to identify these other Levamisole metabolites in the English sport horse samples initiated an investigation for other likely sources of Aminorex, leading to identification of the Brassicaceae as a likely source of post-race trace Aminorex identifications [7].

Based on the wide distribution of *Barbarea vulgaris* in North America [Fig. 5] and the high level of sensitivity of post-race testing, it seems likely that a background level of sporadic inadvertent Aminorex identifications will be ongoing in American racing. One approach to addressing this issue is to identify biomarkers of exposure to Brassicaceae plants, which would allow confident identification of Brassicaceae as the source of these Aminorex identifications. At this time Barbarin, as has recently been synthesized and certified by our research program [9] would appear to be the first biomarker candidate for this role, but further research on administration of Brassicaceae and Barbarin itself to equines is likely required in this area.

A limitation of this research is that, given the worldwide distribution of Brassicaceae, we cannot at this time identify the geographic extent of this potential problem. A further concern is that the pharmacological significance of low concentration urinary Aminorex identifications is unclear. Based on the behavioral observations of Soma et al. [3] it seems that plasma concentrations of Aminorex must be at least 2 ng/mL for pharmacological effect. In fact, the brief five-minute duration of the pharmacological responses to IV administration of 15 mg of Aminorex reported by Soma et al. [3] suggests that these pharmacological responses are transient local high CNS concentration bolus responses, followed immediately by complete loss of pharmacological effect. Consistent with this interpretation, oral 15 mg administration of Aminorex produced no observable pharmacological responses. Given the fact that this orally ineffective dose produced urinary concentrations of Aminorex averaging about 75 ng/mL, it seems reasonable to suggest that urinary concentrations of Aminorex less than 75 ng/mL are unlikely to be associated with pharmacological effects.

With respect to identifying a Screening Limit of Detection (SLOD) for Aminorex in post-race urines, a cut-off / SLOD should be *"high enough to cut-off the environmental*

Fig. 5 Distribution of Yellow Rocket, *Barbarea vulgaris*, in the United States; Data re-constructed from USDA reports [13]. The British Isles show similar distribution, with only parts of Scotland having none reported as of May, 2019 ([14]

noise and low enough to stop performance enhancement" as described in [15]. Given that a sequence of five urinary Aminorex identifications of unknown origins in Massachusetts during 2017 were all less than 20 ng/mL it would seem reasonable to set a urinary interim Screening Limit of Detection of 30 ng/mL (Fenger, personal communication, July 2019), in the absence of a significant number of data points on which to base a more complete analysis.

In presenting this interim 30 ng/mL SLOD we particularly note the unusually long terminal plasma half-life of Aminorex, 46.6 h in one horse in the Soma study. This terminal plasma half-life means that individual horses exposed to an ongoing dietary source of Barbarin will accumulate urinary concentrations over a nine-to-ten-day period, increasing the probability of attaining detectable urinary concentrations of Aminorex. Given this circumstance, it is important that reported Aminorex identifications include best good faith estimates of the concentrations of Aminorex identified. These data will allow evaluation of the contributions of geographically different environmental sources to urinary Aminorex identifications and the above interim Screen Limit of Detection can be adjusted if required, following the model presented by [16].

Other species within the Brassicaceae family may be similarly implicated as likely candidates for the introduction of GlucoBarbarin/Barbarin to animals. The plant *Reseda luteola,* more commonly known as "weld" or Dyer's Rocket is similarly known to produce GlucoBarbarin [17]. This plant is widely distributed throughout Europe and has been introduced to many parts of the United States. Historically used as a source of yellow dye, it was also grown domestically for its sweet aromatic smell. Based on the results herein reported with respect to *Barbarea*

vulgaris it is reasonable to assume that if *Reseda luteola* were to be ingested a measurable level of Aminorex might also be detectable in equine urine.

Conclusion

In conclusion, oral administration of *Barbarea vulgaris* / Yellow Rocket plant material to two horses has resulted in urinary Aminorex identifications. The proximate chemical source of these Aminorex identifications is likely to be GlucoBarbarin/Barbarin, long-recognized components of the Yellow Rocket plant. Given these circumstances and the widespread distribution of such plants in North America and elsewhere, Yellow Rocket or related Brassicaceae plants are likely sources of sporadic low-concentration Aminorex identifications in the sports horse worldwide. Future research will focus on identifying biomarkers of Yellow Rocket or other Brassicaceae to definitively identify botanical origins for Aminorex identifications and the acquisition of enough field data to support or appropriately adjust our proposed 30 ng/mL environmental "cut-off" or Screening Limit of Detection (SLOD) for pharmacologically and forensically insignificant Aminorex identifications in equine drug testing.

Methods

Yellow rocket identification and harvest

Barbarea vulgaris plants in bloom were harvested from a central Kentucky pasture in mid-May, 2018, with the landowner's consent and consistent with State of Kentucky plant harvesting regulations. The harvest plants were identified as *Barbarea vulgaris* / Yellow Rocket by Dr. J. D. Green of the Department of Plant and Soil Sciences of the University of Kentucky. Briefly, stems were ribbed and hairless with shiny dark leaves located in basal rosettes. Basal leaves were stalked with a large terminal lobe and smaller lower lobes. All leaves were alternate with wavy and toothed margins. The flowers were present in terminal clusters above the foliage. The identified plants were cleaned of extraneous material and shipped overnight to the New York State Drug Testing and Research Program for equine administration and forensic analysis and equine administration.

Animal administration and sample collection procedures

The entire plant, including roots, leaves, stems and flowers were ground to a fine texture in an Oster Total Prep 10-C Food Processor. A total of 0.45 kg of wet weight processed plant material was presented to each of two horses. *Barbarea vulgaris* plant material was refused by the horses when presented alone but was readily consumed when mixed with an equivalent amount of sweet feed and fresh cut grass. Blood and urine samples were collected as pre-administration zero-time samples and then at 4, 8, and 12 h post administration.

Mature Standardbred mares weighing between 400 and 540 kg and aged 8 and 11 years were used for these administration experiments. These horses were part of the New York State Drug Testing and Research Program research herd and were in good health and monitored by experienced veterinary clinicians. Mares were maintained in their home box stalls and all sample collections and substance administrations were performed in their home stalls. Blood samples were collected by jugular venipuncture and urine samples by bladder catheterization and draining of the bladder at the indicated time intervals, as previously described [18]. Blood samples were allowed to clot, then centrifuged at 1500 g for 10 m and the serum decanted and stored at -20 °C until analysis. Urine samples were similarly aliquoted, sealed, and stored at -20 °C until analysis. All physical analyses occurred within 20 days of collection. All samples were collected under the supervision of New York State Drug Testing and Research Program personnel and transferred to the New York State Drug Testing and Research Program laboratory where they were logged in as dated and numbered research samples.

Extraction

Aminorex was extracted from 4.0 ml urine samples adjusted with pH 9.5 carbonate buffer using 4.0 ml of ethyl acetate/hexane solvent (9: 1 v/v). The samples were mixed on a rotary mixer and centrifuged for 1 0 min each. The organic phases were transferred to concentration tubes and evaporated to dryness in a 50 °C water bath under nitrogen flow. The residues were reconstituted in methanol for LC/MS analysis.

Liquid chromatography/mass spectrometry

An Agilent Technologies 6150 B Quadrupole LC/MS System and an Agilent 64 70 Triple Quadrupole LC/MS System in positive ionization modes were used for screening and confirmatory analysis respectively. Each instrument used 1290 Infinity Auto Injectors and thermostated column compartments. The 6150B used an Agilent Eclipse AAA, 3 × 100 mm liquid chromatography column. The LC gradient consisted of 100% A (100%/0.1%formic acid) for 5 min and then a linear gradient to 100% B (90% methanol/10% 0.1 formic acid) at 20 min at a flow of 0.4 mL/minute. Column compartment was 40 °C. The 6470 Triple Quadrupole used an Agilent Poroshell EC-120, 3.0 × 100 LC column. An LC gradient of acetonitrile/formic acid in the following composition was used: (90% formic acid/10% acetonitrile) for 1 m, (5% formic acid/95% acetonitrile) for 4.5 min and (90% formic acid/10% acetonitrile) at 5 min. Flow rate was 0.4 ml/minute and column temperature was 50 °C. Limit of Detection was 1 ng/mL and urinary concentrations of Aminorex were estimated to be approximately in the range of 10 ng/ml. No Aminorex

was detected in the post-administration plasma samples, and no Barbarin was detected in either the plasma or urine samples.

Abbreviations

ARCI: Association of Racing Commissioners International; DEA: Drug Enforcement Administration (USA); IACUC: Institutional Animal Care and Use Committee; ISO: International Organization for Standardization; LC/MS: Liquid Chromatography/Mass Spectroscopy; SLOD: Screening Limit of Detection

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Authors' contributions

Pasture sources of Kentucky Yellow Rocket were identified by CF and JM, who identified and collected the *B. vulgaris* plant material growing in Kentucky for this research project. The collected samples were positively identified as *B. vulgaris* by JDG and processed, dried and shipped to GM who administered them to the test horses and analyzed the blood and urine samples for Barbarin and Aminorex. Certified reference standard Barbarin for the analytical work was synthesized, analytically characterized and certified by JM, SK and RE and provided to GM as a reference standard. JM, SK, and RE, GM and TT verified data from the synthetic and analytical chemistry work. All authors contributed scientific expertise to the underlying Barbarin➔Aminorex concept and experimental design, data interpretation, figure preparation, and the writing and editing of the manuscript and all authors approved the final manuscript submitted for publication.

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Availability of data and materials

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

Ethics approval and consent to participate

Animal administration was approved via Institutional Animal Care and Use Committee [IACUC] Protocol Number EHWA0818, as filed by Dr. Clara Fenger at Equine Integrated Medicine, 4904 Ironworks Rd., Georgetown, KY.

Consent for publication

Not applicable

Competing interests

The authors declare that they have no competing interests.

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Publisher's Note

Exhibit

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CONFIDENTIAL CLOSE TO FINAL DRAFT

PEMOLINE, A CENTRAL NERVOUS SYSTEM STIMULANT REPORTEDLY OCCURRING NATURALLY IN EQUINE SAMPLES IN EUROPE AND ELSEWHERE: A REVIEW AND ANALYSIS.

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SUMMARY 299 words

Pemoline, (*RS*)-2-amino-5-phenyl-1,3-oxazol-4(5*H*)-one, is a member of the 4-oxazolidinone group of substances and a central nervous system stimulant closely related structurally and pharmacologically to aminorex. With the increased sensitivity of equine drug testing, low concentration identifications of pemoline have increasingly been identified in horse racing. In 2009 two pemoline and one tetramisole identification in English racing were reported in horses administered levamisole, leading to suggestions that levamisole, known to metabolize to aminorex, could also metabolize to pemoline. In April 2016 the French, German, and South African horseracing laboratories reported frequent identifications of pemoline in equine urine samples, such that these laboratories had "*in-house*" reporting limits below which concentrations urinary pemoline identifications were not reported. In 2016 and again in 2018 the matter of potential pemoline identifications in Indiana horse racing was communicated by the Indiana Horse Racing Commission to Indiana horsemen, with requests that horsemen avoid use of levamisole. Soon thereafter, in late 2018, we became aware that plant barbarin was a potential source of equine urinary aminorex identifications. In Spring 2019 we therefore harvested flowering Kentucky "Yellow Rocket" (*Barbarea vulgaris*), a barbarin-containing invasive plant widely distributed in North America including Kentucky and administered it orally to horses. Urine samples collected from these horses were found to contain aminorex. Aminorex, closely related chemically and pharmacologically to pemoline, is therefore a naturally occurring alkaloid that may be identified in equine urine, suggesting possible similar botanical origins for pemoline, now not infrequently being identified at low ng/mL concentrations in equine urine samples in Europe and elsewhere. Addressing the regulatory implications of these findings, we have therefore reviewed the pharmacological literature on pemoline in the horse and using the Toutain & Lassourd safety factor of 500 now propose 2 ng/mL of pemoline as an Irrelevant Plasma Concentration (IPC) of pemoline in horses.

INTRODUCTION: PEMOLINE AND AMINOREX:

Pemoline (*RS*)-2-amino-5-phenyl-1,3-oxazol-4(5*H*)-one, Figure 1 below, is a member of the 4-oxazolidinone group of substances and is closely related structurally to aminorex and 4-methylaminorex.[1] Like aminorex, pemoline is a central nervous system stimulant, first marketed for use in human medicine in Europe in the nineteen sixties. In 1975 it was approved in the United States for the treatment of Attention-Deficit/Hyperactivity Disorder (ADHD) in persons 6 years of age and older and it has also been used in the treatment of narcolepsy. In 1996 Abbot Laboratories alerted the medical community to cases of serious liver toxicity and deaths associated with its marketed pemoline formulation, Cylert®. This association with a significant incidence of liver toxicity resulted in the withdrawal of pemoline from therapeutic use in the United Kingdom in 1997, Canada in 1999 and the United States in 2005.[2]

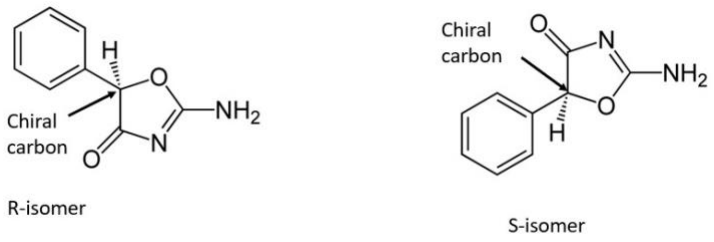


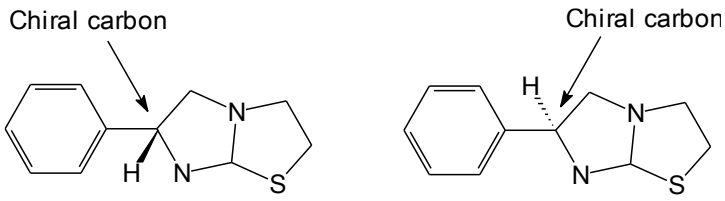
Figure 1: Pemoline, (*R and S*)-2-amino-5-phenyl-1,3-oxazol-4(5*H*)-one, $C_9H_8N_2O_2$, molar mass, 176.175 g·mol^{-1}, pKa 10.5. Chemically, pemoline exists as two enantiomers, the R-enantiomer above left and the S-enantiomer, above right. The pemoline previously approved for therapeutic use in the United States and elsewhere was racemic pemoline.

In horse racing in the United States, pemoline is classified by the Association of Racing Commissioners International (ARCI) as a Drug Class 1, Penalty Class A substance, reflecting its close pharmacological relationship to methylphenidate[3], also a Class 1, Penalty Class A substance. In the United States Horseracing Integrity and Safety Authority (HISA) regulatory system, pemoline is classified as a SO or Banned Substance, with the proviso that "*Pemoline is a metabolite of aminorex, which is a metabolite of levamisole. If there is credible evidence that the detection of pemoline in a horse's sample is the consequence of exposure to levamisole, the classification of pemoline may be revised to S7 (B)*"[4], to our knowledge the penalty classification for an approved therapeutic medication "overage".

We will now review current knowledge of the relationships between levamisole, aminorex and pemoline and the reported detection of pemoline in equine post-race samples world-wide, starting with a 2009 sequence of events in English racing.

PEMOLINES REPORTED IN BRITISH RACING ASSOCIATED WITH LEVAMISOLE ADMINISTRATION:

The first sequence of pemoline identifications that drew attention to a possible relationship between a levamisole administration, Figure 2 below, and post-race detection of pemoline was a circumstance in 2009 in British racing where a number of horses were treated on a veterinarian's advice with Levacide, a levamisole-containing product. Two horses tested "positive" for pemoline, with a third horse testing "*positive"* for tetramisole. Reviewing this situation, we noted the close structural relationship between aminorex, a well-recognized metabolite of levamisole, and pemoline, wherein pemoline would be a simple oxidation product of aminorex. These chemical facts were communicated to the British Horseracing Authority Disciplinary Department which held that "*the Panel was satisfied that the source of both pemoline and tetramisole was a five-day course of 120ml of Levacide for [horse #1] and [horse #2] and [horse #3] and a four-day course for [horse #4], which commenced on August 18, 2009 and was given under veterinary advice."*[5] (ref Gutierrez)



Dexamisole R-enantiomer S-enantiomer **Levamisole**

Figure 2. Tetramisole, *(R and S)*- 6-Phenyl-2,3,5,6-tetrahydroimidazo(2,1-b) thiazole, $C_{11}H_{12}N_2S$, molar mass 204.29 $g \cdot mol^{-1}$. Chemically, tetramisole exists as two enantiomers, the R-enantiomer dexamisole (above left) and the S-enantiomer levamisole (above right). The S-enantiomer, i.e., levamisole is the more biologically active enantiomer.

THE EUROPEAN AND SOUTH AFRICAN EXPERIENCE WITH PEMOLINE

Based on extensive international forensic experience as well as its status as a potential metabolite of levamisole, it now appears that pemoline – like aminorex – may also be a naturally occurring substance detected in equine urine. We first became aware of this possibility in correspondence with analytical colleagues concerning a claimed pemoline identification in Canadian horse racing. Discussions concerning this matter led to a worldwide exchange of correspondence on the matter of low concentration urinary identifications of pemoline in European horses and the then and still unknown origins of these European pemoline identifications. The first communication was on April 8th, 2016, from Dr. Yves Bonnaire, Director of the French Laboratoire des Courses Hippiques, LCH in France. In this communication Dr. Bonnaire set forth as follows.[6]

"Dear colleagues,

We are frequently finding small amount of pemoline in our routine (French and overseas) samples as well as in our experimentation horses (having received no pemoline or tetramisole or any related antiparasitic drug). The apparent concentration is sub nanogram up to 5 ng/ml. The drug is fully characterized (SRM and HRMS). These findings do suggest a natural occurring origin. Is there any other lab(s) having similar experience?

Best regards

Yves" (Our underlining of the "*natural occurring origin"* related wording)

On April 9th, 2016, Dr. Terence Wong of the Hong Kong Jockey Club Laboratory noted that his laboratory was the one that specifically identified pemoline at an estimated concentration of 2.5 ng/mL in a sample received from the French Laboratory concerning a pemoline unrelated matter.[7] On April 12th, 2016, Dr. Bonnaire expanded his response concerning pemoline in LCH samples as follows and noted that the French laboratory was using a 5 ng/mL urinary concentration of pemoline as an "*in house"* reporting limit.[6]

"Now we are screening by Q-exactive and as the sensitivity has increased almost all routine samples do contain pemoline at low level. This is why we do not consider pemoline at low level as potentially reportable as we have too many +ve detection. This molecule is also found in our experimentation samples (as explained in my previous email), we have conducted at the time (long time ago) several analysis which were not conclusive and we decided at the time to adopt an in house reporting level (5ng/ml). This is why several of our negative sample do contain pemoline or pemoline like substances."

Similarly, on April 12th, 2016, Dr. Marc Machnik[8] of the German Sport University reported that his laboratory also detected pemoline at low concentrations in equine urine and that, based on these findings, they have concluded that they should not report pemoline at concentrations below 10 ng/mL in urine samples. Likewise, Dr. Magda Rosemann, a scientist working in the South African equine drug testing laboratory also reported detecting pemoline in their South African samples when they screened these samples at high sensitivity.[9]

PEMOLINE CONCERNS IN INDIANA RACING

Consistent with these reports of urinary identifications of pemoline in European and South African racing horse samples, on May 23rd, 2016 the Indiana Horse Racing Commission (IHRC) issued an "*IHRC Advisory Notice to Horsemen*" entitled "*Horsemen Reminded to Avoid Products Containing Levamisole and Tetramisole*" based on the fact that there is "very *strong evidence in the scientific literature…that the administration of levamisole and potentially tetramisole, to horses results in production of aminorex and pemoline as metabolites".*[10]

Further consistent with the above referenced 2016 "*IHRC Advisory Notice to Horsemen,*" on July 20th, 2018 one author (TT) was contacted by Dr. Michael Mann, DVM, the Indiana Director for the North American Association of Racetrack Veterinarians. He reported verbal warnings from Indiana Horse Racing Commission personnel to a number of trainers at Indiana Grand racetrack concerning the reported presence of trace amounts of pemoline in analytical samples and warning Indiana horsemen about the use of levamisole.[11] The approach taken on this matter in Indiana racing was to not take regulatory action on these apparently low concentration pemoline screening identifications but to simply warn the involved horsemen about the risks of using levamisole, one possible innocent source of low concentration pemoline identifications in equine urine samples.

The problem with these Indiana notifications linking levamisole and pemoline was that the Indiana horsemen were completely unaware of any sources of levamisole in their barns which made their avoidance of levamisole use a moot issue. These regulatory notifications also left the horsemen aware and concerned that their horses were presenting potential analytical identifications of pemoline despite there being, to their knowledge, no known sources of pemoline in the diet or immediate environs of these horses.

Discussions with personnel at the Indiana Racing Commission testing laboratory established that these communications were based on screening identifications of pemoline and not on confirmed identifications. On July 23rd the regulatory significance of the reported lack of confirmation of these trace level screening pemoline identifications was communicated to Dr. Mann[12] and on July 25th the same was formally communicated to the Indiana Horse Racing Commission.[13] Thereafter the matter of these trace level screening identifications of pemoline in racing samples in Indiana has not been presented to Indiana horsemen as a regulatory concern.

In a related analysis of this Indiana pemoline situation on or about July 19th, 2018, one author (TT) sent a report to the Executive Director of the North American Association of Racetrack Veterinarians (NAARV), Dr. Clara Fenger, summarizing the facts and speculations presented above and setting forth that at least two European Laboratories had adopted "*in-house*" reporting limits for pemoline in post-race urine, one at 5 ng/mL and one at 10 ng/mL, as set forth above.[14] This communication also made clear that at that time, July, 2108, the source(s) of the pemoline findings in Europe was or were unknown, and that the existence of this long known, unidentified and possibly natural source of pemoline findings in European racing urine samples needed to be taken into account when evaluating the regulatory significance of low concentration pemoline findings in US racing samples or elsewhere.

LEVAMISOLE AND PEMOLINE IDENTIFICATIONS / "POSITIVES" IN US RACING.

Given the above reported identifications of pemoline in British, French, German and South African post-race urine samples, we elected to review the number of

pemoline identifications reported in US racing. Our first request to the Association of Racing Commissioners International (ARCI) was for a listing of pemoline identifications reported in US racing samples. Only one pemoline identification was reported and when we reviewed this identification it was clear that this identification was a Levamisole-associated pemoline identification.

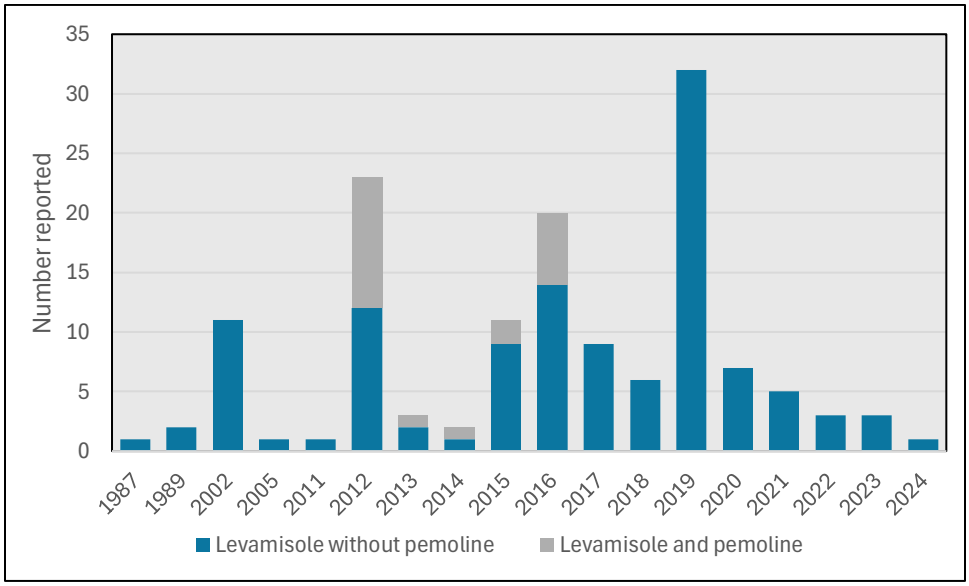


Figure 3/ Numbers of levamisole identifications reported in US racing 1987 to date. Levamisole identifications with no pemoline reported, blue columns, samples containing both levamisole and pemoline, grey columns. Note that in 2012 New York reported 11 of 12 post-race urine samples as "positive" for Levamisole and also "positive" for pemoline. The ARCI has had no reports of pemoline identifications in the absence of levamisole.

We therefore requested from ARCI a listing of all levamisole identifications, which data are presented in graphic form in Figure 3 above. [15] (REF ARCI Holloway) Levamisole identifications started in 1987 but remained low until 2002 when there were 11 identifications. The next significant number of levamisole identifications was in 2012, with 12 identifications in New York racing, and of these 12 identifications 11 were reported as containing pemoline. Then in 2013 there were 2 Levamisole identifications, one of which was reported as containing pemoline, and 1 levamisole identification in 2014, this identification also reported as containing pemoline. The year 2015 saw 9 levamisole identifications with just 1 pemoline identification while 2016 saw 15 levamisole identifications with 5 pemoline identifications. From 2017 on there were 40 plus levamisole identifications reported but no further reports of pemoline identifications. Overall, the ARCI data show a total of 129 levamisole identifications, and 21 of these samples were also reported to contain pemoline, with no ARCI reports of pemoline identifications in the absence of levamisole.

There are a number of possible interpretations of the above ARCI data. One interpretation is that pemoline is a downstream metabolite of levamisole, with the missing pemoline detections in Figure 3 relating to the fact that some regulatory analysts simply chose not to confirm its presence for purposes of regulatory reporting. This interpretation is consistent with the high percentage, i.e., 91% of "positive" samples reported from the 2012 New York samples contained both levamisole and pemoline and also from the 2016 spike in Illinois, which reported 5 identifications of samples containing both levamisole and pemoline, for a full 100% link between levamisole and pemoline identifications in Illinois. Similarly, the 2019 spike in levamisole alone "positive" samples was largely driven by a large number of levamisole "*positive"* samples in Louisiana racing where the analytical focus appears to have been on detecting and quantifying levamisole in both the plasma and urine samples with no other analytes reported present in these Louisiana levamisole identifications.

A second interpretation of the ARCI data is that suggested by the European experience, namely that pemoline is a naturally occurring substance in at least some horse urines and that the pemoline is therefore randomly likely to be found in association with levamisole in post-race urine samples.

A third interpretation is that the detection of pemoline in a levamisole containing sample is due to pemoline being an analytical artefact associated with the urinalysis procedure, most likely related to the enzymatic or other hydrolysis steps prior to analysis.

A fourth but presumably less likely possibility is that a pemoline finding in any sample is due to an administration of pemoline to the horse in question.

AMINOREX, A LEVAMISOLE METABOLITE FOUND IN HORSE URINE FOLLOWING EXPOSURE TO BRASSICACEAE PLANTS

Shortly after the July 2018 communication was sent to Dr. Fenger and the North American Association of Racetrack Veterinarians (NAARV), we became aware of a report by the LGC laboratory in England of identifications of aminorex in sport horse samples in England with no known exposure of these horses to either levamisole or aminorex.[16] Additionally, the LGC report pointed to botanical barbarin, an alkaloidal substance found in members of the Brassicaceae plant family as likely being at least one natural source of these aminorex findings reported by LGC, which analysis was supported by discussions with a number of colleagues.

A compelling Kentucky related consideration in this matter was that the Kentucky pasture plant *Barbarea vulgaris*, colloquially Kentucky "Yellow Rocket", which flowers in Kentucky pastures from late April to early May is a member of this Brassicaceae plant family, thereby giving rise to the possibility that consumption of this plant by horses could give rise to aminorex findings. In Spring 2019, we therefore harvested Kentucky pasture flowering "Yellow Rocket" plants and fed them to a number of horses, suitably disguising the aversive taste of these plants by mixing them with grass and sweet feed.

As reported in the *Irish Veterinary Journal*, analysis of post-administration urine samples from these horses were "*positive"* for aminorex, revealing that consumption of a naturally occurring Kentucky pasture plant could result in urinary aminorex findings in horses.[17]

These various botanical, biochemical and human behavioral activities that may give rise to urinary identifications of levamisole and pemoline, alone or in combination, are summarized in Figure 4 below.

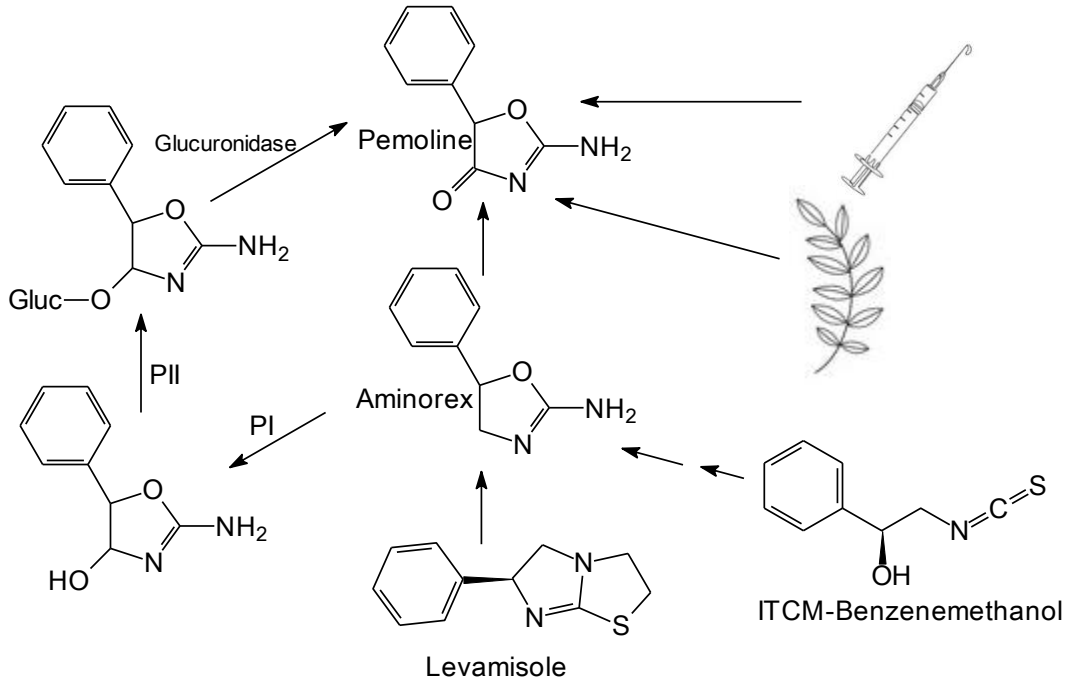


Fig. 4. Possible sources of pemoline in horse urine. Three sources involve aminorex as an intermediate, specifically a) as a breakdown product of levamisole; b) from the proposed barbarin precursor ITCM-benzenemethanol (α-(isothiocyanatomethyl)-benzenemethanol); c) as a glucuronidase-induced artifact from a hypothetical hydroxylated aminorex glucuronide (PI = Phase I metabolism; PII = Phase II metabolism; Gluc = glucuronic acid moiety). Note that the direct aminorex → pemoline conversion has been demonstrated *in vitro,* by use of equine liver microsomes. [18] (Scarth, et al., 2012). The plant image represents derivation from an unidentified plant source, while the syringe represents exogenous administration of pemoline.

CURRENT KNOWLEDGE OF THE RELATIONSHIPS BETWEEN LEVAMISOLE, AMINOREX AND PEMOLINE.

At this time levamisole is well known to metabolize to aminorex and a reported 26 other metabolites, but pemoline has not been reported detected in plasma or urine samples from post-levamisole administration experimental horses. Pemoline, however,

has been identified as an "in vitro" metabolite of aminorex in equine liver microsome experiments by Scarth and colleagues, suggesting that an aminorex/pemoline transformation is at least a biochemical possibility in the horse. [18] (SCARTH REFERENCE). Reporting these findings, Scarth et al. noted that "*No pemoline was detected after incubation with levamisole or tetramisole, but pemoline was detected after dosing with aminorex*". This is presumably a consequence of the small expected amounts of aminorex from levamisole, this in turn resulting in any in vivo generated pemoline being below analytical detectability. The microsomal method of Scarth may also not be optimal for modelling levamisole metabolism, since levamisole is predicted to be a CYP450 1A2 & 2D6 inhibitor (Ref: https://go.drugbank.com/drugs/DB00848) ([19] REF?) and the latter two monooxygenases may very well be responsible for aminorex oxidation to pemoline. Pemoline is frequently detected in post-race equine urine samples in association with levamisole, to the point that it appears that the frequency of detection of pemoline in post-levamisole administration racing samples may well, as a practical matter, be determined principally by the interests/policies of the laboratory performing the post-race analyses.

Additionally, aminorex has also been identified in equine urine samples following administration of the Kentucky pasture plant *Barbarea vulgaris*, consistent with the at times identification of aminorex in equine samples in the absence of known levamisole exposure. These pasture sources of aminorex raise the possibility of similar pasture sources for the pemoline identifications of unknown origins reported in French, German and South African samples and also apparently at times in US samples. As a closely related substance, it appears possible that there is a similar botanical source for the reported findings of low urinary concentrations of pemoline in urine samples from horses racing in France, Germany, and South Africa and also, apparently, in horses racing in Indiana. Given these circumstances, it is appropriate for regulatory authorities to follow the now long in place example of the French and German analytical laboratories and to define an Irrelevant Plasma Concentration (IPC) reporting limit for pemoline in equine blood/plasma/serum samples.

AN IRRELEVANT PLASMA CONCENTRATION (IPC) FOR PEMOLINE

The determination of a reporting limit for blood/plasma/serum samples requires identification of an Irrelevant Plasma Concentration (IPC) for pemoline in equine blood/plasma/serum samples. Studies on the effects of pemoline in horses[15] have shown that administration of pemoline at doses of 2.5 and 5 mg/kg intravenously produced clear-cut dose-related locomotor responses in experimental horses. While no pemoline blood concentration data were available for the 5 mg/kg IV dose, review of the available blood concentration data for the 2.5 mg/kg dose showed that the oral 2.5 mg/kg administration yielded peak plasma concentrations in the order of 1 μg/mL, while the 2.5 mg/kg IV dose was associated with a somewhat higher 1.65 μg/mL peak plasma pemoline concentrations. Based on these data, a blood plasma serum concentration of 1,000 ng/ml is a very conservative Effective Plasma Concentration (EPC) estimate for

pemoline in a racing horse. Dividing this conservative EPC by the also conservative Toutain safety factor of 500 presents an Irrelevant Plasma Concentration for pemoline in horses of 2 ng/mL.[16] Given the fact that pemoline is likely to be found in equine urine at substantially higher concentrations than the corresponding plasma (or serum) concentrations it seems likely that the urinary *"in house"* reporting limits in place in France and Germany, as referenced above, are reasonable and appropriately conservative as Irrelevant Urinary Concentrations (IUCs).[17]

A further consideration is that review of the equine pharmacokinetic data for pemoline in the horse as reported by Igwe and colleagues[15] shows that the mean terminal plasma half-life for pemoline is unusually long at 39.4 hours. Therefore, horses exposed to ongoing dietary or environmental sources of pemoline will require five pemoline half-lives or about 8 days to reach steady state serum or urinary concentrations following exposure to a dietary source of pemoline. Furthermore, the relationship between plasma or serum and urinary concentrations of pemoline is unknown but most likely highly variable due to urinary pH effects, so the most appropriate matrix for pemoline regulation in horse racing is blood/plasma/serum.[17] Overall, given the ongoing experience of our colleagues in Europe, introduction of a 2 ng/mL blood/plasma/serum screening limit for pemoline in US racing should effectively and conservatively address the matter of the detection of pharmacologically irrelevant concentrations of pemoline from botanical or other naturally occurring sources in US equine drug testing.

This derived IPC of 2 ng/mL can be validated by application of pharmacokinetic principles based on the studies of Igwe and Blake (ref). If we assume that European horses are exposed to background sources of pemoline, primarily plant sources, and that if intake is about 1 mg/kg every 48 hours, then the blood concentration will range about 700 ng/mL applying bioavailability ranging 0.833 – 0.863 and clearances ranging 23.8 – 27.4 mL/kg/hr. These calculations suggest that a horse exposed to as little botanical pemoline as 1ug/kg every 48 hours will present a plasma concentration of 0.7 nanograms/ml, well below our suggested and conservative 2ng/ml Irrelevant Plasma Concentration (IPC) for pemoline in a post-race sample.

The situation with regard to an Irrelevant Urinary Concentration (IUC) is complicated by the face that pemoline is a basic substance, pKa 10.5, and that post-race equine urines can be quite acidic, pH values of less than 5.0 occurring at times in Thoroughbred racing horses. Pemoline at a calculated IPC of 2 ng/mL is (2 x 10e-6 ug/L)/176 MW, or 0.011 µmole/L. At a pH as low as 5.0 following exercise, these data can be fit to the Henderson-Hasselbalch equation [19]:

$$pH = pKa + \log([A^-]/[HA])$$

where A- represents free pemoline and HA represents acid-conjugated pemoline, or pemoline +H. At pH 5.0, the ratio of [A-]/[HA] calculates as 0.0000032, or 0.0000032 moles of free pemoline relative to acidified pemoline, i.e. pemoline +H. The inverse

yields 312500 moles pemoline +H relative to free pemoline. In an animal at rest with a plasma pH of 7.4, the log ratio calculates as 0.00089, or 0.00089 moles of free pemoline relative to acidified pemoline. The inverse yields 1123 moles pemoline +H relative to pemoline. Thus, acidification of urine can result in 278-fold higher amounts of pemoline + H on a molar basis, and therefore significantly higher concentrations of pemoline in acidic pH urines [17] [18].

THE ENANTIOMERS OF PEMOLINE DETECTED IN RACING HORSES.

One classic significant scientific and potential regulatory concern is the matter of the specific enantiomers of pemoline (Fig 1) that are being detected in race horse samples. To our knowledge the only form of pemoline commercially available in amounts suitable for equine administration is racemic pemoline, no longer available in the US and many countries given its propensity to cause liver toxicity.[20] Given this reality, it would be of considerable scientific and forensic interest to determine the enantiomeric composition of the pemolines that are being identified in equine urine samples and compare their urinary enantiomeric profiles with the enantiomeric profiles of pemoline recovered from horses administered pharmaceutical pemoline, and – if it can be definitively demonstrated as a source – from horses administered levamisole. This approach has previously been used for identification of aminorex[21] as arising from levamisole, and which approach is facilitated by the commercial availability of chemically pure reference standards of each pemoline enantiomer[22] and Zhu et al,[23] have provided a mechanism for separation of pemoline enantiomers by cyclodextrin-modified micellar electrokinetic chromatography. Additionally, given the fact that pemoline exists as two enantiomers it might also be of interest to review the pharmacology and pharmacodynamics of its enantiomers to determine whether one enantiomer is more pharmacologically active and/or less toxic than the other.

POTENTIAL BOTANICAL SOURCES OF PEMOLINE

Figure 5 provides a summary of the proposed structural relationships between levamisole and its breakdown products aminorex and pemoline (top) as well as for comparison the glucobarbarin isothiocyanate metabolite and its breakdown products, aminorex and barbarin. The latter mechanism proposes involvement of an alkylamine from plant sources. In either mechanism, aminorex would be hypothesized to go on to produce pemoline by an oxidation reaction[24] (symbolized as [O]). However, the matter of finding support for this proposed oxidation is difficult. One could argue in favor of its possibility as follows. Molecular modeling of aminorex with Hyperchem software[25] demonstrates significant electronegativity on the phenyl carbons as well as carbon-4 of the 2-oxazoline ring, providing reasonable targets for electrophilic oxygenation events. Henderson, et al. (1995)[26] studied a compound related to aminorex – 4-methylaminorex – as an analog of psychoactive phenethylamines, and their metabolism studies in the rat suggested that the 4-methyl group of 4-methylaminorex may inhibit metabolism in a manner similar to methyl substitution on the α-group of β-phenylethylamines, e.g. methamphetamine. Furthermore, the aminorex ring opens on hydrolysis to a beta-

hydroxyphenethylurea, a route unavailable to 4-methylaminorex. In this regard, Philip et al.[27] have suggested hydroxy-levamisole and aminorex as levamisole metabolites that

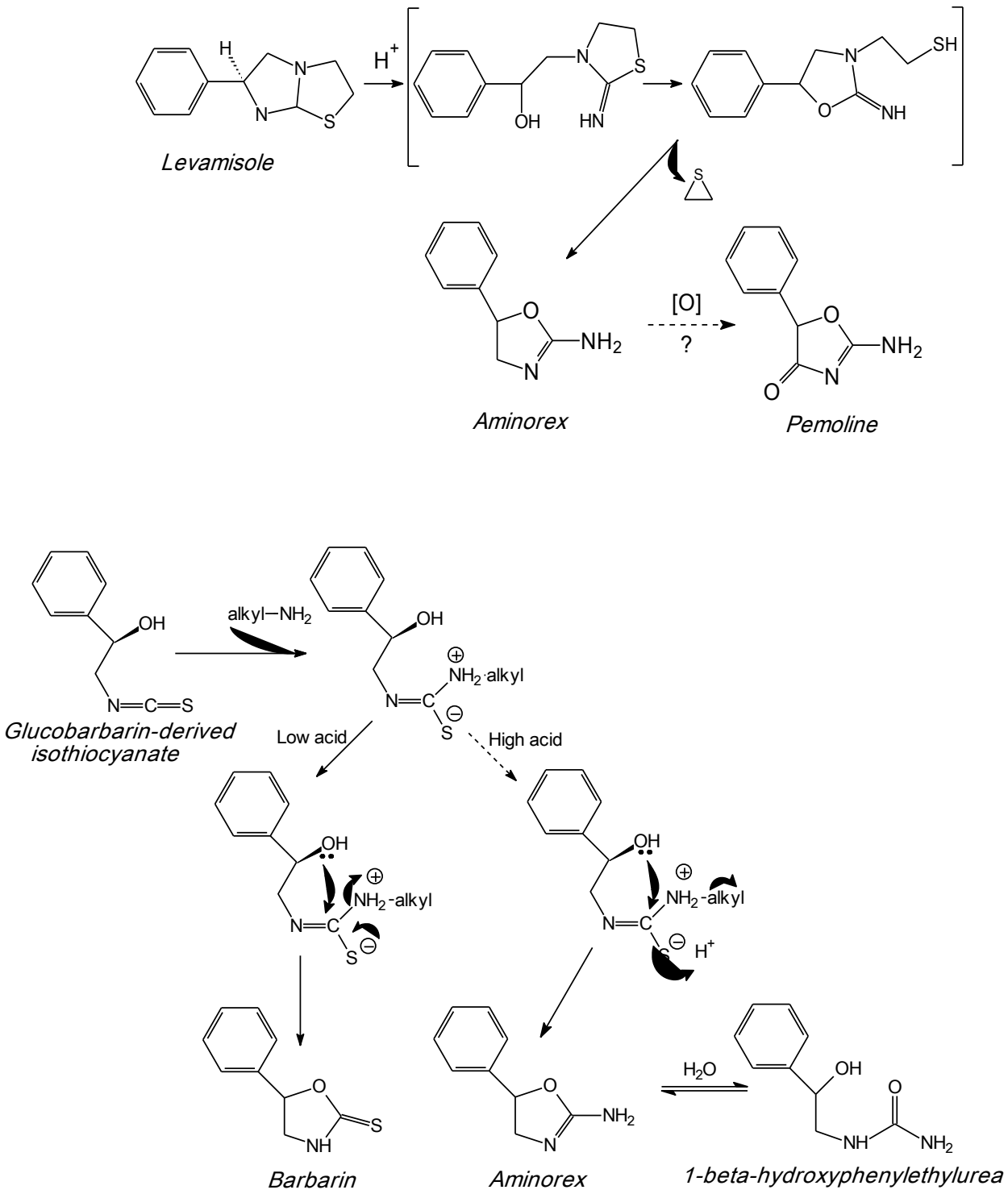


Figure 5. Proposed mechanisms to account for the formation of aminorex and pemoline from levamisole (top, after Gutierrez, et al.[24]) or barbarin and aminorex from the barbarin precursor glucobarbarin and its isothiocyanate breakdown product (bottom).

Aminorex in the latter mechanism could hypothetically also undergo oxidation to pemoline, although it has been shown to ring open to 1-beta-hydroxyphenylethylurea[35].

could be assessed to indicate levamisole administration to horses; however, the former compound ranged to only 0.25% relative abundance compared to the parent drug, and aminorex even less. Igwe & Blake (1983)[15] showed extensive tissue distribution and a particularly long half-life for pemoline in horses after oral administration, suggesting that – if present – it could be present at much lower levels, possibly below the limit of detection of Phillip et al. (2022).[27] The situation is different in humans where the reported half-life is on the order of 11 hours.[28] Owing to the current use of levamisole as an adulterant of illicit cocaine, Hess et al.[29] studied human metabolism of levamisole and claimed to find aminorex but not pemoline. However, the ion source of their ESI(+)-MS/MS detector was set at 400° C; one could argue that this setting acted to limit pemoline detectability in favor of aminorex, since aminorex melts at 137° C, while pemoline melts much higher at 256° C with decomposition.[30] Such decomposition might render pemoline undetectable in sample extracts, where the limit of quantitation was at least 3-fold higher for pemoline than for aminorex. One may conclude that aminorex oxidation to pemoline is a possibility from a chemical perspective, but it is pushed below detectability in sedentary experimental mammals exposed to levamisole or aminorex. The situation may be different for fully oxygenated athletes like racehorses.

On the other hand, stronger arguments can be made against the transition from aminorex to pemoline. A scan of the literature via PubMed or SciFinder shows no reference to oxidation of the 2-oxazoline ring in related compounds to a 4-ketone. Ring oxidation may preferentially proceed to ring-opening type reactions, similar to ones described by Galetto, et al.,[31] for 2-oxazolidinones. The 4-methyl group of 4-methylaminorex, in contrast, protects against any ring opening.[32] Ho, et al.[33] studied levamisole administration to horses and found aminorex, its phenyl ring positional isomer rexamino, and also a different $C_9H_{10}N_2O$ isomer 4-phenyl-2-imidazolidinone, but no pemoline. Plasma and urine elimination rates showed a preponderance of 4-phenyl-2-imidazolidinone compared to the other discovered compounds, leading the authors to suggest that compound as a marker for likely levamisole administration, in contrast to situations where only aminorex may appear from other sources such as plant ingestion, e.g. from yellow rocket.[14] Hundertmark et al.[34] have taken this discussion further in studies of the levamisole constituent tetramisole used in adulterating illicit cocaine. In serum samples from 73 cocaine misusers, only p-hydroxy-tetramisole and 4-phenyl-2-imidazolidinone were identified as tetramisole metabolites when using an assay with high specificity for aminorex, which was not found. Their conclusion is that – despite a low limit of detection in their assay for aminorex – confusion between the isomeric $C_9H_{10}N_2O$ compounds aminorex and 4-phenyl-2-imidazolidinone is a strong possibility, particularly if investigation is highly dependent on GC/MS assays and not LC/MS. If this idea is correct, the interpretations relevant to equine drug testing, therefore, are that perhaps aminorex in fact occurs only from plant sources and not necessarily from levamisole, aminorex is not necessarily a precursor to pemoline, and these European

and elsewhere reported low concentration pemoline findings have most likely arisen from an unidentified botanical source in a manner similar to the barbarin and aminorex relationship (Fig. 5).

CLOSING SUMMARY

This communication presents two clear patterns of pemoline identifications in equine urine samples. The pattern first reported in post-race English regulatory samples and confirmed in New York and Illinois samples presents an at times quite high linkage rate between levamisole administration and pemoline identifications in post-race urine samples. The second and quite unexpected pattern of pemoline identifications is that first reported in France, Germany and South Africa, where pemoline is identified in post-race urine samples and considered to be of natural and presumably botanical origins. Responding to these unexpected and to our knowledge unrelated patterns of pemoline identifications and following the lead of our European colleagues, we therefore propose an interim Irrelevant Plasma Concentration (IPC) Screening Limit / Regulatory Threshold of 2 ng/ml for pemoline in equine blood/plasma/serum samples.

ABBREVIATIONS

ADHD	Attention Deficit Hyperactivity Disorder
ARCI	Association of Racing Commissioners International
DEA	Drug Enforcement Administration
EPC	Effective Plasma Concentration
FDA	Food and Drug Administration
GC/MS	Gas Chromatography/ Mass Spectrometry
HFL	Horseracing Forensic Laboratory
HISA	Horseracing Integrity and Safety Authority.
HIWU	Horseracing Integrity and Welfare Unit.
HRMS	High Resolution Mass Spectrometry
IHRC	Indiana Horse Racing Commission
IPC	Irrelevant Plasma Concentration
IUC	Irrelevant Urinary concentration
LCH	Laboratoire des Courses Hippiques
LC/MS	Liquid Chromatography/ Mass Spectrometry

NAARV North American Association of Racetrack Veterinarians

SRM Selected Reaction Monitoring

SL Screening Limit

US United States

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AUTHORS' CONTRIBUTIONS

TT conceived and directed the project and TT, CKF of the North American Association of Racetrack Veterinarians (NAARV), GAM, Director of the New York Drug Testing and Research Program, RLH of Holland Management Inc., AMB of Caracas, Venezuela and Dubai, United Arab Emirates and LD of Louisiana State University reviewed the data interpretation and analysis and approved the proposed regulatory guidelines from an equine practitioner, researcher, and regulatory scientist's perspective. KB and AFL performed the data searching, chemical structure and reaction evaluations and statistical analyses and TT coordinated and edited all drafts of this manuscript with ongoing contributions from all authors and all authors reviewed approved the final manuscript submitted for publication.

AVAILABILITY OF DATA AND MATERIALS:

The datasets used and/or analyzed during the current study are available in the public domain as referenced in the manuscript or from the corresponding author on reasonable request.

DECLARATIONS:

Ethics approval and consent to participate are not applicable: As a review of the relevant scientific and regulatory literature, no ethics approval or consent to participate was necessary or required and all the authors have consented to publication of this case report and analysis.

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Exhibit

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Review of Environmental Morphine Identifications: Worldwide Occurrences and Responses of Authorities

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Review of Environmental Morphine Identifications: Worldwide Occurrences and Responses of Authorities

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Opium poppies grow wild worldwide, and testing for morphine is now highly sensitive. Currently, many authorities worldwide do not pursue urinary morphine concentrations of <100 ng/ml. This is because such low urinary morphine concentrations are likely to be environmental morphine identifications (EMIs) and are also unlikely to be associated with pharmacological responses. Authors' addresses: Maxwell Gluck Equine Research Center, University of Kentucky, Lexington, KY 40546 (Camargo, Karpiesiuk, Tobin); e-mail: fccama2@uky.edu (Camargo); U.K. Livestock Disease Diagnostic Center, Lexington, KY 40512 (Lehner); Florida Horsemen's Benevolent and Protective Association, Miami, FL 33024 (Stirling); Department of Pharmacology, Trinity College, Dublin, Ireland (Kavanagh); and Connolly's Redmills, Goresbridge, Kilkenny, Ireland (Brennan, Dowling).

1. Introduction

Morphine, the original μ agonist opiate, is derived from the juice of the opium poppy, *Papaver somniferum*. Man has long cultivated *P. somniferum* and facilitated its spread around the world. Morphine has also been detected in rice, beer, lettuce, hay, and human and bovine milk.

In horses, morphine and related opiates stimulate locomotor activity, suppress pain, and seem to prolong endurance. These are potentially useful actions in racing horses, and opiates have a long history of improper use in racing.

In the early 1990s, sensitive enzyme-linked immunosorbent assay (ELISA) tests for morphine and other opiates were introduced, abruptly terminating patterns of opiate abuse that had been in place for 100 yr. However, these tests have also yielded sporadic sequences of low level (<100 ng/ml) morphine identifications in post-race urines around the world.

These low-level morphine identifications first became a problem in Australia, where the morphine was traced to wild *P. somniferum* or *segiterum*. At the same time, low concentration morphine identifications became a problem in Hong Kong, which imported much of its feedstuffs from Australia. The solution adopted in Hong Kong, apparently on an interim basis, was a "reporting limit" for morphine of 100 ng/ml.

NOTES

Fig. 1. Putative *P. somniferum*, yielding morphine, growing wild outside a Dublin, Ireland research laboratory.

A 100 ng/ml reporting limit for morphine is well supported in the scientific literature. The intravenous "No Effect Dose" for locomotor responses to morphine in horses is ~0.1 mg/kg or 50 mg for a 1000-lb horse. This dose yields peak urinary concentrations of morphine glucuronides of ~22,000 ng/ml; therefore, the dose to produce a urinary concentration of 100 ng/ml is ~250 μg, a dose highly unlikely to produce a pharmacological effect. Another consideration is that oral morphine is unlikely to be more than ~20% bioavailable in the horse, further reducing the probability of a pharmacological response from environmental (oral) exposure to morphine.

These conclusions are independently supported by a recent and very conservative pharmacokinetic/pharmacodynamic analysis, which suggested that the "irrelevant" concentration of morphine in equine urine is ~80 ng/ml.

Low concentration identifications of morphine have also been observed in a number of U.S. jurisdictions, leading Ohio and Louisiana to introduce urinary morphine "reporting limits" of 50 and 75 ng/ml, respectively. In late 2002, in the United Kingdom and Ireland, a sequence of low concentration morphine identifications associated with manufactured feed led the British Jockey Club to introduce a 50 ng/ml reporting level for morphine.

In summary, there is a worldwide propensity for sporadic low level environmental morphine identifications (EMIs), apparently from botanical sources of morphine. The most practical and widely used solution has been to set a reporting level for morphine of 50–100 ng/ml, which effectively eliminates the administrative and public relations problems associated with very low-concentration EMIs.

2. Background

The word "opium" is derived from the Greek word for juice, and raw opium is the congealed juice of the opium poppy, *P. somniferum* (Fig. 1).

The juice of *P. somniferum* contains at least 20 alkaloids,[1] and the most pharmacologically useful and widely used of which is morphine. Evidence for the use of opium/morphine for medicinal purposes occurs in the earliest human records, and morphine is still cultivated commercially throughout the world. Some morphine is grown under license for the pharmaceutical industry; some, presumably much more, is grown "off-license" for other markets, and an unknown but possibly substantial fraction of the morphine growing in the world today may be ornamental/feral/wild growth on the part of *P. somniferum* itself and/or its various relatives and/or variants.[2,3]

Morphine produces its pharmacological effects by interacting with μ-opioid receptors. In humans, opioids, including morphine, classically produce analgesia and drowsiness, affect mood, and alter respiratory, cardiovascular, and gastrointestinal functions.[1] The effects of opioids in a variety of systems, including the central nervous system (CNS), are caused by the wide distribution of opioids and their receptors both in the brain and in the periphery.[1]

Peak blood concentrations of morphine found after oral administration of morphine are from five- to seven-fold less than those obtained after its parenteral administration,[4] and thus, the effect of any given dose is less after oral administration than after parenteral administration. Morphine has a significant first-pass metabolism in the liver, and therefore, the effect of a given dose is generally considerably less after oral than parenteral administration. The major urinary metabolites are morphine-3-glucuronides and morphine-6-glucuronides; relatively little morphine (~2–10%) is excreted unchanged in urine.[5]

In horses, morphine produces excitement, increased locomotor activity, and alertness, as described in the late 1970s by Combie et al.[6] These workers also showed that when administered at a dose of 0.1 mg/kg, IV, morphine elimination followed a three-compartment open system with a terminal serum half-life of 87.9 min and a urinary half-life of 101.1 min. Morphine was detected in serum for up to 48 h and in urine for up to 144 h. Urinary "total" morphine peaked at 2 h after dosing at an average of 21,894 ng/ml, and it decreased thereafter. Morphine was detected in hydrolyzed and unhydrolyzed urine up to 144 h after dosing (Fig. 2).[7]

In the early 1990s, highly sensitive ELISA tests for morphine were introduced into racetrack testing,[8,9] and instrumental confirmatory methods also became highly sensitive.[10,11] These tests made it readily possible to detect[9] and confirm[11] concentra-

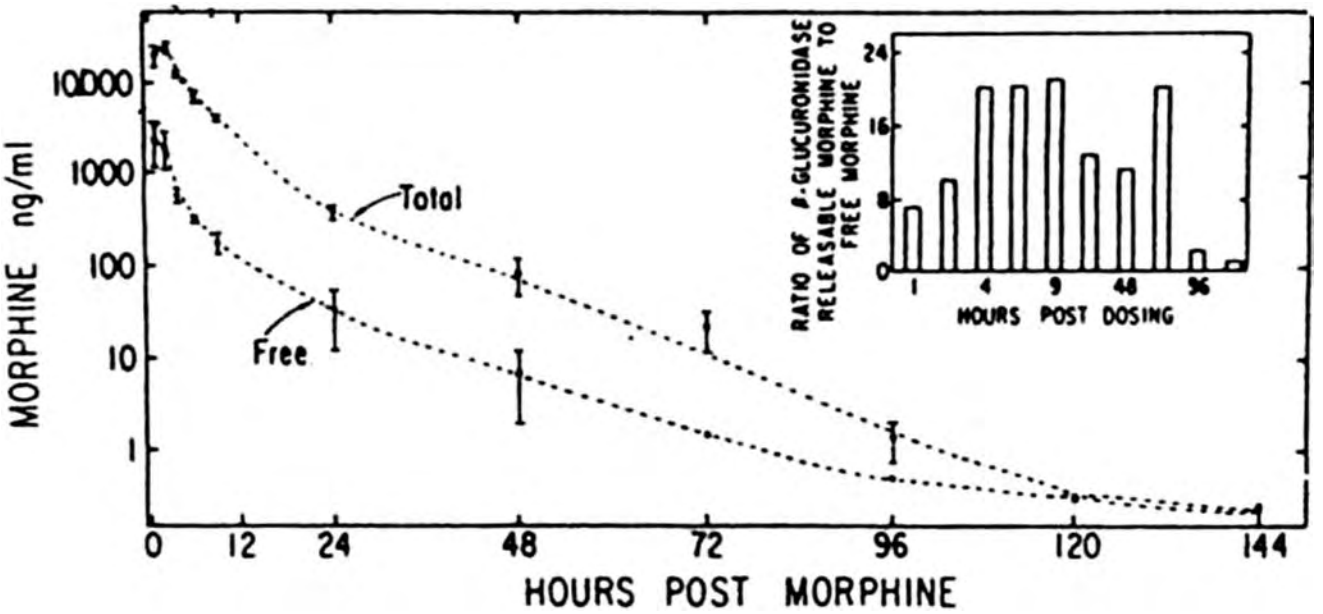

Fig. 2. Urine morphine concentration after administration of 0.1 mg/kg, IV to horses.[10]

tions of morphine as low as 10 ng/ml or less in post-race urines.

Simple mathematics shows that if a dose of morphine of 50 mg/horse yields a urine concentration of >20,000 ng/ml urine, as little as 25 μg/horse will yield a urinary concentration of 10 ng/ml. However, the probability of a pharmacological effect resulting from such a small dose of morphine is, for all practical purposes, zero.

More recently, Kollias-Baker and Sams[12] reported a study in which 10, 5, and 1 g of poppy seeds were administered orally to horses. The horses administered 10 g of poppy seed received 732 μg of morphine; the ones administered 5 g received 366 μg, and finally, the ones administered 1 g received 73.2 μg. No behavioral changes or pharmacological effects were observed in any of the horses.

In these experiments, urinary concentrations of morphine were 213 ng/ml after the 732-μg dose, 119 ng/ml after the 366-μg dose, and 27.6 ng/ml for the 73.2-μg dose, which agrees with our work from 25 yr ago.[7,10] Peak urine concentrations occurred within 4 h of administration. Morphine was detectable by ELISA 24 h after administration in all urine samples of the horses dosed except for one; morphine was not detected in the urine of one horse dosed 73.2 μg of morphine by means of ingestion of 1 g of poppy seeds.

A similar study had been performed by Ginn et al.,[13] where 2 g of poppy seeds were administered with feed twice a day for 3 days to three horses. Urinary excretion after poppy seed administration showed maximum morphine concentration to be up to 120 ng/ml. The morphine concentration declined below their limit of detection at 35–40 h after the last administration of poppy seeds.

Poppy seeds are far from being the only dietary sources of morphine. Trace amounts of morphine, between 280 and 1400 pg/kg, have been detected in bread, rice, vegetable soup, milk, and beer. Higher concentrations (5.6 μg/kg dry weight) have been reported in different types of lettuce.[a] Hazum et al.[14] have found morphine in hay and lettuce at concentrations between 2 and 10 ng/g dry weight, and they "postulate that morphine may be a ubiquitous constituent of plant-derived foods."[14]

Since the introduction of the ELISA[9,15–17] tests in the early 1990s, there have been sporadic low-concentration identifications (<100 ng/ml) of morphine in post-race urine samples in horses racing around the world. Surprisingly, a considerable proportion of these identifications are associated with areas in which morphine has, at one time or another, been cultivated commercially. However, this presentation begins with a report of identifications in Ireland, a country with, to our knowledge, no history of organized commercial cultivation of morphine.

3. Ireland

Ireland, where morphine has never been grown commercially, has recently seen an incident of nine low-level (21–46 ng/ml) morphine "identifications" associated with manufactured racehorse feed. These unexpected findings greatly increased the level of interest in the "wild" opium poppies growing in Ireland. In this regard, our colleagues, Dr. Kavanagh, Dr. Scott, and Dr. Lambert of Trinity College, soon identified significant numbers of "wild" opium-containing poppies growing at various locations in Ireland (Figs. 3 and 4) as well as outside of their Dublin laboratory.

Fig. 3. Putative *P. somniferum*, yielding morphine, growing wild in rural Ireland.[18]

Our colleagues soon showed that these sporadic "wild" sources of morphine have the potential to yield more than sufficient opium/morphine to give rise to low-level identifications of morphine in horse urines.[18] In assessing the significance of these Irish and other similar findings that we will set forth, we must bear in mind that man has been cultivating and using *P. somniferum* for at least 5000 yr, and it is only within the last 100 yr that its cultivation and use have been closely regulated. For most of recorded history, man has cultivated variants of *Papaver* and has carried it with him throughout the old world[19] and to the New World and Australia long before its cultivation was regulated. As such, the finding of wild opium-containing plants related to *P. somniferum,* quietly growing at diverse locations throughout the world, should come as no surprise.[2,3]

We might also note that if the sources of such low-concentration morphine identifications in horses are indeed of local and botanical origin, then one might reasonably expect that these findings would be characteristically seasonal. This possibility may have been observed (it seems to have occurred in California in 2000 and the United Kingdom in 2002) and should be considered during the evaluation of incidents or episode(s) of low-concentration morphine identifications.

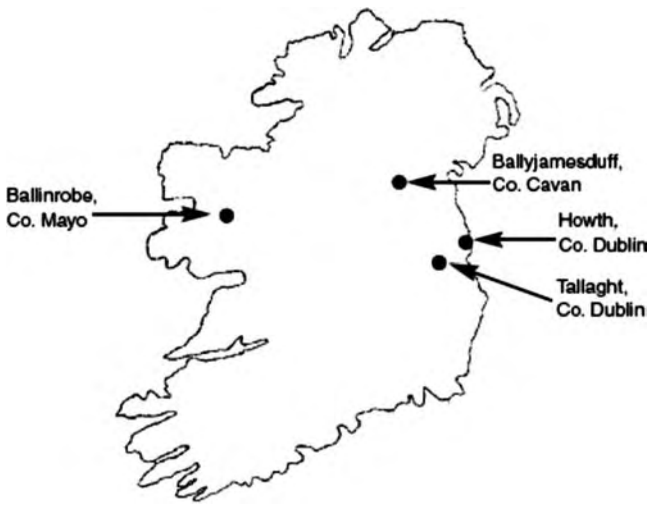

Fig. 4. Geographic locations at which putative *P. somniferum* yielding morphine have been located growing wild in Ireland.[18]

4. United Kingdom

The incident of EMIs in a commercial Irish racehorse feed, apparently from a commercial feed ingredient, also gave rise to a number (~30) of positive identifications for morphine in post-race urine samples from horses racing in the United Kingdom in late 2002 and early 2003. One outcome of these events was that the English Jockey Club, in early 2003, raised the reporting level ("threshold") for morphine (as morphine glucuronides) in post-race urines in horses from the previously estimated 10 ng/ml limit of detection (LOD) to a current 50 ng/ml LOD.

This was an unusual move, and the English Jockey Club only adjusted their morphine reporting level or "threshold" after the repeated assurance of their Chief Veterinarian, Dr. Peter Webbon, that a concentration of 50 ng/ml of morphine, as morphine glucuronides, in a post-race urine would not, or could not, under any circumstances be associated with a performance effect on the racing horse at the time of the race in question.

Additionally, since the introduction of this new reporting level, the Jockey Club authorities, in a letter dated October 28, 2004, noted that "since June 2004 there have been 17 instances where urine samples have indicated the presence of morphine at low levels." However, they were all below 50 ng/ml, so they were reported as negatives. It started with 2 or 3 instances/mo, but in September, there were 8; therefore, the two feed companies that were supplying feed to the trainers concerned were approached. Samples of feed were taken for analysis, but the Horseracing Forensic Laboratory (HFL)[b] "did not find any traces of morphine . . . The Jockey Club has not had any reports from HFL for low levels of morphine for the last four weeks."

We also specifically draw attention to the fact that the Jockey Club letter notes that although these horses were testing "positive" for morphine, analysis

of samples of the feedstuff was unable to definitively identify morphine.

We might add that, based on some earlier research from our group[11] and a review of the scientific and regulatory literature,[20,21] Dr. Webbon was apparently on very safe grounds when he concluded that these concentrations of morphine would not be associated with any effect on the racing performance of a horse.

5. France

In France, in the 1990s, opium poppies were cultivated under license as a French domestic source of medicinal morphine. These opium poppies were then dried in commercial hay drying equipment during their processing. Not unexpectedly, opium poppy residues in these hay driers apparently "cross-contaminated" later shipments of agricultural hay dried in the system. Some of this hay containing trace amounts of morphine was then fed to racehorses, which then yielded morphine "positive" racehorse urines. This gave rise to a number of low-concentration morphine glucuronide identifications in post-race urines in France.

With regard to the French racing authorities, we do not know what the in-house reporting level, if any, for morphine in France is; however, it seems as though the French authorities had, at one time, a problem with low-level concentrations of morphine in their post-race urine samples, and our understanding is that the morphine ELISA test developed at the University of Kentucky was officially recognized as a screening test for morphine in French equine feedstuffs.

6. Australia

The opium poppy has been extensively cultivated in Australia, apparently in Tasmania, as a source of medicinal morphine. There also seems to be a related opium-containing poppies (*P. setigerum*) growing wild in Australia. These feral/wild opium-containing poppies have given rise to identifications of morphine in post-race urines in Australia since at least the mid-1990s. In this regard, at least one Australian jurisdiction had an "in-house" unpublished limit of detection/reporting level for morphine of 100 ng/ml, and personal communication[c] with this authority commented on the difficulty of completely excluding sources of morphine from horsefeed under Australian conditions.

These comments on the technical difficulty encountered in trying to ensure that Australian feed is free of morphine are of interest with respect to the related comments set forth in the British Jockey Club letter referenced above.

7. Hong Kong

As set forth above, low-level morphine identifications are not uncommon in Australia, and at one time, the Hong Kong Jockey Club, which imports much of its hay/fodder from Australia, had an "in-house" unpublished threshold for morphine in post-race urines of 100 ng/ml.

8. United States

In the United States, such low-concentration identifications of morphine have been reported in California, Pennsylvania, Florida, Louisiana, and Ohio (from an unidentified source).

Ohio

The outcome in Ohio was the creation of a reporting level for morphine in post-race urines of 50 ng/ml, as set forth below in the excerpt from The National Horsemen's Benevolent and Protective Association Proposed Policy on Drug Testing and Therapeutic Medication.[22]

Louisiana

The outcome in Louisiana was a published threshold for morphine of 75 ng/ml in post-race urines, as set forth below in the excerpt from The National Horsemen's Benevolent and Protective Association Proposed Policy on Drug Testing and Therapeutic Medication.[22]

An Unnamed Southeastern State

It has been communicated personally to one of the authors[d] that an unnamed southeastern racing state has an in-place unpublished reporting limit for morphine, as morphine glucuronides, of 100 ng/ml.

Pennsylvania and California

Although both Pennsylvania and California have reported low (<50–100 ng/ml) identifications of morphine and there are suggestions that at least some of the California identifications have been seasonal and presumably botanical or environmental in origin, these states have not, to our knowledge, introduced any published or in-house reporting levels or thresholds for morphine in post-race urines.

With regard to the potential for EMIs in California, it is of interest to note that a California website (http://www.calflora.org)[2] refers to *P. somniferum* growing wild in four counties.

Although the state of California has not yet introduced a reporting level for morphine in post-race urines, the California Horse Racing Board (CHRB) has recently ruled in favor of trainers with low-level morphine-positive horses. This was the case of trainers Bob Baffert, a three-time Kentucky Derby winner, and Jesus Mendoza. These trainers had horses that presented low levels of morphine in their post-race urine in the year 2000 at Hollywood Park. Both horses presented morphine urine concentrations <100 ng/ml. On January 10, 2002, the CHRB dismissed Mendoza's case,[23] ascribing the findings to environmental contamination. On March 24, 2005, following the recommendation of an administrative law judge, the CHRB sustained Baffert's appeal and dismissed the complaint,[24] also invoking the likelihood of EMIs in this case. Apparently,

during the spring of 2000, 13 of 95 urine samples tested at the same laboratory were suspect for opioids,[24] a finding which is consistent with seasonal EMIs.

Washington

The state of Washington has recently introduced a threshold/reporting level for morphine of 50 ng/ml of urine.[25]

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

As set forth above, low-concentration identifications of morphine glucuronides have occurred around the world, and a number of jurisdictions now have formal published "thresholds" and "regulatory limits" or in-house "regulatory limits" for morphine. The following excerpt is taken from the National Horsemen's Benevolent and Protective Association's Proposed Policy for Drug Testing and Therapeutic Medication that was published in the January 2003 issue of the Journal of Equine Veterinary Science.[22]

> "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 Association of Racing Commissioners International 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, 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, which shows urinary concentrations of 110 ng/ml after administration to horses of 2-gram doses of poppy seeds containing 3 μg of morphine per dose. These thresholds/regulatory limits are dramatically lower than the 2,000-ng/ml 'cut-off' in place in human workplace medication testing.
>
> Withdrawal Time Guideline: No withdrawal time guidelines, since these are neither relevant nor applicable to dietary and environmental substances/contaminants."[22]

In summary, the regulatory limit in urine of 100 ng/ml of morphine as morphine glucuronides, proposed by the National Horsemen's Benevolent and Protective Association, is an extremely conservative limit; it is apparently in place in a number of jurisdictions worldwide, and there is no possibility whatsoever of a performance effect being associated with these levels of morphine in a post-race urine sample. As such, this proposed reporting limit is well supported by published scientific work and current and evolving international regulatory practice.

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