AGENDA (V1.0)
RCI MODEL RULES COMMITTEE MEETING
Wednesday, May 7, 2025
11:00am – 12:00pm (EDT)
Seelbach Hilton, Louisville, Kentucky, USA

1. Call to Order

2. Pari-Mutuel Auditing & Wagering System Security Committee Recommendation to Add New Section (C) to ARCI-004-0049 ADVANCED DEPOSIT ACCOUNT WAGERING (ADW) entitled “Third-Party Information Security Audits for Advanced Deposit Wagering Companies”. Section (C) would be added to the existing Model Rule ARCI-004-0049 including an Appendix with Information Security Auditing Standards. *(Action Item)*

3. Pari-Mutuel Auditing & Wagering System Security Committee Recommendation to modify ARCI-001-005 TERMS (45); ARCI-004-0105 G. 7 CALCULATION OF PAYOUTS AND DISTRIBTUION OF POOLS; and ARCI-024-035 O. 1 Racing Rules in regard to “No Contest” races. *(Action Item)*

4. Standardbred Committee Recommendation to Amend ARCI-024-025 HORSES PERMITTED TO RACE - subsection (8) would be added to stipulate the number of starts permitted for two-year olds. *(Action Item)*

5. Standardbred Committee Recommendation to Amend ARCI-020-015 JUDGES H – subsection (4) would be added to require Judges to submit accident reports to USTA to maintain in a centralized database. *(Action Item)*

6. Standardbred Committee Recommendation to Amend ARCI-022-020 TRAINERS B (21) to include definition of “equipment” and “safely equipped.” *(Action Item)*

7. Standardbred Committee Recommendation to amend ARCI-001-005 (73) and ARCI-025-020 (4) to add Harness Racing Medication Collaborative (in addition to Racing Medication and Testing Consortium). *(Discussion and Possible Action Item)*

8. Proposal by USTA – Classification/Thresholds - Metformin and Gabpentin *(Discussion and Possible Action Item)*

9. Proposal by USTA – Classification /Thresholds – Benzoylecgonine; Aminorex; Pemoline; Methamphetamine; Synephrine *(Discussion and Possible Action Item)*

10. Classification of Carmoterol *(Action Item)*

11. Other Business

12. Adjourn

(The Chair reserves the right to add or set aside items on this agenda or rearrange their order.)

AGENDA ITEM #2

Proposed Amendment to ARCI -004-0049 – Advanced Deposit Account Wagering (ADW) – add section – Third-Party Information Security Audits for Advanced Deposit Wagering Companies with Appendix

RCI MODEL RULES COMMITTEE
PETITION FOR NEW RULE OR CHANGE TO EXISTING RULE

Please submit the following information to the Chair of the Model Rules Committee at least 45 days in advance of the next scheduled committee meeting to www. rules@arci.com.

Y**our Contact Information:**

Name:	Connie Winn
Organization:	Oregon Racing Commission
Address:	PO Box 366, Gresham, OR 97030
Phone(s):	503-853-5928
Fax #:	
E-mail Address:	Connie.winn@orc.oregon.gov

A. Brief Description of the Issue

There are no specific information security standards in the model rules for online wagering entities.

B. Discussion of the Issue and Problem

Provide background on the issue to build context. Address the following:

- ***What specific problems or concerns are involved in this issue?***
  - *Ensure information security is adequate for online wagering licensees.*
- ***Who does the issue affect?***
  - *Impacts online wagering licensees and states where they operated.*
- ***What existing model rules relate to this issue?***
  - *NA*
- ***Provide relevant quantitative or statistical information if possible.***
  - Standards are derived from
    - *National Institute of Standards & Technology*
    - *International Organization for Standardization*
    - *Gaming Laboratories, International*
    - *Information systems audit and control association*
    - *Center for Information Security*
    - *Payment Card Industry Data Security Standards*
    - *General Data Protection Regulation*
    - *SysAdmin Audit, Network, & Security*
    - *California Consumer Protection Act*
    - *System & Organizational Controls 2*
    - *eCommerce Online Gaming Regulation and Assurance*
    - *Office of the Privacy Commissioner of Canada*

C. Possible Solutions and Impact

Provide possible recommendations to solve the problem. Include details on each proposed solution such as

- ***What solution does this proposal provide?***
  - *Provides a framework and auditing standards and requirements.*
- ***How will the solution fix the problem?***
  - *Ensures adequate controls are in place*
- ***How will the change affect any entities or stakeholders?***
  - *Some licensees may not currently be required to undergo such an audit. This may require them to hire a third-party vendor if they do not already have one.*
- ***How will you or your organization be affected by the proposed change?***
  - *NA – we currently have an ISO Lead Auditor that completes similar audits.*
- ***What are the benefits of the proposed change?***
  - *Provides greater security verification from the online wagering companies therefore providing better protection for the wagering public.*
- ***What are the possible drawbacks of the proposed change?***
  - *Some jurisdictions may choose not to enforce these.*
- ***Identify possible fiscal impact of the recommended change.***
  - *We do not believe there is a fiscal impact to any associations.*

D. Please identify any affected stakeholder groups that expressed support or opposition. (These stakeholders may include the racetracks, breed registries, owners, trainers, jockeys, veterinarians, or others.)

- ***For those stakeholder groups that have expressed an opinion, please list the points on which they agree or disagree, and the arguments they have expressed.***
  - *Xpressbet and AmWest were at the auditing committee meeting and did not have an issue with the rules.*
  - *There were no objections to the ARCI Auditor Conference from any of the regulators that attended.*
  - *We did get a request to add in multi-factor authentication which we have.*
- ***Are there any affected stakeholders that have not been consulted on this proposal?***
  - *No one from North Dakota Racing Commission or their licensees have hosen to participate in any of these discussions.*
- ***Please submit any formal letters of support or opposition by stakeholder groups*.**

E. Attach the model rule language you are proposing. Please show new language with underlined text. If you are proposing that the current model rule language be eliminated, please strikeout the language to be deleted.

Model Rule – Third-Party Information Security Audits for Advanced Deposit Wagering Companies

Section 1. Purpose

This rule establishes the requirement for all online wagering companies, also known as Advanced Deposit Wagering (ADW) companies, to undergo a third-party audit to ensure compliance with industry security and auditing standards.

Section 2. Third-Party Audit Requirement
(a) All ADW companies must engage an independent third-party auditor to conduct an annual audit of their information security and operational controls.
(b) The third-party auditor must possess at least one of the following credentials:

1. *Certified Information Systems Auditor (CISA) Certification*
2. *Lead Auditor for ISO (International Organization for Standardization)*
3. *Certified Information Security Manager (CISM) Certification*

Section 3. Auditing Standards
(a) The audit shall assess compliance with all standards outlined in the ARCI Information Security Auditing Standards.

Section 3. Reporting and Compliance
(a) The results of the third-party audit shall be submitted to the appropriate regulatory body within 30 days of completion.
(b) ADW companies found non-compliant with the required standards must implement corrective measures within 90 days of notification.
(c) Failure to comply with this rule may result in penalties, suspension, or revocation of licensure.

Section 4. Effective Date
This rule shall take effect on January 1, 2026, and apply to all ADW companies operating under the ARCI Model Rules

Proposed ARCI Information Security Auditing Standards

Section	Item	Control or Mitigating Control	Standards
Physical Security	**1.1** Physical Entry	Card, code, lock, biometric scanner or other mitigating controls.	ISO 27001 A.9.1.2, NIST SP 800-53 AC-2, ISACA COBIT 5 DSS05, GLI-12, 28, 24, 33, 26
Physical Security Parameter	Physical barriers based on asset sensitivity and vulnerability (walls, fences, surveillance, doors, etc.).	ISO 27001 A.11.1, NIST SP 800-53 PE-2, ISACA COBIT 5 DSS04	
Security Offices, Rooms, and Facilities	Shield sensitive information, maintain discrete appearance, restrict access.	ISO 27001 A.9.1.3, NIST SP 800-53 PE-3, ISACA COBIT 5 DSS05, GLI-33	
Physical Security Monitoring	Surveillance technology, visitor access logs. Motion detectors and similar tech. Janitorial services and other mitigating controls.	ISO 27001 A.9.4.3, NIST SP 800-53 PE-6, ISACA COBIT 5 DSS05, GLI-33	

Physical & Environmental Threats	Conduct risk assessment to identify potential physical/environmental threats. Ensure smoke detectors, water sensors, and fire suppression systems are in place and inspected.	ISO 27001 A.8.1.2, NIST SP 800-53 PE-11, ISACA COBIT 5 DSS05	
Working in Secure Areas	Restricted access, physical barriers, and mobile phone recording restrictions. Clear emergency procedures.	ISO 27001 A.9.1.2, NIST SP 800-53 PE-2, ISACA COBIT 5 DSS04, GLI-33	
Clear Desk & Screen	Sensitive information must be locked. Automatic screen locks. No visible passwords. Educate employees.	ISO 27001 A.9.4.1, NIST SP 800-53 AC-10, ISACA COBIT 5 DSS04, GLI-33	
Storage Media	Media management policy, encryption, regular offsite backups. Backup power supplies.	ISO 27001 A.10.1, NIST SP 800-53 SC-12, ISACA COBIT 5 DSS01, GLI-28	
Backup Power	Ensure backup power sources are available and routinely tested.	ISO 27001 A.7.4, NIST SP 800-53 PE-11, ISACA COBIT 5 DSS01, GLI-12	
Policies, Procedures, and Documentation	**2.1** Personal Identifiable Information (PII)	Manage and protect PII according to policy.	ISO 27001 A.8.2, NIST SP 800-53 PL-2, ISACA COBIT 5 APO02, GLI 33
Change Management	Follow change management processes, version control, emergency changes, segregation of duties.	ISO 27001 A.12.1, NIST SP 800-53 CM-3, ISACA COBIT 5 DSS01	
Disaster Recovery Plan	Evaluate disaster recovery plan to ensure rapid recovery.	ISO 27001 A.12.3, NIST SP 800-53 CP-9, ISACA COBIT 5 DSS03	
Business Continuity Plan	Assess preparedness against disruptions.	ISO 27001 A.12.2, NIST SP 800-53 CP-4, ISACA COBIT 5 DSS03	
Remote Access	Secure remote access management and MFA implementation.	ISO 27001 A.9.1.1, NIST SP 800-53 AC-17, ISACA COBIT 5 DSS06, GLI 33	
Data Retention Policy	Define and enforce data retention policies.	ISO 27001 A.8.3, NIST SP 800-53 AU-6, ISACA COBIT 5 DSS06, GLI 33	
Governance Policies	Information security governance and compliance.	ISO/IEC 27001, SOC 2, PCI-DSS, GLI-33	

Personal Identifiable Information (PII)	Protection and Management of Personally Identifiable Information (PII)	ISO/IEC 27001:2013 A8.2, A.9, A.18.1, ISO/IEC 27002:2022 A.8.3, A.9.4, A.14.2, A.18.1.A, GDPR Article 5, 32, 33, 35, NIST SP 800-53 AC-17, IR-4, PL-8SC-13, NIST SP 800-122, PCI-DSS, CCPASOC 2, ISO/IEC 29100 Principles 1, 2, 7, GLI-33	
Third Party Policies	Third Party Risk Management Policy	ISO 27001 & ISO 27701, NIST 800-53, PCI-DSS, SOC 2, GDPR/CCPA, GLI-33	
Inventory and Control	**3.1** Enterprise Asset Inventory	Maintain and manage an asset inventory for software and hardware. Ensure version control is in place.	ISO 27001 A.8.1, NIST SP 800-53 CM-8, ISACA COBIT 5 DSS02, GLI-24, GLI-33
Software Inventory	Ensure all software is licensed.	ISO 27001 A.8.1, NIST SP 800-53 CM-8, ISACA COBIT 5 DSS02, GLI-24, GLI-33	
Data Protection	**4.1** Secure Disposal of Data and enforce retention of data.	Secure disposal of data as per policy.	ISO 27001 A.10.1, NIST SP 800-53 SC-12, ISACA COBIT 5 DSS01, GLI-33
Data Encryption in Transit	Encrypt sensitive data during transit.	ISO 27001 A.10.1, NIST SP 800-53 SC-12, ISACA COBIT 5 DSS01, GLI-33	
Data Access Logging	Log access to sensitive data, routinely reviewed and maintain compliance.	ISO 27001 A.8.3, NIST SP 800-53 AU-6, ISACA COBIT 5 DSS06, GLI-33	
Secure Configuration	**5.1** Secure Factory Configuration	Change factory configurations to secure settings.	ISO 27001 A.9.1.1, NIST SP 800-53 CM-2, ISACA COBIT 5 DSS01, GLI-33
Firewalls	Use firewalls at the boundary of dissimilar security domains. Ensure appropriate configuration. Appropriate logs are maintained.	ISO 27001 A.13.1, NIST SP 800-53 SC-7, ISACA COBIT 5 DSS05, GLI-33	
Access and Account Management	**6.1** Account Management	Use unique passwords and follow NIST standards for password complexity.	ISO 27001 A.9.2, NIST SP 800-53 AC-2, ISACA COBIT 5 DSS05, GLI-33
Least Privilege Access	Ensure employees have the least privilege access based on role.	ISO 27001 A.9.2.2, NIST SP 800-53 AC-6, ISACA COBIT 5 DSS05	
Removal or adjustment of access rights.	Access rights are removed or adjusted promptly when an	ISO/IEC 27001 A.9.2.6, NIST SP 800-53, ISACA COBIT DSS05.04, DSS06.03	

	employee leaves for is reassigned.		
Multi-Factor Authentication (MFA)	Enforce MFA for administrative and remote access. **Optional MFA requiring opt out for account holders and required on new devices or other mitigating controls effective January 1, 2026.**	ISO 27001 A.9.4, NIST SP 800-53 IA-2, ISACA COBIT 5 DSS05, GLI-33	
Vulnerability Management	**7.1** Vulnerability Scanning	Establish automated vulnerability scanning and remediation processes.	ISO 27001 A.12.6, NIST SP 800-53 RA-5, ISACA COBIT 5 DSS05, GLI-33
Intrusion Prevention Systems	Use intrusion prevention systems to monitor and block malicious activities.	ISO 27001 A.13.1, NIST SP 800-53 SC-18, ISACA COBIT 5 DSS05, GLI-33	
Audit Logs	**8.1** Audit Log Access Control**.**	Ensure audit logs have access control, are stored properly, and are reviewed regularly.	ISO 27001 A.12.4, NIST SP 800-53 AU-2, ISACA COBIT 5 DSS06, GLI-33
Log Review	Regular review and documentation of audit logs.	ISO 27001 A.12.4, NIST SP 800-53 AU-6, ISACA COBIT 5 DSS06	
Email Security	**9.1** Phishing Training	Conduct routine phishing awareness training for employees.	ISO 27001 A.10.1, NIST SP 800-53 SC-5, ISACA COBIT 5 DSS05, PCI-DSS 12.6, CIS Control 14, 17, GLI-33
Anti-Malware Software	Install and configure anti-malware software with signature updates.	ISO 27001 A.10.1, NIST SP 800-53 SC-5, ISACA COBIT 5 DSS05	
Network Security	**10.1** Network Architecture	Maintain and regularly review network architecture for security vulnerabilities.	ISO 27001 A.13.1, NIST SP 800-53 SC-7, ISACA COBIT 5 DSS05, NIST CSF, CIS control 4, 9, 13, GLI-33
Intrusion detection	Monitoring remote access	Detect unauthorized attempts to access, identify unauthorized access and suspicious activities, and provides software, firmware integrity.	NIST SP 800 53 AC 17, IR-4, SI-4, SI-7, NIST SP 800-94, ISO/IEC 27001 A.12.4.1, PCI-DSS, GDPR Article 32, SANS, GLI-33

Demilitarized Zones (DMZ)	Secure DMZ configuration	NIST SP 800-53 AC-17, SC-12, SC-7, SC-13, CA-3, NIST SP 800-41, NIST SP 800-94, ISO/IEC 27001 A.13.1.1, A.10.1.1, A.9.2.6PCI DSS, CIS 13.3, 9.4NERC CIP CIP-005-6, CIP-007-6, SANS 12	
Application Layer Filtering	Implement application-layer filtering and intrusion detection.	ISO 27001 A.13.1, NIST SP 800-53 SC-7, ISACA COBIT 5 DSS05, GLI-33	
Third-Party Vendors	**11.1** Third-Party Contracts	Include audit rights and security clauses in third-party contracts such as rights to audit, escrow, etc.	ISO 27001 A.15.1, NIST SP 800-53 SA-9, ISACA COBIT 5 DSS04
PCI-DSS	**13.1** PCI-DSS Compliance	Ensure compliance with PCI-DSS standards to protect cardholder data.	ISO 27001 A.15.2, NIST SP 800-53 SC-12, ISACA COBIT 5 DSS04, GLI-33
Wireless Networks	**14.1** WLAN Security	Ensure wireless networks use appropriate authentication, encryption (e.g., AES), and updated standards. Default configs and passwords must be changes. Use appropriate certificate validations.	ISO 27001 A.13.2, NIST SP 800-53 AC-17, ISACA COBIT 5 DSS05
DNS Security	**15.1** Secure DNS Configuration	Use secure primary and secondary DNS servers that are logically and physically separated.	ISO 27001 A.13.2, NIST SP 800-53 SC-19, ISACA COBIT 5 DSS05, GLI-33
Component Hardening	**16.1** Security Hardening	Address known security vulnerabilities by hardening components and removing unnecessary functionality.	ISO 27001 A.9.2, NIST SP 800-53 CM-6, ISACA COBIT 5 DSS01
User Privacy and Data Protection	**17.1** Personal Information Protection	Implement robust controls to ensure that users' personal information (e.g., financial data, identity) is protected.	GDPR Article 25 (Data Protection by Design and by Default), ISO 27001 A.8.2.3 (Handling PII), NIST SP 800-53 AC-17 (Access Control for Sensitive Data), GLI-33, Office of the Privacy Commissioner of Canada

Fraud Detection and Prevention	**18.1** Safeguard against fraud	Implement systems and processes for detecting and preventing fraudulent transactions and activities within the wagering platform.	PCI-DSS 10.2 (Monitoring for Fraudulent Activity), NIST SP 800-53 AC-19 (Access Control for Fraud Prevention), ISO 27001 A.10.1 (Cryptographic Controls for Fraud Detection), GLI-33
Incident Response Plan (IRP) and Communication	**19.1** Incident Management	Develop and maintain an incident response plan specifically addressing security breaches, unauthorized transactions, or any suspicious activities in the platform.	ISO 27001 A.16.1.1 (Incident Management), NIST SP 800-53 IR-4 (Incident Handling), PCI-DSS 12.10 (Incident Response), GLI-33
Authentication and Authorization Controls (for Gamblers)	**20.1** Secure User Access	Implement strong authentication mechanisms (such as multi-factor authentication) for user login to prevent unauthorized access to betting accounts.	NIST SP 800-63 (Digital Identity Guidelines), PCI-DSS 8.3 (User Authentication), ISO 27001 A.9.2 (User Access Control), GLI-33
Mobile Device Security	**21.1** Securing Mobile Access	Ensure that mobile applications used for online wagering are secure, encrypted, and protected from unauthorized access.	NIST SP 800-124 (Mobile Device Security), ISO 27001 A.9.4 (Access Control for Mobile Devices), GLI-33
Application Layer Filtering and Intrusion Detection	**22.1** Traffic Monitoring and Protection	Implement application-layer filtering and intrusion detection to protect systems from attacks and unauthorized access.	ISO 27001 A.13.1 (Network Security), NIST SP 800-53 SC-7 (Boundary Protection), ISACA COBIT 5 DSS05 (Monitor and Evaluate Network Security), GLI-33
Data Minimization and Anonymization	**23.1** Reduce Data Exposure	Minimize the amount of personal data collected from users. Anonymize or pseudonymize sensitive data where possible.	GDPR Article 5 (Data Minimization), ISO 27001 A.8.2.4 (Anonymization), NIST SP 800-53 SC-28 (Data Protection), GLI-33
Server & Database Security	**24.1** Data Storage Protection	Ensure database servers storing financial and personally identifiable information (PII) are configured securely and access is properly controlled.	NIST SP 800-53 SC-28 (Protection of Information at Rest), ISO 27001 A.9.4 (Access Control), PCI-DSS 3.4 (Data Protection), GLI-33

Regular Penetration Testing and Vulnerability Assessments	**25.1** Continuous Security Testing	Perform regular penetration testing and vulnerability assessments to identify and address weaknesses in the security posture of the wagering platform.	ISO 27001 A.12.6 (Vulnerability Management), NIST SP 800-53 RA-5 (Vulnerability Scanning), PCI-DSS 11.3 (Penetration Testing)
Service Level Agreements	Review SLA's for security and compliance clauses.	Uptime and availability, incident response and notification, data protection and privacy, performance monitoring, access controls, change management, backups and disaster recovery, legal and financial penalties.	ISO 27001 & ISO 27701, PCI-DSS, NIST 800-53, SOC 2 Type II, GLI, eCOGRA, GDPR & CCPA, GLI-33
Cybersecurity Insurance and/or Other Mitigating Controls	**26.1** Risk Mitigation through Insurance	Ensure the company holds sufficient cybersecurity insurance to cover the potential risks associated with online wagering operations.	ISO 27001 A.18.1.5 (Cybersecurity Insurance), NIST SP 800-53 CA-9 (Risk Management), GLI-33
Patch Management Policies and Procedures	Verify the existence of a formal patch management policy	Pre-deployment testing, rollback plans are in place, deployment tracking logs, post patch validation and monitoring	ISO 27001 A.12.6.1, NIST 800-40 Rev 3, NIST 800-53 S1-2, PCI DSS 6.1, SOC 2, MOIST 800-137, GLI-33
Pari-mutuel Wagering	Pari-mutuel Integrity	Race data accuracy and fair odds generation, encryption transmission of wagering information, transaction processing controls and audit trails.	ISO 27001, NIST 800-53, SOC 2, PCI-DSS, GLI-33, eCOGRA, GLI-11

F. Do any racing jurisdictions currently have a version of this rule in effect? If yes, please attach copies of those rules. NA

G. Review the RCI Model Rules and identify any other Model Rules this change would affect and submit proposed amendments to those rules to comply with changes that would be made by this proposal. NA

FILING THIS REQUEST WITH RCI DOES NOT GUARANTEE YOUR PROPOSAL WILL BE CONSIDERED BY THE MODEL RULES COMMITTEE. IF YOU HAVE OPPOSITION FROM AN INTERESTED PARTY, YOU ARE STRONGLY ENCOURAGED TO TRY TO REACH CONSENSUS PRIOR TO FILING THIS FORM.

AGENDA ITEM #3

Proposed Amendment to ARCI-022-015 Terms; ARCI-004-0105 Calculation of Payouts and Distribution of Pools; & ARCI-024-035 Racing Rules – "No Contest" Races

RCI MODEL RULES COMMITTEE
PETITION FOR NEW RULE OR CHANGE TO EXISTING RULE

Please submit the following information to the Chair of the Model Rules Committee at least 45 days in advance of the next scheduled committee meeting to www. rules@arci.com.

Your Contact Information:

Name:	Connie Winn
Organization:	Oregon Racing Commission
Address:	PO Box 366, Gresham, OR 97030
Phone(s):	503-853-5928
Fax #:	
E-mail Address:	Connie.winn@orc.oregon.gov

A. Brief Description of the Issue

There are some inconsistencies and clarifications that need to be made. There are also incidents that have occurred that require changes.

B. Discussion of the Issue and Problem

The ARCI Model Rules provide detailed guidance on multi-leg wagers (including Pick-(n) pools) and establish procedures for handling races declared "No Contest", or moved to a different racing surface after betting closes. They also address how to manage multi-leg wagers wherein ~~**horses**~~ **contestants** are scratched, declared non-starters, or 'run for purse money only'. However, the Rules do not explicitly describe how to address multi-leg pools run "For Purse Only". This creates a regulatory gap that can impact payout distributions and the management of carryover amounts.

This became apparent last year when, on March 9, 2024, Tampa Bay Downs experienced a tote system disruption around the time of Race 10 (Florida Oaks). As the tote issue could not be resolved at the time, Race 11 (Tampa Bay Derby) was run as a "For Purse Only" event, and Race 12 was cancelled. All single-race wagers for those races (e.g., WIN, PLACE, SHOW, Exacta, Trifecta, Superfecta, and Super High 5) were refunded. Multi-race wagers involving races 11 & 12 were calculated by declaring all runners as winners for their respective races, for payout purposes only. This included,

- $1 Daily Double: 5/ALL
- 50-cent Pick Three: 3/5/ALL
- 50-cent Pick 4: 1, 2, 4, 6/3/3, 5/ALL
- 50-cent Pick 5: 8/1, 2, 4, 6/3/3, 5/ALL
- 20-cent Ultimate 6: 3/8/1, 2, 4, 6/3/3, 5/ALL

- 50-cent Pick 3 wager (races 10 through 12): 5/ALL/ALL

Provide background on the issue to build context. Address the following:

- ***What specific problems or concerns are involved in this issue?***

- Lack of standardization in payout handling for tracks and tote providers
- Unclear impact on carryovers
  - Operational and technical considerations respecting "For Purse Only" designated races
  - ***Who does the issue affect?***
    - Bettors
    - Racetracks and Tote Providers
    - Regulators and Commissions
  - ***What existing model rules relate to this issue?***
    - Pick (n) Pool (page 114)
    - "No Contest" definition (page 455)
    - Possibly Daily Double, and WIN 3
    - Less commonly operated pools, including Place Pick (n), Exact (n), Choose (n), Pick (n) Position (x), Quinella Double, Twin Quinella, Twin Trifecta, Tri-Superfecta, Twin Superfecta, 123Racing Pick (n) Wager, Racingo
  - ***Provide relevant quantitative or statistical information if possible.***
    - **NA**

C. Possible Solutions and Impact

Provide possible recommendations to solve the problem. Include details on each proposed solution such as

- ***What solution does this proposal provide?***
  - *Language clarifying and accounts for unique circumstances.*
- ***How will the solution fix the problem?***
  - *Cleaning up language*
- ***How will the change affect any entities or stakeholders?***
  - *All entities adopting model rules will be impacted.*
- ***How will you or your organization be affected by the proposed change?***
  - *NA*
- ***What are the benefits of the proposed change?***
  - *Provides clarity and additional options.*
- ***What are the possible drawbacks of the proposed change?***
  - *Some jurisdictions may choose not to enforce these.*
- ***Identify possible fiscal impact of the recommended change.***
  - *We do not believe there is a fiscal impact to any associations.*

D. Please identify any affected stakeholder groups that expressed support or opposition. (These stakeholders may include the racetracks, breed registries, owners, trainers, jockeys, veterinarians, or others.)

- ***For those stakeholder groups that have expressed an opinion, please list the points on which they agree or disagree, and the arguments they have expressed.***
  - *Xpressbet and AmWest were at the auditing committee meeting and did not have an issue with the rules.*
  - *The folks from Canada want to make sure they can comply with these changes.*

- *We checked with the three major tote companies and gained support and additional input.*

- ***Are there any affected stakeholders that have not been consulted on this proposal?***
- ***Please submit any formal letters of support or opposition by stakeholder groups.***

E. Attach the model rule language you are proposing. Please show new language with underlined text. If you are proposing that the current model rule language be eliminated, please strikeout the language to be deleted.

G. Pick (n) Pools

(1) The Pick (n) requires selection of ~~the first-place finisher~~ the winner of ~~in~~ **(n) specified contests** ~~each of a~~ designated **by the association** ~~number of~~ ~~contests~~ **and approved by the commission where (n) is any number greater than (2).**

(2) Each patron placing a Pick-(n) wager must select at least one ~~horse~~ **contestant** in each of the (n) contests comprising the Pick-(n) wager.

(3) The official order of finish for the designated Pick-(n) contests comprise the correct selections for the wager.

(4)~~The~~ **An** association must obtain written approval from the Commission concerning**:**

i. the scheduling of Pick (n) contests,

ii. the designation of one of the methods prescribed in subsection (5F), ~~and~~

ii.i any designated pool share or carryover amounts or percentages

iv. the amount of any cap to be set on the carryover.

v. how the pick (n) pool and carryover, if any, will be distributed on the final day of the race meet or an approved specified mandatory payout date.

(5) Any changes to the approved Pick (n) format require prior approval from the Commission.

(6~~2~~) The Pick (n) pool shall be apportioned under one of the following methods:

(a) Method 1, Pick (n) with No Carryover:

i. The net Pick (n) pool and carryover, if any, shall be distributed as a single price pool to ~~those~~ **the patrons** who selected the **winner of the greatest number of** ~~first-place finisher in each of the~~ Pick (n) contests**.**~~, based upon the official order of finish.~~

ii. If no **patrons correctly select the winner of at least one of the** ~~there are no such wagers, then a designated percentage of the net pool shall be distributed as a single price pool to those who selected the first-place finisher in the greatest number of Pick (n) contests; and the remainder shall be added to the carryover. Where there is no correct selection of the first-place finisher in at least one of the~~

Pick (n) contests, **the pool must be refunded.** ~~based upon the official order of finish, the day's net pool shall be refunded and the previous carryover pool amount, if any, shall be carried over to the next scheduled corresponding pool.~~

(b) Method 2, Pick (n) with 100% Carryover: The net Pick (n) pool and carryover, if any, shall be distributed as a single price pool to those who selected the first-place finisher in each of the Pick (n) contests, based upon the official order of finish. If there are no such wagers, then 100% of that day's net pool shall be added to the carryover. Where there is no correct selection of the first-place finisher in at least one of the Pick (n) contests, based upon the official order of finish, the day's net pool shall be refunded and the previous carryover pool amount, if any, shall be carried over to the next scheduled corresponding pool.

(c) Method 3, Pick (n) with Minor Pool and Carryover: The major share of the net Pick (n) pool and the carryover, if any, shall be distributed to those who selected the first-place finisher in each of the Pick (n) contests, based upon the official order of finish. The minor share of the net Pick (n) pool shall be distributed to those who selected the first place finisher in the second greatest number of Pick (n) contests, based upon the official order of finish. If there are no wagers selecting the first-place finisher of all Pick (n) contests, the minor share of the net Pick (n) pool shall be distributed as a single price pool to those who selected the first-place finisher in the greatest number of Pick (n) contests; and the major share shall be added to the carryover. Where there is no correct selection of the first-place finisher in at least one of the Pick (n) contests, based upon the official order of finish, the day's net pool shall be refunded and the previous carryover pool amount, if any, shall be carried over to the next scheduled corresponding pool.

(d) Method 4, Pick (n) with No Minor Pool and No Carryover: The net Pick (n) pool shall be distributed as a single price pool to those who selected the first-place finisher in the greatest number of Pick (n) contests, based upon the official order of finish. If there are no winning wagers, the pool is refunded.

(e) Method 5, Pick (n) with Minor Pool and No Carryover: The major share of the net Pick (n) pool shall be distributed to those who selected the first place finisher in the greatest number of Pick (n) contests, based upon the official order of finish. The minor share of the net Pick (n) pool shall be distributed to those who selected the first-place finisher in the second greatest number of Pick (n) contests, based upon the official order of finish. If there are no wagers selecting the first-place finisher in a second greatest number of Pick (n) contests, the minor share of the net Pick (n) pool shall be combined with the major share for distribution as a single price pool to those who selected the first-place finisher in the greatest number of Pick (n) contests. If the greatest number of first-place finishers selected is one (1), the major and minor shares are combined for distribution as a single price pool. If there are no winning wagers, the pool is refunded.

(f) Method 6, Pick (n) with Minor Pool and No Carryover: The major share of net Pick (n) pool shall be distributed to those who selected the first-place finisher in each of the Pick (n) contests, based upon the official order of finish. The minor share of the net Pick (n) pool shall be distributed to those who selected the first-place finisher in the second greatest number of Pick (n) contests, based upon the official order of finish. If there are no wagers selecting the first-place finisher in all Pick (n) contests, the ~~**entire**~~ net Pick (n) pool shall be distributed as a single price pool to those who selected the first-place finisher in the greatest number of Pick (n) contests. If there are no wagers selecting the first-place finisher in a second greatest number of Pick (n) contests, the minor share of the net Pick (n) pool shall be combined with the major share for distribution as a single price pool to those who selected the first-place finisher in each of the Pick (n) contests. If there are no winning wagers, the pool is refunded.

(g) Method 7, Pick (n) with Carryover and "Unique Winning Ticket" Provision: The net Pick (n) pool and carryover, if any, shall be distributed to the holder of a unique winning ticket that selected the first-place finisher in each of the Pick (n) contests, based upon the official order of finish. If there is no unique ticket selecting the first-place finisher in each of the Pick (n) contests, or if there are no wagers selecting the first-place finisher of all Pick (n) contests, the minor share of the net Pick (n) pool shall be distributed as a single price pool to those who selected the first-place finisher in the greatest number of Pick (n) contests, and the major share shall be added to the carryover. Associations may suspend previously approved unique winning ticket wagering with the prior approval of the Commission. Any carryover shall be held until the suspended unique winning ticket wagering is reinstated. Where there is no correct selection of the first-place finisher in at least one of the Pick (n) contests, based upon the official order of finish, the day's net pool shall be refunded and the previous carryover pool amount, if any, shall be carried over to the next scheduled corresponding pool. In obtaining authorization for operating the Pick (n) pool under this subsection, associations must clearly identify which definition under paragraph 16(b) will be relied upon for determining the existence of a unique winning ticket.

(h) Method 8, Pick (n) with the Pool split into three shares, one share having a Carryover: The share percentages are determined by the pool host and approved by the Commission. The first share of the net Pick (n) pool and the carryover, if any, shall be distributed to those who selected the first-place finisher in each of the Pick (n) contests, based upon the official order of finish. The second share of the net Pick (n) pool shall be distributed to those who selected (n-1) of the Pick (n) contests, based upon the official order of finish and a third share of the Pick (n) pool shall be distributed to those who selected (n-2) of the Pick (n) contests, based upon the official order of finish. If there are no wagers selecting the first-place finisher of all Pick (n) contests, the first share shall be added to the carryover. If there are no wagers selecting (n-1) of the Pick (n) contests, this second share shall be added to the carryover. If there are no wagers selecting (n-2) of the Pick (n) contests, this third share shall be added to the carryover. Where there is no correct selection of the first-place finisher in at least one of the Pick (n) contests,

based upon the official order of finish, the day's net pool shall be refunded and the previous carryover pool amount, if any, shall be carried over to the next scheduled corresponding pool.

(i) Method 9, Pick (n) with the pool split into three shares, with Carryovers, and a Unique Winning Ticket Provision: The share percentages are determined by the pool host and approved by the Commission. The first share of the net Pick (n) pool and the first share carryover, if any, shall be distributed to those who selected the first-place finisher in each of the Pick (n) contests, based upon the official order of finish. The second share of the net Pick (n) pool shall be distributed to those who selected the first-place finisher in the second greatest number of Pick (n) contests, based upon the official order of finish. If there are no wagers selecting the first-place finisher of all Pick (n) contests, the second share of the net Pick (n) pool shall be distributed as a single price pool to those who selected the first-place finisher in the greatest number of Pick (n) contests, and the first share shall be added to the first share carryover. The third share and the third share carryover, if any, shall be distributed to the holder of a unique winning ticket that selected the first-place finisher in each of the Pick (n) contests, based upon the official order of finish. If there is no unique winning ticket selecting the first-place finisher in each of the Pick (n) contests, the third share shall be added to the third share carryover. For greater certainty, the holder of a unique winning ticket shall receive both the first share, and first share carryover, if any as well as the third share, and the third share carryover, if any. Where there is no correct selection of the first-place finisher in at least one of the Pick (n) contests, based upon the official order of finish, the day's net pool shall be refunded and the previous carryover pool(s) amount(s), if any, shall be carried over to the next scheduled corresponding pool. In obtaining authorization for operating the Pick (n) pool under this subsection, associations must clearly identify which definition under paragraph 16(b) will be relied upon for determining the existence of a unique winning ticket.

(~~3~~**6**) If there is a dead heat for first in any of the Pick (n) contests involving:

(a) contestants representing the same betting interest, the Pick (n) pool shall be distributed as if no dead heat occurred.

(b) contestants representing two or more betting interests, the Pick (n) pool shall be distributed as a single price pool with each winning wager receiving an equal share of the profit.

(**4**~~6~~**7**) If a wagering interest is "scratched" for a Pick (n) contest, or is designated to run for purse money only, the association shall use the actual favorite, as evidenced by total amounts wagered in the Win pool at the host association for the contest at the close of wagering on that contest, and shall be substituted for the scratched betting interest for all purposes, including pool calculations. In the event that the Win pool total for two or more favorites is identical, the substitute selection shall be the betting interest with the lowest program number. The totalizator shall produce reports showing each of the wagering combinations with substituted betting interests which became winners as a result of the substitution, in addition to the normal winning combination. Notwithstanding the provisions of this subsection, an association may

also obtain authorization from the Commission to allow patrons to select an alternate wagering interest in any of the Pick (n) contests.

(~~5~~**8**) Subject to subsection (9), (10), or (12), the Pick (n) pool shall be cancelled and all Pick (n) wagers for the individual performance shall be refunded if:

(a) at least two contests included as part of a Pick 3 are cancelled or declared "no contest."

(b) at least three contests included as part of a Pick 4, Pick 5 or Pick 6 are cancelled or declared "no contest."

(c) at least four contests included as part of a Pick 7, Pick 8 or Pick 9 are cancelled or declared "no contest."

(d) at least five contests included as part of a Pick 10 are cancelled or declared "no contest."

(~~6~~**9**) Subject to subsection (9), (10), or (12), if at least one contest included as part of a Pick (n) is cancelled or declared "no contest", but not more than the number specified in subsection (5) of this rule, the net pool shall be distributed as a single price pool to those whose selection finished first in the greatest number of Pick (n) contests for that performance. Such distribution shall include the portion ordinarily retained for the Pick (n) carryover but not the carryover from previous performances.

(~~7~~**10**) If the condition of the course warrants a change of racing surface in any of the legs of the Pick (n) races, and such change was not known to the public prior to the closing of wagering for the Pick (n) pool, the stewards shall declare the changed leg(s) a “no contest” for Pick (n) wagering purposes only. A “no contest” race is not to be considered as a contested race. ***In addition, any race designated as “For Purse Only” shall also be treated as a “No Contest” for Pick (n) wagering purposes, with all runners considered winners.***

(~~8~~**11**) The Pick (n) carryover may be capped at a designated level approved by the Commission so that if, at the close of any performance, the amount in the Pick (n) carryover equals or exceeds the designated cap, the Pick (n) carryover will be frozen until it is won or distributed under other provisions of this rule. After the Pick (n) carryover is frozen, 100 percent of the net pool, part of which ordinarily would be added to the Pick (n) carryover, shall be distributed to those whose selection finished first in the greatest number of Pick (n) contests for that performance.

(~~9~~**12**) A written request for permission to distribute the Pick (n) carryover on a specific performance may be submitted to the Commission. The request must be for a specified date no greater than one (1) year from the date the request is submitted and contain justification for the distribution, an explanation of the benefit to be derived, and the intended date and performance for the distribution.

(13~~0~~) Should the Pick (n) carryover be designated for distribution on a specified date and performance in which there are no wagers selecting the first-place finisher in each of the Pick

(n) contests, the ~~entire~~ **net** pool shall be distributed as a single price pool to those whose selection finished first in the greatest number of Pick (n) contests. The Pick (n) carryover shall be designated for distribution on a specified date and performance only under the following circumstances:

(a) Upon written approval from the Commission as provided in subsection (8) of this rule.

(b) Upon written approval from the Commission when there is a change in the carryover cap, a change from one type of Pick (n) wagering to another, or when the Pick (n) is discontinued.

(c) On the closing performance of the meet or split meet.

(1~~1~~**4**) A written request for permission to transfer the Pick (n) carryover to another Pick (n) pool operated by the same pool host may be submitted to the Commission. The request must contain ~~justification for the transfer, including an explanation of the benefit to be derived,~~ a description of the method by which the pool host will present the information to the public that identifies the racetrack(s) for which the pool will be operated and the intended date(s) and performance(s) of the transfer.

(1**5**~~2~~) Unless otherwise stated in writing by the Commission under subsection (9), on the last Pick (n) race on the final day of the meeting, the net pool, including any applicable carryover, shall be distributed as a single price pool to those who selected the first-place finisher in the greatest number of Pick (n) contests, based upon the official order of finish.

(1**6**~~3~~) Notwithstanding subsections (9) and (11), if for any reason the Pick (n) carryover must be held over to the corresponding Pick (n) pool of a subsequent meet, the carryover shall be deposited in an interest-bearing account approved by the Commission. The Pick (n) carryover plus accrued interest shall then be added to the net Pick (n) pool of the following meet on a date and performance so designated by the Commission.

(1**7**~~4~~) With the written approval of the Commission, the association may contribute to the Pick (n) carryover a sum of money up to the amount of any designated cap.

(1**8**~~5~~) The association may suspend previously-approved Pick (n) wagering with the prior approval of the Commission. Any carryover shall be held until the suspended Pick (n) wagering is reinstated. An association may request approval of a Pick (n) wager or separate wagering pool for specific performances.

(**19**~~6~~) As it relates to any distribution method under section 2 which contains a unique winning ticket provision:

a. A written request for permission to distribute the Pick (n) unique winning ticket carryover on a specific performance may be submitted to the Commission. The request must contain justification for the distribution, an explanation of the benefit to be derived, and the intended date and performance for the distribution. Should the Pick (n) unique winning ticket net pool and any applicable carryover be designated for distribution on a specified date and performance

in which there is no unique winning ticket, the ~~entire~~ **net** pool shall be distributed as a single price pool to those who selected the first-place finisher in the greatest number of Pick (n) contests.

b. Associations must clearly identify which selection under clauses (i) and (ii) below will be relied upon ~~for determining~~ **to determine** the existence of a unique winning ticket:

i. there is one and only one winning ticket that correctly selected the first place finisher in each of the Pick (n) contests, based upon the official order of finish, to be verified by the unique serial number assigned by the tote company that issued the winning ticket; or

ii. the total amount wagered on one and only one winning combination selecting the first-place finisher in each of the Pick (n) contests, based up on the official order of finish, is equal to the minimum allowable wager.

F. Do any racing jurisdictions currently have a version of this rule in effect? If yes, please attach copies of those rules. NA

G. Review the RCI Model Rules and identify any other Model Rules this change would affect and submit proposed amendments to those rules to comply with changes that would be made by this proposal. NA

FILING THIS REQUEST WITH RCI DOES NOT GUARANTEE YOUR PROPOSAL WILL BE CONSIDERED BY THE MODEL RULES COMMITTEE. IF YOU HAVE OPPOSITION FROM AN INTERESTED PARTY, YOU ARE STRONGLY ENCOURAGED TO TRY TO REACH CONSENSUS PRIOR TO FILING THIS FORM.

AGENDA ITEM #4

Proposed Amendment ARCI-024-025 Horses Permitted to Race – stipulate permitted number of starts for two-year olds

RCI MODEL RULES COMMITTEE
PETITION FOR NEW RULE OR CHANGE TO EXISTING RULE

Please submit the following information to the Chair of the Model Rules Committee at least 45 days in advance of the next scheduled committee meeting to www. rules@arci.com.

Y**our Contact Information:**

Name:	Michael Tanner/ Michele Kopiec/ TC Lane
Organization:	USTA
Address:	
Phone(s):	
Fax #:	
E-mail Address:	Michele.kopiec@ustrotting.com

A. Brief Description of the Issue: *There is currently no restriction on the number of starts for a two-year old.*

B. Discussion of the Issue and Problem

Provide background on the issue to build context. Address the following:

- ***What specific problems or concerns are involved in this issue?*** *Limits the number of starts for a two-year old by requiring two full days in between races.*
- ***Who does the issue affect?*** *Standarbred Industry*
- ***What existing model rules relate to this issue?*** *None that can be found.*
- ***Provide relevant quantitative or statistical information if possible.***

C. Possible Solutions and Impact

Provide possible recommendations to solve the problem. Include details on each proposed solution such as

- ***What solution does this proposal provide?*** *Protects two-year olds from being raced too frequently.*
- ***How will the solution fix the problem?*** *Self-explanatory*
- ***How will the change affect any entities or stakeholders?*** *Judges will need to verify that two "off" days between races for two-year olds is maintained by USTA reports*
- ***How will you or your organization be affected by the proposed change?*** *As above*
- ***What are the benefits of the proposed change?*** *As above*
- ***What are the possible drawbacks of the proposed change?*** *None known*
- ***Identify possible fiscal impact of the recommended change.*** *None – current reports already in use to assist judges in identifying days between starts for all horses.*

D. Please identify any affected stakeholder groups that expressed support or opposition. (These stakeholders may include the racetracks, breed registries, owners, trainers, jockeys, veterinarians, or others.)

- ***For those stakeholder groups that have expressed an opinion, please list the points on which they agree or disagree, and the arguments they have expressed.***
- ***Are there any affected stakeholder groups that have not been consulted on this proposal?***
- ***Please submit any formal letters of support or opposition by stakeholder groups*.**

This proposal was unanimously approved by the USTA Board of Directors and was circulated to the entire membership prior to the approval of the regulation.

E. Attach the model rule language you are proposing. Please show new language with underlined text. If you are proposing that current model rule language be eliminated, please strikeout the language to be deleted.

Two-Year-Old—Two-year-olds must have at least two days in between race days. No two-year-old shall be permitted to start in a heat or race exceeding one mile in distance and no two-year-old shall be permitted to race in more than one heat or dash in any single day. Starting any two-year-old in violation of this rule shall result in the horse being disqualified from the second start.

F. Do any racing jurisdictions currently have a version of this rule in effect? If yes, please attach copies of those rules. *Pennsylvania has similar rule currently in effect.*

G. Review the RCI Model Rules and identify any other Model Rules this change would affect and submit proposed amendments to those rules to comply with changes that would be made by this proposal. *None that can be found.*

FILING THIS REQUEST WITH RCI DOES NOT GUARANTEE YOUR PROPOSAL WILL BE CONSIDERED BY THE MODEL RULES COMMITTEE. IF YOU HAVE OPPOSITION FROM AN INTERESTED PARTY, YOU ARE STRONGLY ENCOURAGED TO TRY TO REACH CONSENSUS PRIOR TO FILING THIS FORM.

AGENDA ITEM #5

Proposed Amendment to ARCI-020-015 Judges - require Judges to submit accident/incident reports to USTA for purposes of maintaining a central database

RCI MODEL RULES COMMITTEE
PETITION FOR NEW RULE OR CHANGE TO EXISTING RULE

Please submit the following information to the Chair of the Model Rules Committee at least 45 days in advance of the next scheduled committee meeting to www. rules@arci.com.

Y**our Contact Information:**

Name:	Michael Tanner/ Michele Kopiec/ TC Lane
Organization:	USTA
Address:	
Phone(s):	
Fax #:	
E-mail Address:	Michele.kopiec@ustrotting.com

A. Brief Description of the Issue: *From the Standardbred standpoint, the industry does not utilize a centralized database to report injuries or incidents.*

B. Discussion of the Issue and Problem

Provide background on the issue to build context. Address the following:

- ***What specific problems or concerns are involved in this issue?*** *Fills a current lapse/lack in data collection.*
- ***Who does the issue affect?*** *Standarbred Industry*
- ***What existing model rules relate to this issue?*** *N/A*
- ***Provide relevant quantitative or statistical information if possible.***

C. Possible Solutions and Impact

Provide possible recommendations to solve the problem. Include details on each proposed solution such as

- ***What solution does this proposal provide?*** *Fills the void*
- ***How will the solution fix the problem?*** *Self-Explanatory*
- ***How will the change affect any entities or stakeholders?*** *Will be required labor to provide data to the USTA, much similar to the Fines and Suspensions platform that the industry currently utilizes.*
- ***How will you or your organization be affected by the proposed change?*** *Industry will now be able to collect information in a centralized area.*
- ***What are the benefits of the proposed change?*** *Self-Explanatory*
- ***What are the possible drawbacks of the proposed change?*** *None known.*
- ***Identify possible fiscal impact of the recommended change.*** *USTA has already built the database/platform.*

D. Please identify any affected stakeholder groups that expressed support or opposition. (These stakeholders may include the racetracks, breed registries, owners, trainers, jockeys, veterinarians, or others.)

- ***For those stakeholder groups that have expressed an opinion, please list the points on which they agree or disagree, and the arguments they have expressed.***
- ***Are there any affected stakeholder groups that have not been consulted on this proposal?***
- ***Please submit any formal letters of support or opposition by stakeholder groups*.**

This proposal was unanimously approved by the USTA Board of Directors and was circulated to the entire membership prior to the approval of the regulation.

E. Attach the model rule language you are proposing. Please show new language with underlined text. If you are proposing that current model rule language be eliminated, please strikeout the language to be deleted.

Duties of the Judges— Conduct an investigation of any accidents to determine the cause thereof, and the judges shall completely fill out an accident report and transmit it to the USTA. Judges shall report to the association the details of the incident for each horse involved. In connection with the investigation of an accident the judges shall have the authority to require any driver or other person involved in the accident to submit to testing for the personal use of alcohol and/or drugs.

F. Do any racing jurisdictions currently have a version of this rule in effect? If yes, please attach copies of those rules. *Not to our knowledge, although in most jurisdictions, they require an "incident or daily report" to be filed. This would be similar, although centralized.*

G. Review the RCI Model Rules and identify any other Model Rules this change would affect and submit proposed amendments to those rules to comply with changes that would be made by this proposal.

FILING THIS REQUEST WITH RCI DOES NOT GUARANTEE YOUR PROPOSAL WILL BE CONSIDERED BY THE MODEL RULES COMMITTEE. IF YOU HAVE OPPOSITION FROM AN INTERESTED PARTY, YOU ARE STRONGLY ENCOURAGED TO TRY TO REACH CONSENSUS PRIOR TO FILING THIS FORM.

AGENDA ITEM #6

Proposed Amendment to ARCI-022-020 Trainers to include definition of "equipment" and "safely equipped"

RCI MODEL RULES COMMITTEE
PETITION FOR NEW RULE OR CHANGE TO EXISTING RULE

Please submit the following information to the Chair of the Model Rules Committee at least 45 days in advance of the next scheduled committee meeting to www. rules@arci.com.

Y**our Contact Information:**

Name:	Michael Tanner/ Michele Kopiec/ TC Lane
Organization:	USTA
Address:	
Phone(s):	
Fax #:	
E-mail Address:	Michele.kopiec@ustrotting.com

A. Brief Description of the Issue: *Definition of "equipment" and horse safely equipped.*

B. Discussion of the Issue and Problem

Provide background on the issue to build context. Address the following:

- ***What specific problems or concerns are involved in this issue?*** *Defines of "equipment" and horse safely equipped in regard to trainer responsibility.*
- ***Who does the issue affect?*** *Standarbred Industry*
- ***What existing model rules relate to this issue? ARCI-022-020 TRAINERS, TRAINER RESPONSIBILITY (21) - ensuring that the trainer's horses are properly prepared and equipped***
- ***Provide relevant quantitative or statistical information if possible.***

C. Possible Solutions and Impact

Provide possible recommendations to solve the problem. Include details on each proposed solution such as

- ***What solution does this proposal provide?*** *Defines equipment.*
- ***How will the solution fix the problem?*** *Self-explanatory*
- ***How will the change affect any entities or stakeholders?*** *As above*
- ***How will you or your organization be affected by the proposed change?*** *As above*
- ***What are the benefits of the proposed change?*** *As above*
- ***What are the possible drawbacks of the proposed change?*** *None known*
- ***Identify possible fiscal impact of the recommended change.*** *None*

D. Please identify any affected stakeholder groups that expressed support or opposition. (These stakeholders may include the racetracks, breed registries, owners, trainers, jockeys, veterinarians, or others.)

- ***For those stakeholder groups that have expressed an opinion, please list the points on which they agree or disagree, and the arguments they have expressed.***
- ***Are there any affected stakeholder groups that have not been consulted on this proposal?***
- ***Please submit any formal letters of support or opposition by stakeholder groups*.**

This proposal was unanimously approved by the USTA Board of Directors and was circulated to the entire membership prior to the approval of the regulation.

E. Attach the model rule language you are proposing. Please show new language with underlined text. If you are proposing that current model rule language be eliminated, please strikeout the language to be deleted.

(21) ensuring that the trainer's horses are properly prepared and equipped. <u>It shall be the responsibility of the trainer to see that each horse under his supervision is safely equipped for each race and if it is determined by the judges that a horse has been raced with unsafe or faulty equipment the judges may impose a fine, suspension or both. The definition of equipment is what a horse wears to race. The definition does not include the saddle pads that are required to be attached to the harness prior to the respective race.</u>

F. Do any racing jurisdictions currently have a version of this rule in effect? If yes, please attach copies of those rules. *All states require trainers as responsible parties for horse.*

G. Review the RCI Model Rules and identify any other Model Rules this change would affect and submit proposed amendments to those rules to comply with changes that would be made by this proposal. *None that can be found.*

FILING THIS REQUEST WITH RCI DOES NOT GUARANTEE YOUR PROPOSAL WILL BE CONSIDERED BY THE MODEL RULES COMMITTEE. IF YOU HAVE OPPOSITION FROM AN INTERESTED PARTY, YOU ARE STRONGLY ENCOURAGED TO TRY TO REACH CONSENSUS PRIOR TO FILING THIS FORM.

AGENDA ITEM #7

Proposed Addition to ARCI-001-005 Purpose & ARCI-025-020 Medications & Prohibited Substances to add the Harness Racing Medication Collaborative (HRMC)

RCI MODEL RULES COMMITTEE
PETITION FOR NEW RULE OR CHANGE TO EXISTING RULE

Please submit the following information to the Chair of the Model Rules Committee at least 45 days in advance of the next scheduled committee meeting to www. rules@arci.com.

Your Contact Information:

Name:	Michael Tanner/ Michele Kopiec/ TC Lane
Organization:	USTA
Address:	
Phone(s):	
Fax #:	
E-mail Address:	Michele.kopiec@ustrotting.com

A. Brief Description of the Issue: *To establish the Harness Racing Medication Collaborative (HRMC) as the identifying party for medication regulation in Standardbred racing.*

B. Discussion of the Issue and Problem

Provide background on the issue to build context. Address the following:

- ***What specific problems or concerns are involved in this issue?*** *Certain current medication thresholds are not representative to the harness racing industry - only the HRMC can speak for the Standardbred breed regarding medication issues*
- ***Who does the issue affect?*** *Standarbred Industry*
- ***What existing model rules relate to this issue?*** *ARCI-001-005 Purpose (73) Regulatory Limit; ARCI-025-020 Medication and Prohibited Substances (4)*
- ***Provide relevant quantitative or statistical information if possible.*** *HRMC consists of key industry leaders including multiple expert Standardbred veterinarians. Below are a few recaps of the scientific research that has been completed since the inception of HRMC in 2018:*
  Metformin and Gabapentin
  Lasix use
  Clenbuterol and Betamethasone

C. Possible Solutions and Impact

Provide possible recommendations to solve the problem. Include details on each proposed solution such as

- ***What solution does this proposal provide?*** *Provides recommendations for Standardbreds, as the physical characteristics of the Standardbred and Thoroughbred breeds are significantly different, and what thresholds apply to one breed may not apply to the other.*

- ***How will the solution fix the problem?*** *Self-explanatory*
- ***How will the change affect any entities or stakeholders?*** *HRMC recommendations will be used instead of RMTC.*
- ***How will you or your organization be affected by the proposed change?*** *As above*
- ***What are the benefits of the proposed change?*** *As above*
- ***What are the possible drawbacks of the proposed change?*** *None known*
- ***Identify possible fiscal impact of the recommended change.*** *None at this time.*

D. Please identify any affected stakeholder groups that expressed support or opposition. (These stakeholders may include the racetracks, breed registries, owners, trainers, jockeys, veterinarians, or others.)

- ***For those stakeholder groups that have expressed an opinion, please list the points on which they agree or disagree, and the arguments they have expressed.***
- ***Are there any affected stakeholder groups that have not been consulted on this proposal?***
- ***Please submit any formal letters of support or opposition by stakeholder groups*.**

This proposal was unanimously approved by the USTA Board of Directors and was circulated to the entire membership prior to the approval of the regulation.

E. Attach the model rule language you are proposing. Please show new language with underlined text. If you are proposing that current model rule language be eliminated, please strikeout the language to be deleted.

(73) Regulatory Limit is the concentration of a specified regulatory analyte that has been defined and published by the Racing Medication and Testing Consortium and Harness Racing Medication Collaborative and adopted by the commission such that exceeding the specified concentration is either an overage or a positive test.

(4) Any drug or metabolite thereof found to be presenting a pre- or post-race sample which is not classified in the most current RCI Uniform Classification Guidelines for Foreign Substances shall be assumed to be a RCI Class 1 Drug and the trainer and owner shall be subject to those penalties as set forth in schedule "A" unless satisfactorily demonstrated otherwise by the Racing Medication and Testing Consortium or Harness Racing Medication Collaborative, with a penalty category assigned.

F. Do any racing jurisdictions currently have a version of this rule in effect? If yes, please attach copies of those rules. *Not at this time.*

G. Review the RCI Model Rules and identify any other Model Rules this change would affect and submit proposed amendments to those rules to comply with changes that would be made by this proposal. *Two are listed above, there may be others which ARCI will need to define.*

FILING THIS REQUEST WITH RCI DOES NOT GUARANTEE YOUR PROPOSAL WILL BE CONSIDERED BY THE MODEL RULES COMMITTEE. IF YOU HAVE OPPOSITION FROM AN INTERESTED PARTY, YOU ARE STRONGLY ENCOURAGED TO TRY TO REACH CONSENSUS PRIOR TO FILING THIS FORM.

AGENDA ITEM #8

Proposal from U.S. Trotting Association - Classification/Thresholds – Metformin & Gabapentin

RCI MODEL RULES COMMITTEE
PETITION FOR NEW RULE OR CHANGE TO EXISTING RULE

Please submit the following information to the Chair of the Model Rules Committee at least 45 days in advance of the next scheduled committee meeting to www. rules@arci.com.

Your Contact Information:

Name:	Michael Tanner/ Michele Kopiec/ TC Lane
Organization:	USTA
Address:	
Phone(s):	
Fax #:	
E-mail Address:	Michele.kopiec@ustrotting.com

A. Brief Description of the Issue: *Two medications that are extensively used in human medicine, Metformin and Gabapentin, have been appearing as likely contaminants passed in unchanged form from humans.*

B. Discussion of the Issue and Problem

Provide background on the issue to build context. Address the following:

- ***What specific problems or concerns are involved in this issue?*** *Metformin is the third-most prescribed drug in the United States for humans, with dozens, if not more, of environmental contamination positives having been called/detected in the past year. Published research indicates that a screening limit of 5 ng/ml in plasma would be appropriate. A screening limit of 8 ng/ml in plasma for Gabapentin has been implemented in Ohio. – **can't find reference*
- ***Who does the issue affect?*** *Standarbred Industry*
- ***What existing model rules relate to this issue?*** *Uniform Classification Guidelines*
- ***Provide relevant quantitative or statistical information if possible. ***need published research from HRMC***

C. Possible Solutions and Impact

Provide possible recommendations to solve the problem. Include details on each proposed solution such as

- ***What solution does this proposal provide?*** *Addresses the issue of environmental contamination of two commonly prescribed human medications, gives thresholds recommended by HRMC for Standardbreds.*
- ***How will the solution fix the problem?*** *Provides thresholds for substances that are currently no-threshold.*
- ***How will the change affect any entities or stakeholders?*** *Self-explanatory*
- ***How will you or your organization be affected by the proposed change?*** *As above*

- ***What are the benefits of the proposed change?*** *As above*
- ***What are the possible drawbacks of the proposed change?*** *None known*
- ***Identify possible fiscal impact of the recommended change.*** *None*

D. Please identify any affected stakeholder groups that expressed support or opposition. (These stakeholders may include the racetracks, breed registries, owners, trainers, jockeys, veterinarians, or others.)

- ***For those stakeholder groups that have expressed an opinion, please list the points on which they agree or disagree, and the arguments they have expressed.***
- ***Are there any affected stakeholder groups that have not been consulted on this proposal?***
- ***Please submit any formal letters of support or opposition by stakeholder groups.***

The USTA Board of Directors has declared that the Harness Racing Medication Collaborative has been established as the entity to develop thresholds and/or screening levels for medications in use in harness racing.

E. Attach the model rule language you are proposing. Please show new language with underlined text. If you are proposing that current model rule language be eliminated, please strikeout the language to be deleted.

Metformin, at 5 ng/ml in plasma
Gabapentin, at 8 ng/ml also plasma

F. Do any racing jurisdictions currently have a version of this rule in effect? If yes, please attach copies of those rules. *Pennsylvania has similar rule currently in effect.*

G. Review the RCI Model Rules and identify any other Model Rules this change would affect and submit proposed amendments to those rules to comply with changes that would be made by this proposal. *Uniform Classification Guidelines*

FILING THIS REQUEST WITH RCI DOES NOT GUARANTEE YOUR PROPOSAL WILL BE CONSIDERED BY THE MODEL RULES COMMITTEE. IF YOU HAVE OPPOSITION FROM AN INTERESTED PARTY, YOU ARE STRONGLY ENCOURAGED TO TRY TO REACH CONSENSUS PRIOR TO FILING THIS FORM.

Irish Veterinary Journal

CASE REPORT Open Access

Gabapentin, a human therapeutic medication and an environmental substance transferring at trace levels to horses: a case report

Kimberly Brewer[1], Jacob Machin[2], George Maylin[3], Clara Fenger[4], Abelardo Morales-Briceño[5] and Thomas Tobin[2*]

Abstract

Gabapentin, 1-(Aminomethyl)cyclohexaneacetic acid, MW 171.240, is a frequently prescribed high dose human medication that is also used recreationally. Gabapentin is orally absorbed; the dose can be 3,000 mg/day and it is excreted essentially unchanged in urine. Gabapentin is stable in the environment and routinely detected in urban wastewater. Gabapentin randomly transfers from humans to racing horses and is at times detected at pharmacologically ineffective / trace level concentrations in equine plasma and urine. In Ohio racing between January 2019 and July 2020,18 Gabapentin identifications, all less than 2 ng/ml in plasma, were reported. These identifications were ongoing because the horsemen involved were unable to pin down and therefore avoid the source of these identifications. Given that 44 ng/ml or less is an Irrelevant Plasma Concentration (IPC) of Gabapentin in horses, we proposed a 5 ng/ml plasma interim Screening Limit of Detection for Gabapentin identifications in Ohio racing, and an essentially similar 8 ng/ml plasma Screening Limit of Detection was suggested by a scientific advisor to the Ohio Horse Racing Commission. As such, an analytical Screening Limit of 8 ng /ml in plasma is an appropriate and pharmacologically conservative analytical "cut-off" or Screening Limit of Detection (SLOD) for Gabapentin in equine competitive events to avoid the calling of *"positive"* identifications on random unavoidable trace level identifications of this widely prescribed human therapeutic medication in equine forensic samples.

Keywords: Gabapentin, Environmental presence, Horses, Plasma concentration, Screening Limit of Detection, 8 ng/mL

Background

Gabapentin, 1-(Aminomethyl)cyclohexaneacetic acid, MW 171.240, is the 11th most frequently prescribed human medication and Gabapentin is also available and used recreationally in the United States. This review starts with the matter of a Standardbred racehorse shipped from Ontario, Canada, to Scioto Downs, Ohio, racing on September 7th, 2019. The horse won its race and post-race blood, and urine samples were collected and sent to the Analytical Toxicology Laboratory of the Ohio Department of Agriculture (ODA). The primary "A" post-race blood sample was subjected to a "*preliminary analysis*" which "*seemed to indicate the suspected presence of Gabapentin",* which "*suspected presence*" was then confirmed [1]. The B split sample analysis confirmed the Ohio laboratory identification of Gabapentin and

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reported the serum concentration as 273 pg/ml [2]. The connections of the horse /responsible persons were completely unaware of any possible exposure of this horse to Gabapentin. Furthermore, this low concentration plasma Gabapentin *"positive"* was consistent with the year 2018 and thereafter experience of Ohio Harness Horsemen, who were presented with a sequence of about twenty or more low plasma concentration Gabapentin *"positives"*, starting in 2018 and apparently ending in 2020. These low concentration Gabapentin *"positives"* present as a classic series of innocent trace level identification *"positives"* resulting from random inadvertent exposure of these horses to environmental Gabapentin, as we will now detail.

The ongoing gabapentin "positives" in Ohio racing

To our knowledge the first Gabapentin *"positive"* of this Ohio sequence came from a race on January 6th, 2018, followed by two more, these races on Oct. 23rd and Nov. 12th, 2018, for total of three Gabapentin *"positives"*in Ohio racing in 2018 [3]. These Gabapentin *"positives"* resumed in May 2019 and continued for a total of 18 through to July 30th, 2020, constituting a significant fraction of the total number of Gabapentin *"positives"* called in US racing, as presented in Fig. 1. In Ohio the basic penalty imposed was purse redistribution, a US$ 500.00 fine, and a 15 day DisQualification (DQ), although on occasion the fine and DQ times were increased. Ohio horsemen were therefore fully aware of these Gabapentin *"positives"* and the associated penalties but were obviously unable to take any action to avoid these low concentration Gabapentin *"positives"* and the resultant penalties.

The best available quantitative data relating to these Ohio Gabapentin*"positives"* are the estimated concentrations reported present in a sequence of samples called *"positive"* in Ohio from January 1st, 2019, to July 30th, 2020, as presented in Fig. 2, these data provided under a Freedom Of Information (FOI) request to the Ohio Dept. of Agriculture [4]. These *"estimated"* concentrations range from 230 pg/ml to 1,800 pg/ml, with the majority of the *"positives"* being less than 1 ng/ml in plasma. These ongoing 1 ng/ml or so Gabapentin *"positives"* in equine plasma, continued over a period of approaching 1 year or more in the face of penalties for horsemen, completely consistent with and speaking to the horsemen involved being unaware of the origins of these Gabapentin *"positives"* [3].

The classic example in horse racing of *"positives"* of unknown origin is the sequence of racing chemistry *"positives "*for Aminorex, an amphetamine related substance, many as it happens also in Ohio racing, which were eventually found to be caused by the unexpected metabolic transformation of Levamisole, an equine anthelmintic and immune stimulant, to Aminorex [5]. Identification and communication of the Levamisole origins of these Aminorex *"positives"* led to an immediate reduction in the frequency of these Aminorex *"positives",* but sporadic *"positives"* continued. More recently Barbarin, an Aminorex related substance present in Brasicaceae pasture plants /weeds, in Kentucky *Barbarea vulgaris,* colloquially "Yellow Rocket ", has been

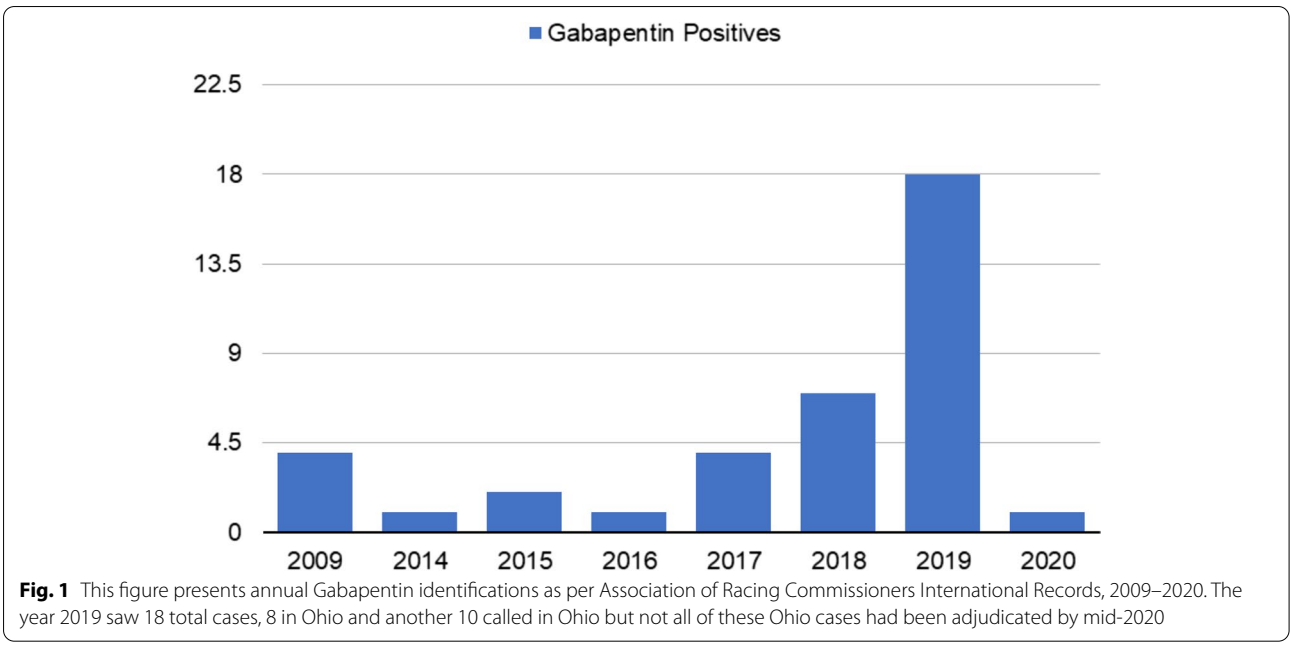

Fig. 1 This figure presents annual Gabapentin identifications as per Association of Racing Commissioners International Records, 2009–2020. The year 2019 saw 18 total cases, 8 in Ohio and another 10 called in Ohio but not all of these Ohio cases had been adjudicated by mid-2020

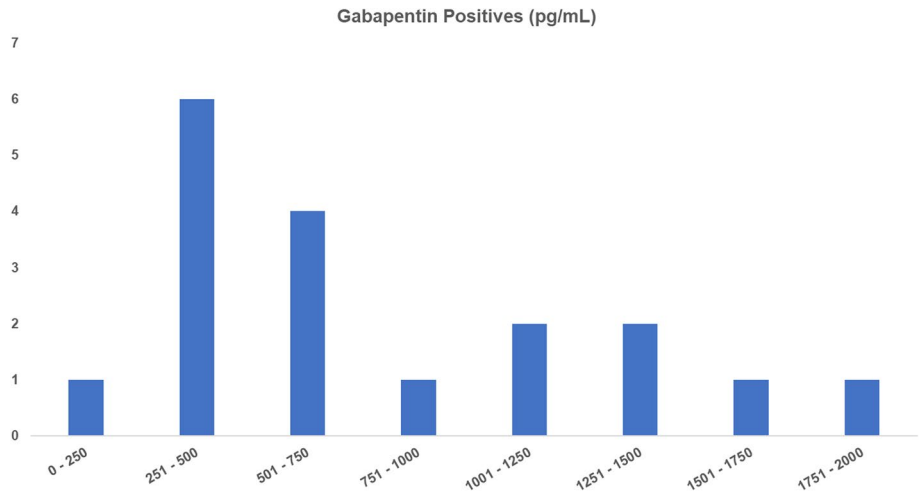

Fig. 2 "Estimated" plasma concentrations in pg/ml of the Gabapentin *"positives"* reported in Ohio racing, January 1st, 2019, to July 30th, 2020, data courtesy of a Freedom of Information (FOI) request to the Ohio Department of Agriculture

shown to be a plant source of Aminorex "positives", presumably explaining at least some of the recent Aminorex *"positives"*reported in American racing and also in European sport horses [6].

Gabapentin as an environmental substance

With regard to the origins of these recent low plasma concentration Ohio Gabapentin *"positives"*, environmental Gabapentin resulting from excretion of human prescription Gabapentin is a / the obvious candidate. Gabapentin, Fig. 3 above, is a human anticonvulsant medication used to treat neuropathic pain, hot flashes and restless leg syndrome and a number of other human conditions. Gabapentin is the 11thmost frequently prescribed medication in the US, it is at times prescribed in combination with opiates, and it is reportedly also used recreationally by humans, presumably at times also in combination with opiates [7]. Gabapentin is also prescribed for similar conditions in veterinary medicine, as we will detail later.

With regard to human prescription use of Gabapentin, the prescribing records for Franklin County, Ohio, show that in September of 2019 a total of about 30,000 prescriptions were written for Gabapentin. If we assume an average prescribed dose of 2 g/day, this is 2 million milligrams / day or 2 kg/day prescribed each and every day in Franklin County, the Ohio county in which the Scioto Downs racetrack is located (https://www.ohiopmp.gov/Stats.aspx) [8]. With regard recreational use of Gabapentin, starting in 2017, three Ohio neighboring states, namely Kentucky, Michigan, and West Virginia, and also non-neighboring Tennessee and Alabama, elected to classify Gabapentin as a Drug Enforcement Administration (DEA) Schedule V Controlled Substance, but to our knowledge Gabapentin remains a non-DEA regulated substance in Ohio.

The second factor driving the Ohio environmental presence of Gabapentin is the unusually large daily human dose of Gabapentin, the maximum prescribed daily dose being in the order of 2,400–3,600 mg/day, taken in 3 divided doses, making it one of the larger daily dose medications administered to humans.

The third factor concerning Gabapentin is how it is handled by the human body. Chemically, Gabapentin is an amino acid and orally administered Gabapentin is absorbed from the intestine by the Large neutral

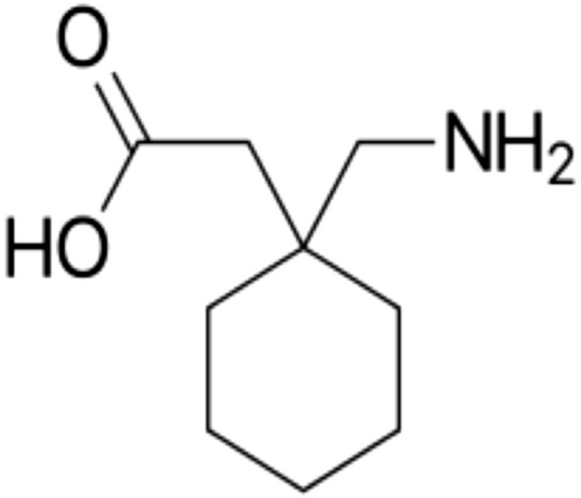

Fig. 3 Chemical structure of Gabapentin, 1-(aminomethyl) cyclohexaneacetic acid, $C_9H_{17}NO_2$, molecular weight 171.24

Amino acid Transporter (LAT-1) a specific amino acid transporter, following which it distributes throughout the body, distributing primarily in body water. This active transporter uptake system means that small oral doses of environmental contamination Gabapentin are likely to be close to fully absorbed, while with higher doses the fraction absorbed and therefore the relative bioavailability declines [9].

Like all amino acids Gabapentin is a zwitterion, a hybrid molecule containing both acidic, i.e., the negatively charged COOH group and also the positively charged basic NH2 group. Gabapentin is therefore not significantly metabolized by the intracellular drug metabolizing systems that modify drug molecules for excretion and Gabapentin is excreted largely unchanged [9]. This means that individuals taking 2,400 mg/day or more of Gabapentin contribute essentially 2.4 g/day or more of Gabapentin per day to their environment. A further concern is that Gabapentin is chemically stable in the environment and is one of many human pharmaceuticals routinely detected in urban wastewater [10].

Given these circumstances, namely 1/ the high daily dose administered to humans, up to 2.4 or more grams/day, both prescribed and recreational Gabapentin, 2/ the fact that essentially all 2.4 g or more are eliminated unchanged by the human, 3/ that it is chemically stable and persists in the environment and 4/ that it is orally absorbed, it is not surprising that inadvertent transfer of Gabapentin from humans prescribed Gabapentin to horses occurs, as has previously been reported [11].

Racehorse identifications directly linked to humans prescribed gabapentin

Review of horse racing regulatory records show that a number of equine Gabapentin *"positives"* have been directly linked to humans prescribed Gabapentin. In Charles Town, West Virginia, a horse racing on April 20th, 2018, was called "positive" for Gabapentin, 3 ng/ml in plasma, 86 ng/ml in urine. The horse's groom was prescribed and taking 2,000 mg /day of Gabapentin [11]. In a second Charles Town matter, on June 8th, 2019, the plasma concentration was 16.7 ng/ml, again linked to an employee in contact with the horse taking prescription Gabapentin [11].

Similar incidents have occurred in California. In one matter two racehorses tested "positive" for Gabapentin, horse #1 on April 14th, 2019, 9–10 ng/ml in urine and Horse #2, April28th, 2109, 5–6 ng/ml in urine. The horse's groom was prescribed Gabapentin, 400 mg TID, and the groom acknowledged urinating in the stall of horse #1. Horse #1 was claimed in the April 19th race and horse #2 was moved into the horse #1 stall on or about April 14th. On April 28th the trainer was notified of the Gabapentin positive in horse #1, on which day horse #2 was racing, and which horse also tested Gabapentin "positive", reported on May 25th, 2019 [12]. Similarly, a July 10th, 2020 "positive" in California involving 2 ng/ml of Gabapentin in blood was also linked to an individual working around the horses being prescribed Gabapentin and urinating in the stall in question [12]. Simply put, there are a significant number of cases linking Gabapentin "positives" to individuals prescribed Gabapentin and it is also of interest that in all of these cases the plasma concentrations of Gabapentin were below the Toutain Irrelevant Plasma Concentration (IPC) for Gabapentin, consistent with the "positives" being well below a pharmacologically significant concentration, as we will now set forth [13].

Human urinary concentrations of gabapentin

Pharmacologically, Gabapentin is a low potency medication, so relatively high plasma concentrations are required for pharmacological effect. In humans, the plasma concentrations required for pharmacological effect are in the order of 2 to 15 ug/ml. Given these relatively high plasma concentrations and the fact that Gabapentin is excreted unchanged, the concentrations of Gabapentin found in human urine can be quite significant, as reported by Heltsley et al. (2011) [14].

Reporting on the concentrations of Gabapentin found in human urine samples, Heltsley et al. (2011) [14] identified a median concentration of 259.8 μg /ml, a mean concentration of 430.9 μg/ml and a high end concentration of 35,345 μg/ml, no less than 35 mg/ml in urine. Given that an average human urinary void volume is about 300 ml, an individual excreting Gabapentin at 35 mg/ml in urine could theoretically contribute approaching 10 g or so of Gabapentin per urine voiding into a horse stall or other equine related environment.

Irrelvant plasma concentrations (IPC) of gabapentin in horses

The pharmacology of Gabapentin in the horse has been described by Terry et al. (2010) [15]. The dose of Gabapentin used was 20 mg/kg, or 9 g to a 453 kg horse, close to the 10 g or so quantity in the above referenced high concentration single human urine voiding. Following IV administration, the median peak plasma concentration was 73 ug/ml and sedation was observable in all horses out to 150 min post IV administration, at which time the mean plasma concentrations of Gabapentin were well above 10 ug/ml. No effects of Gabapentin on heart rate,

rhythm or blood pressure were observed after either Oral or IV administrations.

Using these Terry data and applying the well-established Toutain Irrelevant Plasma Concentration (IPC) model, we can estimate the IPC for Gabapentin in the horse. Calculation of the IPC using the Toutain model, and the Terry data gives an Effective Plasma Concentration (EPC) of about 22 micrograms/ml for Gabapentin in horses. Dividing this concentration by 10 to account for horse to horse variability and then by 50 to ensure no pharmacological effect, one obtains the Toutain IPC of 44 nanograms/ml, we note a somewhat conservative estimate, as discussed by Machin et al., 2018 [16]. We also note that this IPC value is well above the 5 ng/ml in plasma suggested interim Screening Limit of Detection proposed by Brewer et al., [11] and the "in house" 8 ng/ml plasma "Screening Limit" for Gabapentin suggested by Dr. Richard A. Sams in response to a request from the Ohio State Racing Commission [17].

These proposed screening limits are also well supported by a Tukey outlier analysis [16] performed on the Fig. 2 Ohio State Gabapentin data, presented in Fig. 4 below. In this analysis the fences are 2.2 ng/ml for a moderate outlier and 3.3 ng/ ml for an "extreme" outlier, fully consistent with the proposed Screening Limits for Gabapentin in equine plasma referenced above.

This review and analysis shows that unusually large amounts of unchanged Gabapentin are excreted into the environment by humans prescribed Gabapentin and also presumably by humans using it recreationally. In those Gabapentin "*positive* " cases where specific human sources in contact with the horse in question were identified, the concentrations of Gabapentin identified in the plasma and urine samples generally ran above 3 ng/ml. On the other hand, in all of these Ohio January 1st, 2019, to July 30th, 2020 "*positives*", where to our knowledge no specific human source has been identified, all of the plasma concentrations are lower, with the highest Ohio *"positive"* concentration being 1.8 ng/ml, the median being 0.5 ng/ml and the concentration in question in the September 7th, 2019, Scioto Downs racehorse sample being an extremely modest 0.28 ng/ml. As such, this claimed Ohio Gabapentin *"positive"*, at 0.28 ng/ml of Gabapentin in plasma, is an exceptionally low concentration claimed "*positive* " identification. This low concentration claimed present in this horse, and all of the similarly Irrelevant Plasma Concentrations claimed identified in the Ohio *"positives"* of Fig. 2 are completely consistent with innocent, inadvertent and essentially unavoidable exposure of these horses to trace level environmental amounts of Gabapentin. Of equal importance, there is no possibility whatsoever of an effect on the outcome of the race in question associated with these low concentration claimed plasma *"positives"*, which interpretation is fully supported by the independent analysis and Screening Limit presented in this matter by the Ohio State Racing Commission expert [17].

As this communication was in final draft a report appeared in The Irish Times [18] detailing a circumstance were Gabapentin transferred from a dog to a racing

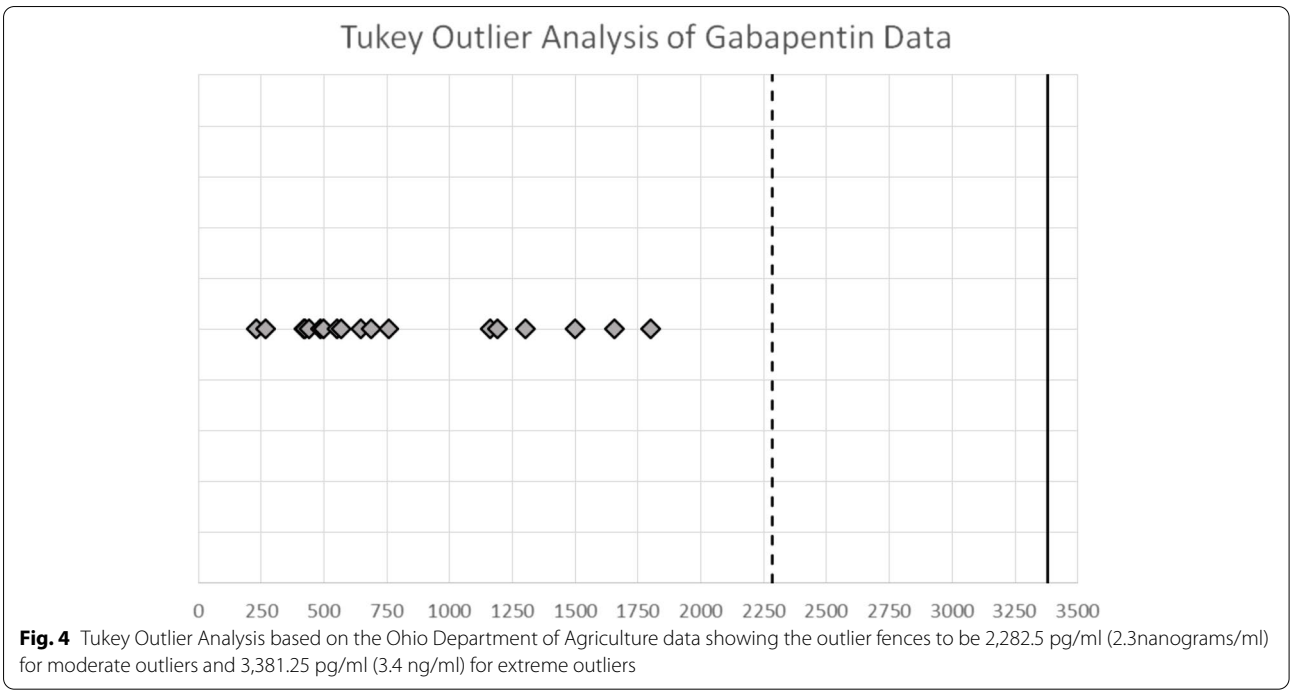

Fig. 4 Tukey Outlier Analysis based on the Ohio Department of Agriculture data showing the outlier fences to be 2,282.5 pg/ml (2.3nanograms/ml) for moderate outliers and 3,381.25 pg/ml (3.4 ng/ml) for extreme outliers

horse which horse then tested "positive " for Gabapentin. Gabapentin is used as a therapeutic medication in canine medicine and the dog in this case was a large, 45 kg or so Rhodesian Ridgeback, which had been prescribed Gabapentin for a back injury, presumably at a dose rate comparable to that used in humans. The dog had access to the stable in which the horse was kept, which facts were communicated to the regulatory authority in this matter, the Irish Horseracing Regulatory Board (IHRB). As reported in The Irish Times [18], this possibility was investigated by dosing dogs with Gabapentin and an "*extensive laboratory investigation"* was carried out by the English testing laboratory, LGC, which investigation apparently showed *"sufficient scientific evidence for the IHRB to accept (*the presented*) explanation for the post-race result as likely".* The investigation concluded that the medication was *"unknowingly administered*" to the horse after *"excretion from the dog in the stable"* a somewhat unexpected but fully understandable sequence of events with respect to the well understood transfer of Gabapentin from humans to horses and now from at least one dog prescribed Gabapentin to a racing horse.

Conclusions

Gabapentin is a high dose human prescription medication that is also used recreationally by humans. Gabapentin is a DEA class 5 scheduled substance in Kentucky, Michigan, and West Virginia, but not in Ohio. Gabapentin is not metabolized by humans, so the full 3 g/day or so human dose is excreted unchanged into the environment, at times at remarkably high concentrations in human urine. Gabapentin is chemically stable and persists in contaminated environments. As such, inadvertent transfer from humans using Gabapentin to horses occurs, as is clear from the data analyzed and reviewed in this case report.

More importantly, the amounts of environmental Gabapentin transferring to horses are usually minimal and all of these referenced Ohio equine plasma concentrations are an order of magnitude or more below the best available estimates of the conservative Toutain Irrelevant Plasma Concentration (IPC) of Gabapentin in horses, calculated at about 44 ng/ml in plasma. These findings therefore strongly support the proposed Ohio State Racing Commission in place Screening Limit Of Detection (SLOD) of 8 ng/ml for Gabapentin in equine plasma and we note that this Screening Limit Of Detection is actually five-fold more conservative than the itself quite conservative 44 ng/ml Toutain calculated Irrelevant Plasm Concentration (IPC) for Gabapentin in equine plasma.

Abbreviations

IPC: Irrelevant Plasma Concentration; SLOD: Screening Limit of Detection; OHRC: Ohio Horse Racing Commission; ODA: Ohio Department of Agriculture; DQ: DisQualification; FOI: Freedom Of Information; LAT-1: Large Neutral Amino acid Transporter; DEA: Drug Enforcement Administration; EPC: Effective Plasma Concentration; IHRB: Irish Horseracing Regulatory Board.

Acknowledgements

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Authors' Note

Recent more extensive review of the Ohio State Racing Commission (OSRC) gabapentin identification records shows that one 2018 postrace equine sample presented the highest recorded gabapentin concentration, 89.4 ng/ml in plasma, along with detections of four other substances, hydrochlorothiazide, O-desmethyltramadol and oxazepam and nordiazepam. An individual was identified with medical prescriptions for gabapentin, hydrochlorothiazide, Tramadol, and a benzodiazepine who frequently urinated in the stall in question. As outlined to the OSRC, this matter presents as a classic case of urinary driven substance transfer from the individual prescribed these medications to the horse housed in the stall, in which stall the individual frequently urinated. To our knowledge this is the to date largest number of human prescription medications inadvertently transferring from a single human urine source to a racing horse.

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 Emirates Endurance Village reviewed the data interpretation and analysis and approved the proposed interim SLOD from an equine practitioner, researcher, and regulatory scientist's perspective. JM and KB performed the data 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 review.

Competing interests

The authors cite no competing interests or specific funding other than those referenced in acknowledgements. All authors contributed to the review, analysis and drafting of the manuscript and all reviewed and approved the final product for publication. Authors KB, CF, AMB, GAM and TT are researchers and participants in areas of equine forensic science and have presented

and at times testified as experts such matters. The corresponding author, TT, and author CF have at times advised parties with respect to matters involving Gabapentin identifications.

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References

1. "Final Test Report", dated October 9th, 2109, submitted by the Analytical Toxicology Laboratory of the Ohio Department of Agriculture to the Ohio State Racing Commission.
2. "Positive Letter" dated November 21st, 2019, from Travis Mays PhD Texas A& M Veterinary Medical Diagnostic Laboratory to Mr. Bill Crawford, Executive Director, Ohio State Racing Commission.
3. Personal communication 8/13/2020 to TT by Mr. Kerry Holloway Association of Racing Commissioners International of an updated listing of ARCI recorded Gabapentin regulatory "positives".
4. Ohio Department of Agriculture response of 9/2/2020 to public records request 200808 by James Gallagher, 471 East Broad St, Columbus, Ohio, document entitled "Gabapentin Positives in Plasma (January 1, 2019, to July 30, 2020).
5. Gutierrez J, Eisenberg RL, Koval NJ, et al. Pemoline and Tetramisole 'positives' in English racehorses following Levamisole Administration. Ir Vet J. 2010;63(8). https://doi.org/10.1186/2046-0481-63-8-498
6. Maylin G, Fenger C, Machin J, et al. Aminorex identified in horse urine following consumption of Barbarea vulgaris; a preliminary report. Ir Vet J. 2019;72(1). https://doi.org/10.1186/s13620-019-0153-5
7. Goodman CW, Brett AS. A clinical overview of off-label use of gabapentinoid drugs. JAMA Intern Med. 2019;179(5):695. https://doi.org/10.1001/jamainternmed.2019.0086.
8. Pharmacy OSB of. Reports and statistics gabapentin prescriptions per 100K residents by month . Ohio Automated Rx Reporting System: OARRS. https://ohiopmp.gov/Stats.aspx. Accessed 12 Mar 2022.
9. del Amo EM, Urtti A, Yliperttula M. Pharmacokinetic role of L-type amino acid transporters LAT1 and LAT2. Eur J Pharm Sci. 2008;35(3):161–74. https://doi.org/10.1016/j.ejps.2008.06.015.
10. Gurke R, Rößler M, Marx C, et al. Occurrence and removal of frequently prescribed pharmaceuticals and corresponding metabolites in wastewater of a sewage treatment plant. Sci Total Environ. 2015;532:762–70. https://doi.org/10.1016/j.scitotenv.2015.06.067.
11. Brewer K, Fenger C, Machin J, Catignani M, Tobin T. Gabapentin: a classic human medication transferring in trace amounts to racing horses . The Horseman's J. 2020. https://issuu.com/thehorsemensjournal/docs/fall2020. Accessed 12 Mar 2022.
12. Personal communications to TT by Mr. Darryl Vienna, Esq., 5256 S. Mission Road, Suite 703 - #808, Bonsall, CA 92003 communicated 6/11/2021 concerning Gabapentin "positives" in California horseracing.
13. Toutain PL, Lassourd V. Pharmacokinetic/pharmacodynamic approach to assess irrelevant plasma or urine drug concentrations in postcompetition samples for drug control in the horse. Equine Vet J. 2010;34(3):242–9. https://doi.org/10.2746/042516402776185985.
14 Heltsley R, DePriest A, Black DL, Robert T, Caplan YH, Cone EJ. Urine drug testing of chronic pain patients. iv. prevalence of gabapentin and pregabalin. J Anal Toxicol. 2011;35(6):357–9. https://doi.org/10.1093/anatox/35.6.357.
15 Terry RL, McDonnell SM, Van Eps AW, et al. Pharmacokinetic profile and behavioral effects of gabapentin in the horse. J Vet Pharmacol Ther. 2010;33(5):485–94. https://doi.org/10.1111/j.1365-2885.2010.01161.x.
16. Machin J, Brewer K, Catignani M, et al. An interim screening limit of detection for naproxen in equine plasma: a review and analysis. Comp Exerc Physiol. 2020;16(2):153–60. https://doi.org/10.3920/cep190044.
17. Sams RA. "Reporting Recommendations for Specific Substances". Memorandum provided to M. Ryzmek at the Ohio State Racing Commission on or about November 19th, 2019.
18. O'Connor B. Coolmore vet loses "winner" due to drug in family dog The Irish Times Friday June 17th 2022. 2022. https://www.irishtimes.com/sport/racing/2022/06/17/.

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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 [11]. 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].

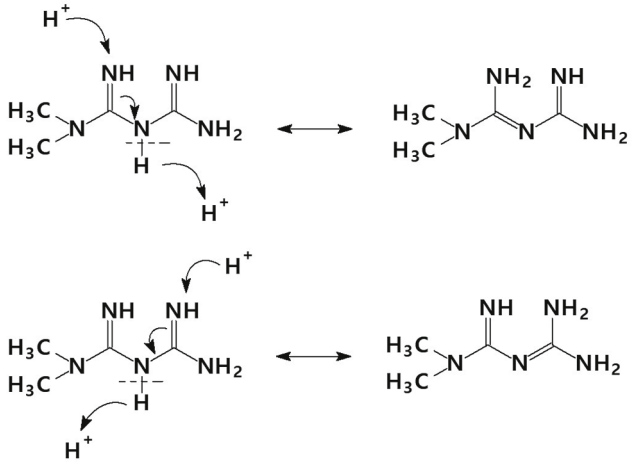

Fig. 1 Equilibrium structures of metformin, *N,N*-dimethylbiguanide, $C_4H_{11}N_5$, molar mass 129.167 g/mol. In acid, protonation of the C-2 imine induces release of a proton from the central nitrogen resulting in a 2-3 double bond (top). Protonation of the C-4 imine induces release of a proton from 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-

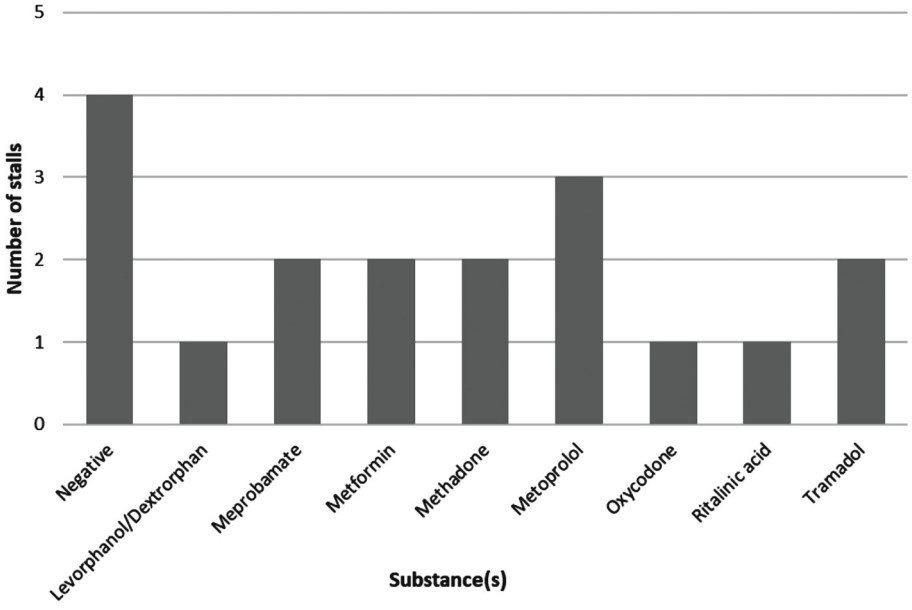

Fig. 2 Human therapeutic substances found in the "ship-in" stalls at Charles Town races. | *Humantherapeutische Substanzen, die in den „Ship-in"-Ständen bei den Rennen in Charles Town gefunden wurden.*

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			
6/11/2023	Guadalupe Munoz Elizondo	Quinton's Charmer	NM	Plasma/ serum	162 pg/ml Plasma 242.5 ng/ml urine			Work, not race
6/24/2023	Javier Morzan	Lady Liv	Delaware Park	Plasma/ serum	253 pg/ml; Corrected 222 pg/ml		Suspended	
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.

References

1 Ambrosio-Albuquerque EP, Cusioli LF, Bergamasco R, Sinópolis Gigliolli AA, Lupepsa L, Paupitz BR, Barbieri PA, Borin-Carvalho LA, de Brito Portela-Castro AL (2021) Metformin environmental exposure: A systematic review. Environm Toxicol Pharmacol 83, PMID 103588, DOI 10.1016/j.etap.2021.103588
2 AORC (2007) AORC Draft Glossary of Terms, available at https://www.aorc-online.org/documents/draft-aorc-glossary-of-terms-11-11-2007/, accessed October 24, 2023
3 ARCI, Association of Racing Commissioners International (2023). Model Rules & Standards. Association of Racing Commissioners International, available at https://www.arci.com/model-rules-standards/, accessed November 1, 2023
4 Bradley PM, Journey CA, Button DT, Carlisle DM, Clark JM, Mahler BJ, Nakagaki N, Qi SL, Waite IR, VanMetre PC (2016) Metformin and Other Pharmaceuticals Widespread in Wadeable Streams of the Southeastern United States. Environm Sci Technol Lett 3, 243–249, DOI 10.1021/acs.estlett.6b00170
5 Bradley PM, Journey CA, Romanok KM, Barber LB, Buxton HT, Foreman WT, Furlong ET, Glassmeyer ST, Hladik ML, Iwanowicz LR, Jones DK, Kolpin DW, Kuivila KM, Loftin KA, Mills MA, Meyer MT, Orlando JL, Reilly TJ, Smalling KL, Villeneuve DL (2017) Expanded Target-Chemical Analysis Reveals Extensive Mixed-Organic-Contaminant Exposure in U.S. Streams. Environm Sci Technol 51, 4792–4802, DOI 10.1021/acs.est.7b00012
6 Brewer K, Fenger C, Machin J, Catignani M, Tobin T (2020) Gabapentin: Classic Human Medication Transferring in TRACE Amounts to Racing Horses. The Horsemen's Journal 67, 44–47
7 Brewer K, Machin J, Maylin G, Fenger C, Morales-Briceño A, Tobin T (2022) Gabapentin, a human therapeutic medication, and an environmental substance transferring at trace levels to horses: a case report. Irish Vet J 75, 19, DOI 10.1186/s13620-022-00226-5
8 Das S, Behera SK, Srinivasan A, Xavier AS, Selvarajan S, Kamalanathan S, Sahoo JP, Nair NS (2018) Effect of metformin on exercise capacity: A meta-analysis. Diab Res Clin Pract 144, 270–278, DOI 10.1016/j.diabres.2018.08.022
9 Durham AE, Rendle DI, Newton JE (2008) The effect of metformin on measurements of insulin sensitivity and beta cell response in 18 horses and ponies with insulin resistance. Equine Vet J 40, 493–500, DOI 10.2746/042516408X273648
10 FEI (2023) FEI Clean Sport Prohibited Substances Database, available at prohibitedsubstancesdatabase.feicleansport.org, accessed November 1, 2023
11 Fenger C, Catignani M, Machin J, Tobin T (2017) An In-Depth Look at Stall Contamination: A Total of 28 Substances Were Identified in Charles Town Ship-In Stalls as a Mix of Human Medications and Recreational Substances with Some Actual Equine Medications. The Horsemen's Journal 64, 41–44
12 Fenger C, personal communication to T Tobin (2023)
13 Gerken DF, Sams RA, McKeever KH, Hinchcliff KW, Ashcraft S (1991) Urinary pH effects on the renal clearance of lidocaine and phenylbutazone in exercising horses. Toxicologist 297, abstract
14 Graham GG, Punt J, Arora M, Day RO, Doogue MP, Duong JK, Furlong TJ, Greenfield JR, Greenup LC, Kirkpatrick CM, Ray JE, Timmins P, Williams KM (2011) Clinical Pharmacokinetics of Metformin. Clin Pharmacokin 50, 81–98, DOI 10.2165/11534750-000000000-00000
15 HIWU (2023) Update on ADMC Cases and the Harmonization Among HIWU-Accredited Laboratories of Metformin's Limit of Detection in Blood. Available at https://www.hiwu.org/news/update-on-admc-cases-and-the-harmonization-among-hiwu-accredited-laboratories-of-metformin-s-limit-of-detection-in-blood, accessed October 24, 2023.
16 Holloway K. Personal Communication to T Tobin (2023)
17 Horn JR, Hansten PD (2017) Metformin and Organic Cation Transporter Interactions. Pharmacy Times, 83. Available at https://www.pharmacytimes.com/view/metformin-and-organic-cation-transporter-interactions, accessed October 10, 2023.
18 Houston T, Chay S, Woods WE, Combs G, Kamerling S, Blake JW, Edmundson AG, Vessiney R, Tobin T (1985) Phenylbutazone and its metabolites in plasma and urine of thoroughbred horses: population distributions and effects of urinary pH. J Vet Pharmacol Ther 8, 136–49, DOI 10.1111/j.1365-2885.1985.tb00937.x
19 Hustace JL, Firshman AM, Mata JE (2009) Pharmacokinetics and bioavailability of metformin in horses. Am J Vet Res 70, 665–668, DOI 10.2460/ajvr.70.5.665

20 Lewis S (2022) The Top 50 Drugs Prescribed in the United States. Available at https://www.healthgrades.com/right-care/patient-advocate/the-top-50-drugs-prescribed-in-the-united-states, accessed October 10, 2023
21 Ningrum VDA, Ikawati Z, Sadewa AH, Ikhsan MR (2018) Patient-factors associated with metformin steady-state levels in type 2 diabetes mellitus with therapeutic dosage. J Clin Transl Endocrinol 12, 42–47, DOI 10.1016/j.jcte.2018.05.001
22 Shenfield G (2013) Metformin: Myths, misunderstandings and lessons from history. Austr Prescrib 36, 38–39, DOI 10.18773/austprescr.2013.017
23 Toutain PL, Lassourd V (2010) Pharmacokinetic/pharmacodynamic approach to assess irrelevant plasma or urine drug concentrations in postcompetition samples for drug control in the horse. Equine Vet J 34, 242–249, DOI 10.2746/042516402776185985
24 Vienna D. Personal Communication T Tobin (2023).
25 WADA (2017, November). Diabetes Mellitus - World Anti-Doping Agency. WADA-World Ant-Doping Program. https://www.wada-ama.org/sites/default/files/wada-tpg-diabetes-mellitus-v4-en.pdf, accessed November 14, 2023

Erweiterte Zusammenfassung

Metformin als Umweltsubstanz wird auf Pferde übertragen – Fallbericht und Analyse

Metformin ist ein häufig verschriebenes orales Antihyperglykämiemittel und derzeit ein Medikament der ersten Wahl bei der Behandlung von Typ-2-Diabetes beim Menschen, mit insgesamt 92 Millionen Verschreibungen in den USA im Jahr 2022. Die Tagesdosis pro Mensch kann bis zu 2,5 Gramm/Tag betragen, die weitgehend unverändert in die Umwelt ausgeschieden werden. Metformin ist chemisch stabil und ein weit verbreiteter Umweltstoff. Metformin hat daher das Potenzial, in Spuren von Blut- und Urinproben von Pferden als Ergebnis einer zufälligen Exposition gegenüber Metformin in der Umwelt nachgewiesen zu werden. Unter diesen Umständen haben wir die wissenschaftliche Literatur überprüft und eine irrelevante Blut-/Plasma-/Serumkonzentration von Metformin von 5 Nanogramm/ml berechnet. Wir schlagen daher nun diese Plasmakonzentration von Metformin als vorläufige Screening-Nachweisgrenze (SLOD) für Metformin vor, unterhalb derer eine Blut-/Plasma-/Serum-Identifizierung von Metformin nicht als geeignet für regulatorische Maßnahmen angesehen werden sollte.

Schlüsselwörter: Metformin, Umweltverschmutzung, antihyperglykämisches Mittel, Screening-Nachweisgrenze, Rennpferde

AGENDA ITEM #9

Proposal from U.S. Trotting Association – Classification/Thresholds – Benzoylecgonine; Aminorex; Pemoline; Methamphetamine; Synephrine

RCI MODEL RULES COMMITTEE
PETITION FOR NEW RULE OR CHANGE TO EXISTING RULE

Please submit the following information to the Chair of the Model Rules Committee at least 45 days in advance of the next scheduled committee meeting to www. rules@arci.com.

Your Contact Information:

Name:	Michael Tanner/ Michele Kopiec/ TC Lane
Organization:	USTA
Address:	
Phone(s):	
Fax #:	
E-mail Address:	Michele.kopiec@ustrotting.com

A. Brief Description of the Issue: *To establish threshold levels for multiple substances with levels that have been recommended by the HRMC.*

B. Discussion of the Issue and Problem

Provide background on the issue to build context. Address the following:

- ***What specific problems or concerns are involved in this issue?*** *Screening limits for certain drugs, listed below, have been researched and threshold levels recommended by published papers, see below.*
- ***Who does the issue affect?*** *Standarbred Industry*
- ***What existing model rules relate to this issue?*** *Uniform Classification Guidelines*
- ***Provide relevant quantitative or statistical information if possible:***
    - **Benzoylecgonine (BZE) – 1 ng/ml in plasma**. Supported by a recently released paper co-authored by HRMC members Drs. Fenger, Maylin, and Tobin.
    - **Aminorex – 30ng/ml in urine.** Based upon previously published peer-reviewed studies. This recommendation solely addresses an environmental contamination threshold which could be exceeded by the administration of Levamisole.
    - **Pemoline – 2ng/ml in plasma.** Supported by a paper published in late 2024 by Drs. Fenger, Maylin, and Tobin.
    - **Methamphetamine – 1 ng/ml in plasma**. Supported by previously published (and peer-reviewed) papers.
    - **Synephrine – 50 ng/ml in urine**. Based upon previously published peer-reviewed studies.

C. Possible Solutions and Impact

Provide possible recommendations to solve the problem. Include details on each proposed solution such as

- ***What solution does this proposal provide?*** *Addresses the testing levels for multiple substances in the Standardbred industry.*
- ***How will the solution fix the problem?*** *Provides thresholds.*
- ***How will the change affect any entities or stakeholders?*** *Self-explanatory*
- ***How will you or your organization be affected by the proposed change?*** *As above*
- ***What are the benefits of the proposed change?*** *As above*
- ***What are the possible drawbacks of the proposed change?*** *None known*
- ***Identify possible fiscal impact of the recommended change.*** *None*

D. Please identify any affected stakeholder groups that expressed support or opposition. (These stakeholders may include the racetracks, breed registries, owners, trainers, jockeys, veterinarians, or others.)

- ***For those stakeholder groups that have expressed an opinion, please list the points on which they agree or disagree, and the arguments they have expressed.***
- ***Are there any affected stakeholder groups that have not been consulted on this proposal?***
- ***Please submit any formal letters of support or opposition by stakeholder groups.***

The USTA Board of Directors has declared that the Harness Racing Medication Collaborative has been established as the entity to develop thresholds and/or screening levels for medications in use in harness racing.

E. Attach the model rule language you are proposing. Please show new language with underlined text. If you are proposing that current model rule language be eliminated, please strikeout the language to be deleted.

Benzoylecgonine (BZE) – 1 ng/ml in plasma.
Aminorex – 30ng/ml in urine
Pemoline – 2ng/ml in plasma
Methamphetamine – 1 ng/ml in plasma
Synephrine – 50 ng/ml in urine

F. Do any racing jurisdictions currently have a version of this rule in effect? If yes, please attach copies of those rules.

G. Review the RCI Model Rules and identify any other Model Rules this change would affect and submit proposed amendments to those rules to comply with changes that would be made by this proposal. *Uniform Classification Guidelines*

FILING THIS REQUEST WITH RCI DOES NOT GUARANTEE YOUR PROPOSAL WILL BE CONSIDERED BY THE MODEL RULES COMMITTEE. IF YOU HAVE OPPOSITION FROM AN INTERESTED PARTY, YOU ARE STRONGLY ENCOURAGED TO TRY TO REACH CONSENSUS PRIOR TO FILING THIS FORM.

Compendium Pferdeheilkunde (2025) 1 (Januar/Februar) 13–20

Trace level Benzoylecgonine detections in equine plasma/serum – a proposed interim plasma level regulatory "cut-off"

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Summary: Cocaine is a widely used human recreational substance routinely transferring at pharmacologically irrelevant trace level amounts to non-user humans and horses. Horses and humans rapidly demethylate cocaine to benzoylecgonine which is excreted in urine at concentrations approaching two thousand times higher than the corresponding plasma cocaine concentrations. In human workplace drug testing, these usually pharmacologically irrelevant trace level urinary detections of benzoylecgonine are handled by application of a 100 ng/ml urinary *"cut-off"*, as presented by the Substance Abuse and Mental Health Services Administration (SAMHSA). In horse racing several American states have long used published urinary *"cut-offs"* for benzoylecgonine of from 50 to 150 ng/ml. More recently, on September 13th 2023, the Horseracing Integrity and Welfare Unit (HIWU) *"called"* a plasma benzoylecgonine *"positive"* at 76 picograms/mL, thereby raising the matter of defining a benzoylecgonine *"cut-off"* in equine plasma equivalent to the long in place 100 ng/ml or thereabouts urinary *"cut-offs"* for benzoylecgonine used in equine medication regulation. We therefore reviewed the relevant published scientific data and proposed 1.0 nanogram/ml of benzoylecgonine as an equine plasma/serum benzoylecgonine *"cut-off"* equivalent to a 100 ng/ml urinary *"cut-off"*. We communicated this analysis to the Pennsylvania Horsemen's Benevolent and Protective Association (PAHBPA) on September 14th and 24th 2023 as preliminary draft communications. Soon thereafter, on or about November 17th 2023, the HIWU authority withdrew its case in the above referenced matter and also a second case involving another Pennsylvania trainer with a presumably similar benzoylecgonine plasma/serum identification. We specifically note that trace level amounts of cocaine are found on US currency, leading to widespread exposure of individuals and horses to pharmacologically irrelevant amounts of cocaine which is excreted as its benzoylecgonine metabolite at readily detectable but pharmacologically irrelevant concentrations in human and equine urine. Given the long in place and well established 100 nanogram/ml SAMHSA "cut-off" for urinary cocaine in human workplace testing we now formally present 1.0 ng/ml plasma *"cut-off"* level as a plasma/serum regulatory *"cut-off"* for trace level identifications of benzoylecgonine in equine plasma/serum which *"cut-off"* was also communicated by the Horseracing Integrity and Welfare Unit on May 14th, 2024.

Keywords: horse, equine, trace level, doping, Benzoylecgonine, plasma/serum, cut-off

Citation: Lehner AF, Brewer K, Dirikolu L, Morales Briceño A, Holland R, Maylin G, Fenger C, Tobin T (2025) Trace level Benzoylecgonine detections in equine plasma/serum – a proposed interim plasma level regulatory "cut-off". Comp Pferdehlk 1, 13–20

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Background

Cocaine (Methyl (1R,2R,3S,5S)-3-(benzoyloxy)-8-methyl-8-azabicyclo[3.2.1] octane-2-carboxylate, MW 303.358, Figure 1) is a plant alkaloid with marked central nervous stimulant activities and widespread use as a human recreational substance. [1–3] In the United States (US) cocaine is a Schedule II substance under the Controlled Substances Act and cocaine is also the second most popular illegal recreational drug in the US, the US being the world's largest consumer of cocaine. As such, cocaine is widely distributed in the US, and paper money in the US frequently presents with detectable amounts of cocaine. [4–8] Given these circumstances, most individuals in the US are at essentially all times inadvertently and unknowingly exposed to small environmental amounts of cocaine that may be sufficient to at times give rise to detectable urinary concentrations of its major urinary metabolite BenZoylEcgonine, (BZE, Figure 1) which substance is chemically a zwitterion and is therefore excreted at unusually high and readily detectable concentrations in both human and equine urine, as we will now detail. [1,2]

As set forth in Figure 2, the BenZoylEcgonine molecule has two pKa values, one at 2.15 on the carboxyl group and another between 11.4–11.7 on the basic bridge nitrogen.[9,10] Figure 2 shows how the charge distribution on the BenZoylEcgonine molecule changes depending on pH and note that the middle structure with net charge of 0 represents the zwitterion form, nominally electrically neutral but neutral because it carries one positive charge and also one negative charge. Given that post-race urines can range from pH 4.5 to 9.5 in Thoroughbred horses and pH 5.5 to 9.5 in Standardbred horses,[11] the likelihood is that BZE in horse urine will nearly always be present in the electrically neutral but actually double charged zwitterion state.

This benzoylecgonine zwitterion/double charge effect means that the interaction between water/H_2O molecules, which carry a positive charge on each hydrogen atom and a negative charge on the oxygen atom is particularly strong, as set forth in Figure 2. In horse urine therefore the positively charged nitrogen of the BZE molecule binds with the negatively charged oxygen in the urinary water molecules. Similarly, the negatively charged COOH group of the benzoylecgonine molecule binds with the positively charged hydrogens in the urinary water molecules. Together these interactions mean that benzoylecgonine is trapped in aqueous horse urine at the pH values of equine urines and can be concentrated approaching a 2,000-fold or more concentration in equine urine than the concentration of the parent cocaine molecule in plasma, as shown by *Lehner* and colleagues[12] and as we will now detail.

In place urinary "cut-offs" for Benzoyllecgonine

Given these circumstances, humans and horses associated with humans in the United States are always at risk of having trace level concentrations of cocaine or more particularly its highly concentrated and readily detectable BenZoylEcgonine metabolite present at readily detectable concentrations in their urine. The solution to this problem, long in place in human drug testing, is a defined regulatory "cut-off", the classic example being the procedure used by the Substance Abuse and Mental Health Services Administration (SAMHSA), which involves an *"Initial test cut-off"* of 150 ng/ml of BZE in urine,

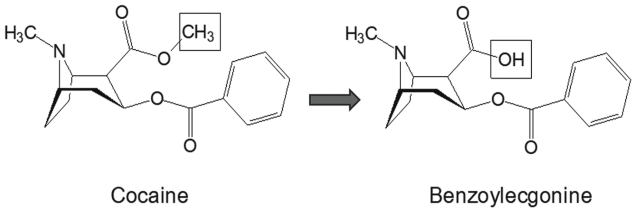

Fig. 1 Chemical structures of cocaine and its major demethylated urinary metabolite BenZoylEcgonine (BZE), the removed methyl group indicated by the box at left. Chemically, BZE is a zwitterion, able to carry a positive charge on the basic bridge nitrogen and a negative charge on its carboxyl group. | *Chemische Strukturen von Kokain und seinem wichtigsten demethylierten Harnmetaboliten BenZoylEcgonin (BZE), der entfernten Methylgruppe, die im Kasten links angezeigt wird. Chemisch gesehen ist BZE ein Zwitterion, das eine positive elektrostatische Aufladung am Grundstickstoff der Brücke und eine negative elektrostatische Aufladung an seiner Carboxylgruppe tragen kann.*

followed by a 100 ng/ml *"Confirmatory test cutoff"* for benzoylecgonine in urine.[13]

Adapting this *"cut-off"* approach to trace levels detections of cocaine and or its major urinary metabolite benzoylecgonine in horse racing regulation, a number of US Racing Authorities have presented and communicated regulatory *"cut-offs"* for BenZoylEcgonine in equine urine. Reviewing this matter in 2006 and also in 2012 *Tobin* and colleagues noted 7 US racing authorities with communicated thresholds/*cut-offs* for BenZoylEcgonine in urine, and at least one unnamed North American jurisdiction using a 50 ng/ml benzoylecgonine *"in house"* screening limit.[1,2] Overall, the urinary BenZoylEcgonine *"cut-off"* or their equivalent being utilized in horse racing regulation have been broadly similar to the current SAMSHA *"Confirmatory test cutoff concentration of 100 ng/ml of benzoylecgonine in urine, the range being from 50 ng/ml to 150 ng/ml"*.[1,2,13]

Plasma concentrations equivalent to these 100 ng/ml urinary "cut-offs" for Benzylecgonine

On or about September 13th 2023 the US Horseracing Integrity and Welfare Unit (HIWU) reported the to our knowledge first ever BenZoylEcgonine *"positive"* in equine plasma, a claimed

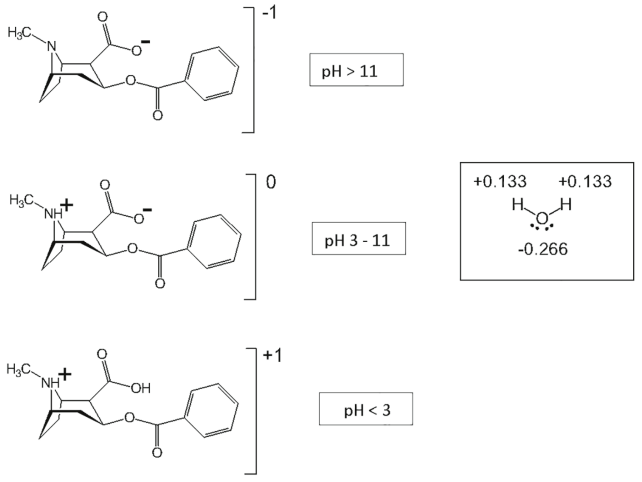

Fig. 2 Left, charges on BZE functional groups depending on environmental pH. The bracket superscript -1, 0 or +1 indicates the net molecular charge depending on whether a proton has been added or subtracted from the tertiary amine or carboxyl functional groups. Right, molecular structure of water. Partial positive charges on H-atoms and negative charge on O-atom were determined by semi-empirical CNDO geometry optimization via a Polak-Ribiere conjugate gradient algorithm in Hyperchem 8.0 software (Hypercube, hypercubeusa.com). The double dots represent non-bonding lone electron pairs. Note the net neutrality of the water molecule. | *Links: elektrostatische Aufladungen an BZE-Funktionsgruppen in Abhängigkeit vom pH-Wert der Umgebung. Die hochgestellte Klammer -1, 0 oder +1 gibt die molekulare Nettoladung an, je nachdem, ob den tertiären Amin- oder Carboxyl-Funktionsgruppen ein Proton hinzugefügt oder davon abgezogen wurde. Rechts, molekulare Struktur von Wasser. Teilweise positive Aufladungen an H-Atomen und negative Aufladung an O-Atomen wurden durch semiempirische CNDO-Geometrieoptimierung über einen konjugierten Polak-Ribiere-Gradientenalgorithmus in der Software Hyperchem 8.0 (Hypercube, hypercubeusa.com) bestimmt. Die Doppelpunkte stellen nichtbindende freie Elektronenpaare dar. Beachten Sie die Nettoneutralität des Wassermoleküls.*

apparently 76 picogram/ml detection in a post-race equine plasma/serum sample taken from a horse racing on August 16th at Penn National Race Course in Pennsylvania. We were asked by the Pennsylvania Horsemen's Benevolent and Protective Association (PAHBPA) for our best analysis of the pharmacological significance of this claimed identification.[14] Given that there was at that time to our knowledge no scientifically determined or published regulatory *"cut-off"* for BenZoylEcgonine in plasma in human or equine forensic science we reviewed the available scientific literature on the relationship between plasma and urinary concentrations of BenZoylEcgonine in horses with a view to identifying a blood/plasma/serum concentration of BenZoylEcgonine equivalent to the above previously communicated and long in place regulatory *"cut-offs"* for BenZoylEcgonine in both human and equine urine.[1,2,13]

To our knowledge the only published data on the relationship between IV doses of cocaine and plasma and urinary concentrations of cocaine and benzoylecgonine in the horse are those communicated by *Lehner* et al. 2000[12] and replotted in Figures 3 and 4, respectively. In this *Lehner* reported research administration of a 100 mg IV dose of cocaine to horses yielded peak urinary concentrations of benzoylecgonine of 9,200 ng/ml at one-hour post administration, as presented in Figure 3.

Furthermore, when *Lehner* et al.[12] looked at the corresponding plasma concentrations of cocaine and BZE in this horse administered 100 mg of cocaine IV (Figure 4) they saw that the plasma concentrations of cocaine closely followed a classic two compartment pharmacokinetic model. As per this model, cocaine administered IV first redistributes rapidly, with a 12-min distribution half-life, followed by a later elimination phase with a 151-minute half-life. The BZE metabolite peaked at 30 minutes post-administration at a calculated 79 ng/ml and thereafter declined with a terminal plasma half-life of about 7.22 hours over the following eight hours. These data suggest a relatively consistent and slowly declining equine plasma concentration of BZE starting about 30 minutes after a 100 mg IV administration of cocaine. This pharmacokinetic analysis was carried out using a commercial software, Phoenix WinNonLin software (Phoenix Inc WinNonLin Software, Version 8.3 Certara, Princeton, NJ, USA). A two compartmental model for cocaine and a noncompartmental model for BZE were used for calculation of pharmacokinetic parameters. The results for cocaine demonstrate an excellent fit between the observed and predicted time courses for plasma cocaine following its IV administration (Fig. 4b) and Table 1 presents the calculated pharmacokinetic parameters for cocaine and its metabolite BZE.

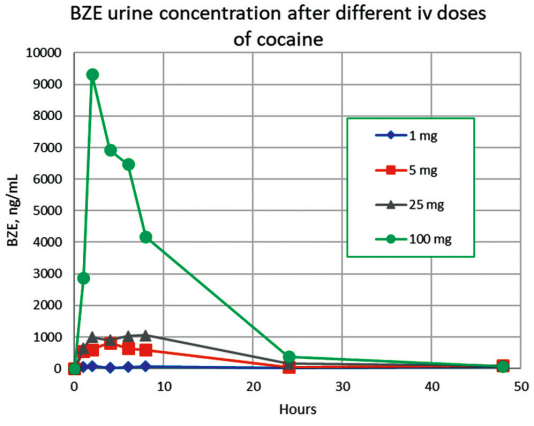

Fig. 3 Urinary concentrations of benzoylecgonine (BZE) following IV administration of the indicated doses of cocaine, replotted from *Lehner* et al.[12]. | *Urinkonzentrationen von Benzoylecgonin (BZE) nach intravenöser Verabreichung der angegebenen Kokaindosen, neu aufgetragen von Lehner et al.*[12].

Based on the fact that a 100 mg IV dose of cocaine yielded a peak urinary BenZoylEcgonine concentrations of 9,200 ng/ml, a 1 mg administration would be expected to produce a urinary concentration of 92 ng/ml of BenZoylEcgonine and a plasma concentration of BenZoylEcgonine of 0.79 ng/ml. Adjusting the 1 mg IV dose up to 1.087 mg gives a urinary BenZoylEcgonine concentration of 100 ng/ml, the currently in place SAMSHA cutoff, and this IV dose would give rise to a plasma BenZoylEcgonine concentration of a fraction under 0.86 ng/ml. Rounding this value up to 1 ng/ml, it seemed reasonable based on the data reviewed to propose a 1 ng/ml screening limit for BenZoylEcgonine in equine plasma as an interim SAMHSA equivalent *"cut-off"* or Screening Limit of Detection (SLOD) for BenZoylEcgonine in equine plasma/serum in equine regulatory testing.

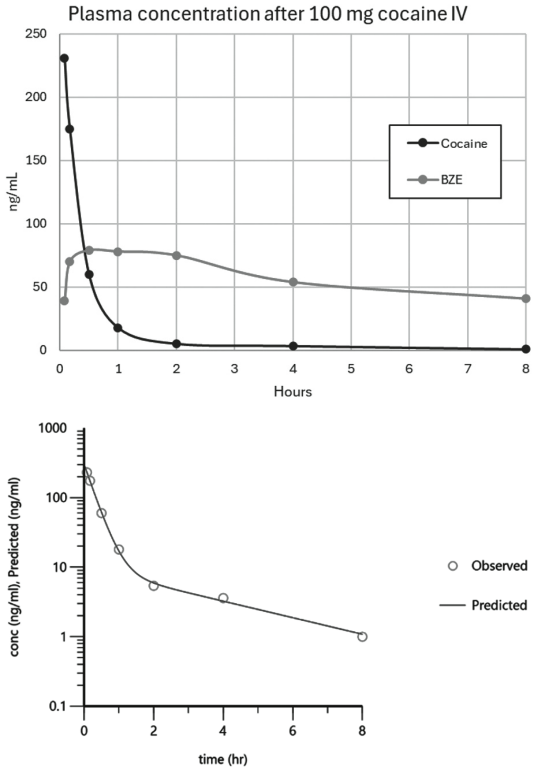

Fig. 4 Figure 4a, above, presents plasma concentration of cocaine and BenZoylEcgonine following IV administration of a 100 mg dose of cocaine, replotted from *Lehner* et al.[12]. Figure 4b, bottom, shows that these plasma cocaine concentration data are well fit by a classic two compartment pharmacokinetic model. | *Abbildung 4a oben zeigt die Plasmakonzentration von Kokain und BenzoylEcgonin nach intravenöser Verabreichung einer 100-mg-Dosis Kokain, neu aufgetragen von Lehner et al.*[12]. *Abbildung 4b unten zeigt, dass diese Plasma-Kokainkonzentrationsdaten gut mit einem klassischen Zwei-Kompartiment-Pharmakokinetikmodell übereinstimmen.*

This proposed plasma Screening Limit/Threshold/"cut-off" was communicated to the Pennsylvania Horsemen's Benevolent and Protective Association in outline form on September 14th 2023 and in a more complete form on September 24th 2023. We are unclear as to what if any actions were taken with respect to these communications and the by then apparently two trainers involved in the matter of HIWU plasma/serum BenZoylEcgonine identifications, but on November 7th 2023 HIWU[15] announced that it had withdrawn its cases against the two Pennsylvania trainers that had HIWU reported identifications of the cocaine metabolite BenZoylEcgonine in blood. Additionally, HIWU also noted that with regard to these BenZoylEcgonine matters it had *"developed a new blood testing specification"* and *"that the levels found in the horses in question" "did not exceed the new specification"*, as reported on November 17th in the Bloodhorse.[16]

Subsequently, on May 14th 2024 the Horseracing Integrity and Safety Authority (HISA) formally communicated a Screening Limit/Threshold/Minimum Reporting Level (MRL) for BenZoylEcgonine in equine serum or plasma of 1.0 ng/ml,[17] in good agreement with our previously proposed and communicated 1.0 ng/ml plasma concentration as an interim plasma level regulatory *"cut-off"* for trace level identifications of BenZoylEcgonine in equine plasma/serum samples.

Discussion

This decision by HIWU to continue application of the long in place 100 ng/ml analytical *"cut-off"* for BenZoylEcgonine in equine urine and their application of its equivalent plasma *"cut-off"* of 1 ng/ml is well supported by decades of practical equine regulatory and forensic experience. To the best of our knowledge the source and driving factor in trace level urinary detections of cocaine/benzoylecgonine in racing horses is inadvertent transfer from recreational users or environmental contamination associated with such recreational users. Clear-cut evidence for such racing related exposure to trace level amounts of environmental cocaine in a racing environment comes from the identification of cocaine/benzoylecgonine in the Charles Town Racing *"ship-in"* stalls. When the Charles

Table 1 Principal pharmacokinetic parameters calculated for cocaine as administered drug at 100 mg (approximately 0.2 mg/kg) dosing following a two-compartment model and for BZE as its metabolite following a noncompartmental model. | *Wichtigste pharmakokinetische Parameter, berechnet für Kokain als verabreichtes Arzneimittel bei einer Dosierung von 100 mg (ungefähr 0,2 mg/kg) nach einem Zwei-Kompartiment-Modell und für BZE als seinen Metaboliten nach einem Nicht-Kompartiment-Modell.*

Parameter	Units	Estimate	Definition
		Cocaine	
AUC0-inf	hr*ng/ml	121.234	Area under curve from zero to infinity
K10_HL	hr	0.278	Elimination half-life from central compartment
Alpha	1/hr	3.407	Distribution rate constant
Beta	1/hr	0.274	Elimination rate constant
Alpha_HL	hr	0.203	Distribution half-life
Beta_HL	hr	2.533	Terminal elimination half-life
A	ng/ml	292.195	Y-intercept of distribution phase
B	ng/ml	9.704	Y-intercept of elimination phase
Cmax	ng/ml	301.899	Maximum plasma concentration
Cls	ml/hr	8.25E+11	Systemic clearance
AUMC	hr*hr*ng/ml	154.75	Area under moment curve
MRT	hr	1.2765	Mean residence time
Vss	ml	1.05E+12	Volume distribution at steady state
		Benzoylecgonine	
Lambda_z	1/hr	0.0959	Elimination rate constant
HL_Lambda_z	hr	7.229	Elimination half-life
Tmax	hr	0.50	Time to maximum concentration
Cmax	ng/ml	79.00	Maximum plasma concentration
AUCINF_pred	hr*ng/ml	882.06	AUC zero to infinity, predicted
AUMCINF_pred	hr*hr*ng/ml	9278.1	AUMC zero to infinity, predicted
MRTINF_pred	hr	10.519	MRT zero to infinity, predicted

Town Racing authorities swabbed their *"ship-in"* stalls over possible concerns about Naproxen contamination of their *"ship-in"* stalls, of 21 *"ship-in"* stalls tested 10 were found to have detectable concentrations of cocaine/benzoylecgonine, just fractionally below 50% of the stalls tested, as set forth in Figure 5. Simply put, both the *"ship-in"* stalls and presumably also the horses racing at Charles Town Racing were at risk of exposure to trace level amounts of cocaine and or benzoylecgonine from human recreational users in contact with both the Charles Town Racing *"ship-in"* stalls and also with the horses racing from these stalls.[18]

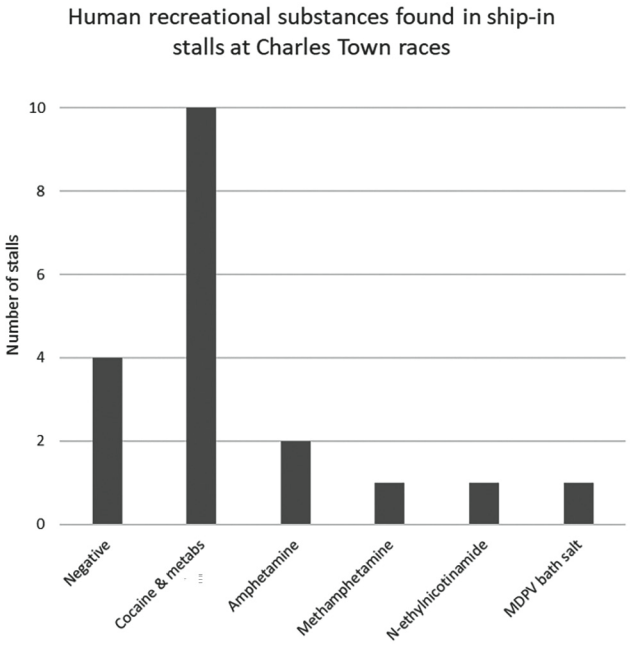

Fig. 5 Human recreational substances identified in "ship-in" stalls at Charles Town Races. Of 21 "ship-in" stalls drug tested at Charles Town Races, 10 stalls tested "positive" for cocaine and metabolites. Reproduced with permission from reference 18. | *Menschliche Freizeitsubstanzen, die in „Ship-in"-Ständen bei Charles Town Races identifiziert wurden. Von 21 „Ship-In"-Ständen, die bei Charles Town Races auf Drogen getestet wurden, wurden 10 Stände „positiv" auf Kokain und Metaboliten getestet. Wiedergabe mit Genehmigung aus Referenz 18.*

The second matter of concern is that this random trace level exposure of racing horses to pharmacologically irrelevant amounts of cocaine can readily give rise to detectable levels of benzoylecgonine in post-race urine samples. As presented in Figure 3, administration of an IV dose of 100 mg of cocaine to a horse yielded a urinary concentration of 9,280 ng/ml of benzoyl ecgonine with just 4 nanograms/ml of parent cocaine present in this urine sample, and which urinary parent cocaine concentration is similar to the post-distribution phase plasma concentrations of cocaine presented in Figure 3. Based on these figures a reasonable estimate of the urinary concentrations of cocaine and benzoylecgonine following administration of 1 mg of cocaine IV would be 98 nanograms/ml benzoylecgonine and a full 40 picograms/ml or so of parent cocaine. Furthermore, there is no reason to expect that 1 mg of cocaine will produce a pharmacological effect in a horse, so urinary detections of benzoylecgonine at concentrations in the order of 92 ng/ml or less are completely unlikely to be associated with a pharmacological effect.

These evaluations of the pharmacological and forensic significance of urinary identification of benzoylecgonine in the order of 100 nanograms/ml are well supported in the scientific literature. The most clear-cut study in this area is that of Professor *Queiroz-Neto* and colleagues[3] who identified 10 mg of cocaine administered IV as the Highest No-Effect Dose (HNED) for cocaine in their experimental horses following IV administration and noted that this administration produced urinary concentrations of benzoylecgonine in the order of 550 ng/ml, in good general agreement with the data of Figure 3. Simply put, urinary concentrations of benzoylecgonine of 100 ng/ml or less are unlikely to be associated with a pharmacological response and are fully consistent with exposure of the horse to inadvertent transfer of trace level amounts of cocaine from human recreational users.

Identifications of benzoylecgonine in post-race urine samples were relatively unusual until the introduction of more sensitive immunoassay-based testing technologies in horse racing in the late nineteen eighties.[19] In a 1988/1989 sequence of events in California racing involving detections of *"cocaine"*, almost certainly actually nothing more than pharmacological-

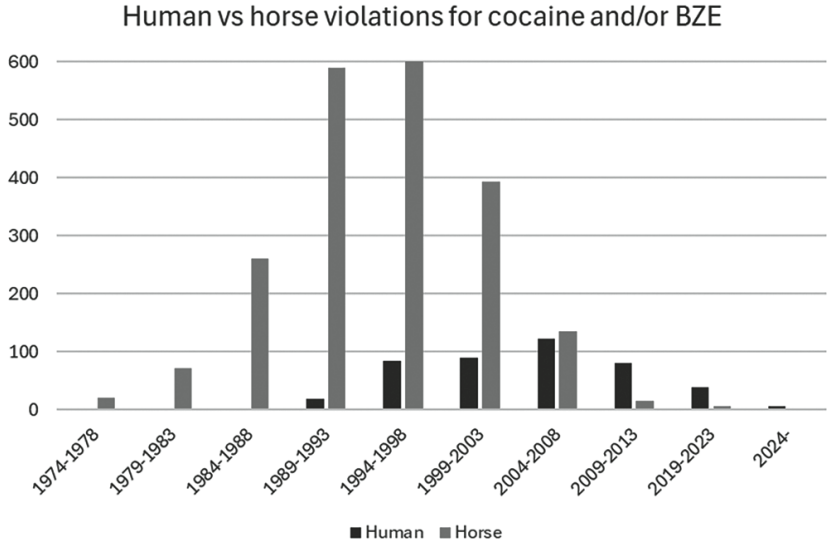

Fig. 6 Number of ARCI-labeled Medication/Drug Violations for humans or animals for cocaine and/or benzoylecgonine in 5-year increments from 1974–2024. These reported violations are about 94% equine and 6% canine and have been labeled as "Horse" in the graph. Only 2024 data are available for what would be the 2024–2028 5-year range. | *Anzahl der ARCI-gekennzeichneten Medikamenten-/Drogenverstöße bei Menschen oder Tieren wegen Kokain und/oder Benzoylecgonin in 5-Jahres-Schritten von 1974 bis 2024. Diese gemeldeten Verstöße betreffen etwa 94% Pferde und 6% Hunde und wurden in der Grafik als „Pferd" gekennzeichnet. Für den 5-Jahres-Bereich 2024–2028 sind nur Daten für 2024 verfügbar.*

ly irrelevant urinary traces of the benzoylecgonine metabolite, reportedly at concentrations in the order of 30 nanograms/ml, were reported as soon as immunoassay-based testing for cocaine/benzoylecgonine was introduced in California. These then novel events took some time to sort out but to our knowledge the ultimate solution was introduction of an unpublished *"in-house" "cut-off"* for benzoylecgonine, presumably in the order of 100 nanograms/ml.

This matter of trace level detections of benzoylecgonine detections in human urine samples had already been addressed in human workplace drug testing. The first solution presented in or about 1988 was the initial Substance Abuse and Mental Health Services Administration (SAMHSA) screening level *"cut-off"* for benzoylecgonine in workplace drug testing of 300 ng/ml in urine linked to a 150 nanogram/ml confirmation *"cut-off"*,to our knowledge introduced in or about 1988. More recently this *"cut-off"* has been adjusted to a 150 ng/ml screening *"cut-off"* and a 100 ng/ml confirmation *"cut-off"*, as described above.

Consistent with this human workplace approach to the matter of trace level benzoylecgonine detections in urine samples the to our knowledge first formally communicated regulatory *"cut-off"* for benzoylecgonine in horse racing was the 150 ng/ml *"cut-off"* introduced on July 1 [st], 1999 by the Ohio Horse Racing Commission.[20] Since then, a number of other jurisdictions have communicated *"cut-offs"* for benzoylecgonine in horse racing, as communicated by *Camargo* et al.[2] and also by *Tobin* et al.[1]

The ability of introduction of these "cut-offs" to handle the matter of random environmental exposure of racing horses to trace level amounts of cocaine are evident from the history of such cocaine/benzoylecgonine detections in racing horses. According to data for the period 1974–2024 provided by the Association of Racing Commissioners International (ARCI),[21] reported cocaine or benzoylecgonine violations peaked in horse/dog racing during the years 1989–1998 (Fig. 6). In the horseracing community human exposure was primarily reported in individuals identified as persons with intent to sell, although drug testing was also occasionally linked to cocaine or benzoylecgonine use. This activity in humans peaked during a different period than that in horses, namely in the years 2004–2008, basically a decade beyond the highpoint of racehorse exposure. Diminished reporting rates in horses were apparently due to the introduction of regulatory "cut-offs" in horseracing, which eliminated the calling of trace level benzoylecgonine identifications associated with random environmental exposure. The decrease in use by grooms, jockeys or trainers with a peak in the early 2000s is most likely related to ongoing shifts in the popularity of abused drugs, especially as new drugs or drug combinations become available. For example, cocaine hit a high in the 1979–1988 period at 6.25% of the population and declined to 2.14% for the 2010–2017 period.

Closing comments

In closing, in the United States cocaine is the second most widely used human recreational substance with trace amounts of cocaine readily transferring inadvertently to racing horses. In horses cocaine is rapidly metabolized to benzoylecgonine, which zwitterion metabolite is excreted in equine urine at up to 2,000-fold or so greater concentrations than the corresponding plasma cocaine concentrations. The ready detectability of these pharmacologically irrelevant urinary concentrations of benzoylecgonine has led to the introduction of regulatory *"cut-off"* concentrations for benzoylecgonine in equine urine in the order of 100 ng/ml. With the recently increased sensitivity and capability of testing for benzoylecgonine in blood we now propose 1 ng/ml of benzoylecgonine in blood/plasma/serum as a plasma/serum concentration "cut-off" for benzoylecgonine equivalent to the long in place 100 nanogram/mL urinary "cut-off" for benzoylecgonine in place in SAMSHA work place drug testing and also historically in place in many US racing jurisdictions, and which plasma/serum concentration "cut-off" for benzoylecgonine has to the best of our knowledge been adopted by the US Horseracing Integrity and Welfare Unit.

Abbreviations

ARCI	Association of Racing Commissioners International
BZE	BenZoylEcgonine
HISA	Horseracing Integrity and Safety Authority
HIWU	Horseracing Integrity and Welfare Unit
IV	Intra-Venous
MRL	Minimum Reporting Level
PA	HBPA Pennsylvania Horsemen's Benevolent and Protective Association
SAMSHA	Substance Abuse and Mental Health Services Administration
SLOD	Screening Limit of Detection (SLOD)
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 guideline from an equine practitioner, researcher, and regulatory scientist's perspective. KB, AFL and LD performed the data searching, chemical structure evaluations and statistical and pharmacokinetic 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.

References

1 Tobin T, Brewer K, Stirling K (2012) World Rules for Equine Drug Testing and Therapeutic Medication Regulation 2012 Policy of the National Horsemen's Benevolent and Protective Association. Nicholasville, Kentucky, Wind Publications

2 Camargo FC, Hughes C, Lehner AF, Tobin T (2006) Trace Level Benzoylecgonine Identifications in Post-Race Urines: Probable Sources and the Regulatory Significance of Such Identifications. In: Proceedings of the 52nd Annual Convention of the American Association of Equine Practitioners: San Antonio, Texas, December 2–6, 2006. Lexington, Ky: American Association of Equine Practitioners, 331–336

3 Queiroz-Neto A, Zamur G, Lacerda-Neto JC, Tobin T (2002) Determination of the highest no-effect dose (HNED) and of the elimination pattern for cocaine in horses. J Appl Toxicol 22, 117–121, DOI 10.1002/jat.836

4 Sams RA (1997) Review of possible sources of exposure of horses to natural products and environmental contaminants resulting in regulatory action. In: Convention Report: 40th annual AAEP Convention: Vancouver, British Columbia, December 4–7, 1997, American Association of Equine Practitioners, 220–223

5 Kollias-Baker C (2002) A review of possible environmental sources of drug positives. In: Proceedings of the 48th Annual Convention of the American Association of Equine Practitioners, Orlando, Florida, December 4–8, 2002. Orlando FL, American Association of Equine Practitioners, 186–189

6 Oyler J, Darwin WD, Cone EJ (1996) Cocaine contamination of United States paper currency. J Appl Toxicol 20, 213–216, DOI 10.1093/jat/20.4.213

7 Negrusz A, Perry J, Moore C (1998) Detection of cocaine on various denominations of United States currency. J Forensic Sci 43, 626–629, DOI 10.1520/jfs16193j

8 Taddei L, Benoit M, Sukta A, Peterson J, Gaensslen RE, Negrusz A. Detection of various performance enhancing substances in specimens collected from race horses in Illinois: A five-Year experience. Appl Toxicol 35, 438–443, DOI 10.1093/anatox/35.7.438

9 Billings K (2003) Development of a simple method to detect and quantify benzoylecgonine, a cocaine metabolite, in urine [Internet]. Illinios Wesleyan University; [cited 2024 Sept 12]. Available from: https://digitalcommons.iwu.edu/chem_honproj/5/

10 Cone EJ, Huestis MA (2007) Interpretation of oral fluid tests for drugs of abuse. Ann N Y Acad Sci 1098, 51–103, DOI 10.1196/annals.1384.037

11 Stanley SD, Sams RA, Harkins JD, Mundy GD, Boyles J, Woods WE, Tobin T (1995) Frequency distribution of post race urine pH from Standardbreds compared with Thoroughbreds: Research and Regulatory Significance. Equine Vet J 27, 471–473, DOI 10.1111/j.2042–3306.1995.tb04429.x

12 Lehner AF, Hughes CG, Woods WE, Karpiesiuk W, Harkins JD, Dirikolu L (2001) A liquid chromatographic-electrospray tandem MS/MS method for quantitation of equine cocaine Metabolites. In: Proceedings of the 13th International Conference of Racing analysts and Veterinarians, Cambridge, United Kingdom 2000. Newmarket, Suffolk: R & W Publications; 2001, 413–419

13 Reilly J (2024) Standard drug testing cut-off levels. SAMHSA Certified Labs [Internet]. National Drug Screening; 2024 [cited 2024 Sept 12]. Available from: https://www.nationaldrugscreening.com/blogs/standard-drug-testing-cut-off-levels-from-our-samhsa-certified-labs/

14 Personal communication to T Tobin by Mr. Todd Mostoller, Executive Director of the Pennsylvania Horsemen's Benevolent and Protective Association

15 Update on ADMC cases and regulation of cocaine under the ADMC program [Internet]. Horseracing Integrity and Welfare Unit; 2023 [cited 2024 Sept 12]. Available from: https://www.hiwu.org/news/update-on-admc-cases-and-regulation-of-cocaine-under-the-admc-program

16 Bloodhorse (2023) HIWU drops cases against trainers Brion, Hendriks [Internet]. BloodHorse; 2023 [cited 2024 Sept 12]. Available from: https://www.bloodhorse.com/horse-racing/articles/273440/hiwu-drops-cases-against-trainers-brion-hendriks

17 Regulations - horseracing integrity and Safety Authority/Prohibited List (Rule Series 4000) [Internet]. Horse Racing Integrity and Safety Authority; [cited 2024 Sept 12]. Available from: https://hisaus.org/regulations

18 Fenger C, Catignani M, Machin J, Tobin T (2017) An in-depth look at stall contamination [Internet]. National HBPA; [cited 2024 Sept 12]. Available from: https://uknowledge.uky.edu/cgi/viewcontent.cgi?article = 1033&context = gerc_facpub

19 Crist S (1989) Cocaine case proves testers are gaining [Internet]. The New York Times; 1989 [cited 2024 Sept 12]. Available from: https://www.nytimes.com/1989/02/28/sports/on-horse-racing-cocaine-case-proves-testers-are-gaining.html

20 Therapeutic Substances [Internet]. Ohio State Racing Commission; [cited 2024 Sept 12] Available from: https://racing.ohio.gov/thoroughbred/02-therapeutic-substances

21 Personal communication to T Tobin by Mr. Kerry Holloway Association of Racing Commissioners International

Spurennachweis von Benzoylecgonin in Plasma/Serum von Pferden – Vorschlag eines vorläufigen regulatorischen "Cut-off" für den Plasmagehalt

Kokain ist eine weit verbreitete menschliche Freizeitsubstanz, die routinemäßig in pharmakologisch irrelevanten Spurenmengen auf Nichtkonsumenten und Pferde übertragen wird. Pferde und Menschen demethylieren Kokain schnell zu Benzoylecgonin, das im Urin in Konzentrationen ausgeschieden wird, die fast zweitausendmal höher sind als die entsprechenden Plasma-Kokainkonzentrationen. Bei Drogentests am Arbeitsplatz werden diese normalerweise pharmakologisch irrelevanten Nachweise von Benzoylecgonin im Urin im Spurenbereich durch die Anwendung eines "Grenzwertes" von 100 ng/ml im Urin gehandhabt, wie von der Behörde für Drogenmissbrauch und psychische Gesundheit (SAMHSA) festgelegt. Im Pferderennsport gelten in einigen US-Bundesstaaten seit Langem veröffentlichte Grenzwerte für Benzoylecgonin im Urin von 50 bis 150 ng/ml. Kürzlich, am 13. September 2023, hat die Horseracing Integrity and Welfare Unit (HIWU) einen Benzoylecgonin-Plasmawert von 76 Pikogramm/ml als „positiv" bezeichnet und damit die Frage aufgeworfen, ob ein Benzoylecgonin-„Cut-off" im Pferdeplasma definiert werden sollte, der den seit langem geltenden „Cut-offs" für Benzoylecgonin im Urin von 100 ng/ml oder darüber entspricht, die bei der Regulierung von Pferdemedikamenten verwendet werden. Wir haben daher die relevanten veröffentlichten wissenschaftlichen Daten überprüft und 1,0 Nanogramm/ml Benzoylecgonin als "Grenzwert" für Benzoylecgonin im Plasma/Serum von Pferden vorgeschlagen, was einem "Grenzwert" im Urin von 100 ng/ml entspricht. Wir haben diese Analyse am 14. und 24. September 2023 als vorläufigen Kommunikationsentwurf an die Pennsylvania Horsemen's Benevolent and Protective Association (PAHBPA) weitergeleitet. Kurz darauf, etwa am 17. November 2023, zog die HIWU-Behörde ihren Fall in der oben genannten Angelegenheit sowie einen zweiten Fall zurück, in dem es um einen anderen Trainer aus Pennsylvania mit einer vermutlich ähnlichen Benzoylecgonin-Plasma-/Serum-Identifizierung ging. Wir weisen ausdrücklich darauf hin, dass Spuren von Kokain auf US-Geld gefunden werden, was zu einer weit verbreiteten Exposition von Einzelpersonen und Pferden gegenüber pharmakologisch irrelevanten Mengen an Kokain führt, das als Benzoylecgonin-Metabolit in leicht nachweisbaren, aber pharmakologisch irrelevanten Konzentrationen im Urin von Menschen und Pferden ausgeschieden wird. In Anbetracht des seit langem bestehenden und gut etablierten SAMHSA-„Cut-off"-Werts von 100 Nanogramm/ml für Kokain im Urin bei Tests am Arbeitsplatz beim Menschen stellen wir nun offiziell einen Plasma-/Serum-„Cut-off"-Wert von 1,0 ng/ml als regulatorischen „Cut-off"-Wert für die Identifizierung von Benzoylecgonin im Plasma/Serum von Pferden vor, der am 14. Mai 2024 auch von der Horseracing Integrity and Welfare Unit mitgeteilt wurde.

Schlüsselwörter: Pferd, Spurennachweis, Doping, Benzoylecgonin, Plasma, Serum, Cut-off

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Trace-level detections of methamphetamine 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 mucous 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 mucous membrane concentration of methamphetamine and the systemic circulation of the horse. Based on published scientific data, mucous 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.

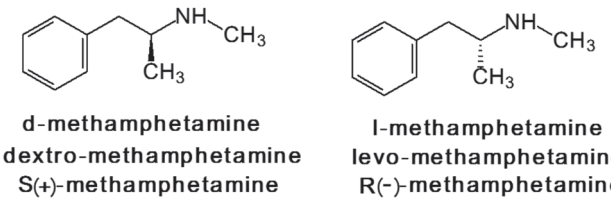

Fig. 1 Methamphetamine (N-methyl-1-phenylpropan-2-amine) exists as two mirror image enantiomers, d-methamphetamine and l-methamphetamine. d-Methamphetamine, the more pharmacologically active enantiomer, is a US DEA Schedule II controlled substance available in the US as the prescription medication Desoxyn®. 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 mucous 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

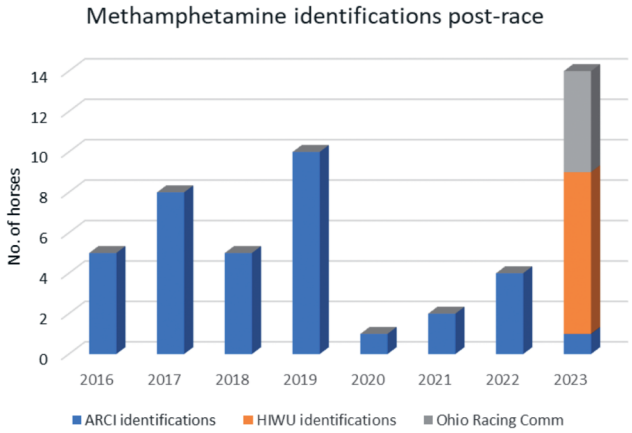

Fig. 2 Methamphetamine identifications reported in US racing, 2016 to October 2023, as per the Association of Racing Commissioners International (ARCI). Methamphetamine identifications reported since May 22nd, 2023, as per the Horse Racing Integrity and Welfare Unit (HIWU) are represented by the orange 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.

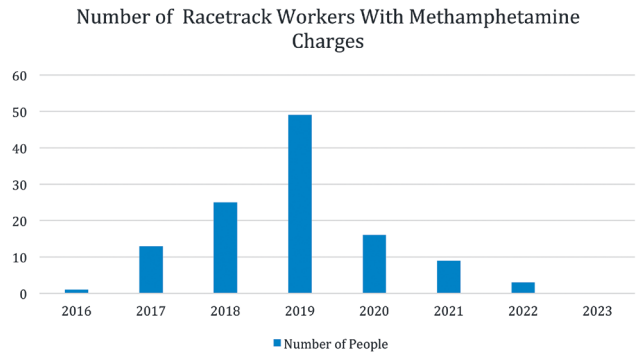

Fig. 3 Number of racetrack workers per year recorded as having methamphetamine related charges per year in Association of Racing Commissioners International (ARCI) methamphetamine records.[4] | *Anzahl der Rennstreckenarbeiter pro Jahr, bei denen in den Methamphetamin-Aufzeichnungen der Association of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

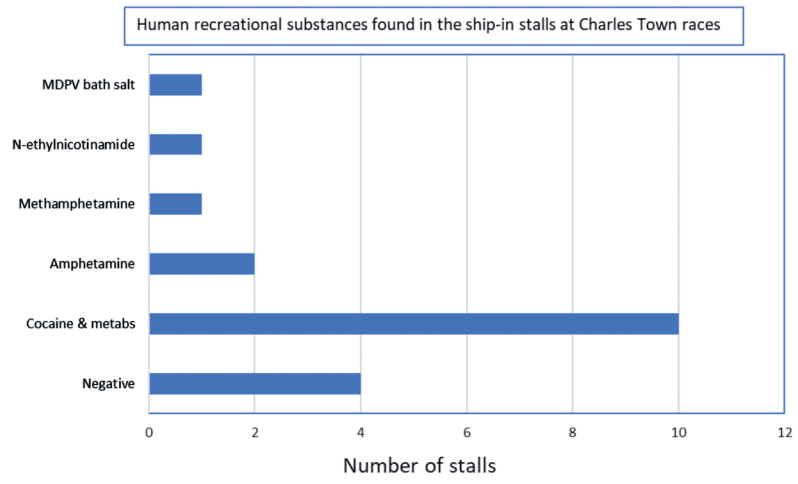

Fig. 4 Listing of the human recreational substances identified in equine ship-in stalls at the Charles Town racecourse, where 79% of the stalls were found to contain substances of regulatory interest. MDPV is 3,4-methylenedioxypyrovalerone; N-ethylnicotinamide is a metabolite of the stimulant nikethamide; 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, 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 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 Klink	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].

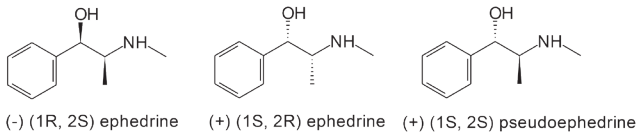

Fig. 5 Ephedrine stereoisomers that are used as methamphetamine precursors. (-)1R, 2S-ephedrine (left) is marketed as pharmaceutical grade ephedrine; (+)1S, 2R-ephedrine (middle) constitutes its mirror image as would be found in racemic ephedrine. A different ephedrine stereoisomer, (+)1S, 2S-pseudoephedrine (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, breed, location, lab, testing matrix, and claimed concentration. | *Vom Ohio Department of Agriculture Analytical Toxicology Laboratory im Zeitraum 2022–2023 gemeldete Methamphetamin-Identifizierungen bei Trabrennen mit Angabe des Renndatums, des Namens des Trainers, des Namens des Pferdes, der Rasse, des Standorts, das Labor, der Testmatrix und der angegebenen Konzentration (sofern verfügbar).*

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	Dashintothebeach	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 (-)-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-

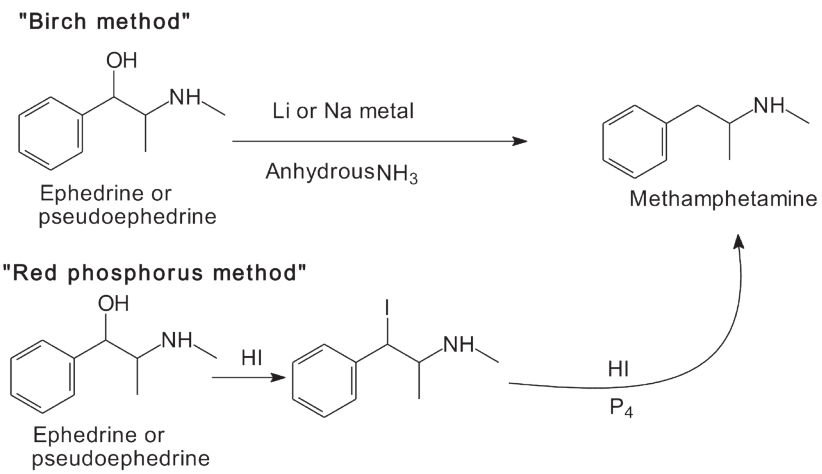

Fig. 6 Common routes to methamphetamine starting from ephedrine or pseudoephedrine. The "Birch method" is a chemical reduction method relying on reaction with ammonia in the presence of alkali metal catalysts. The "Red phosphorus method" requires hydrogen iodide and the phosphorus allotrope known as 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

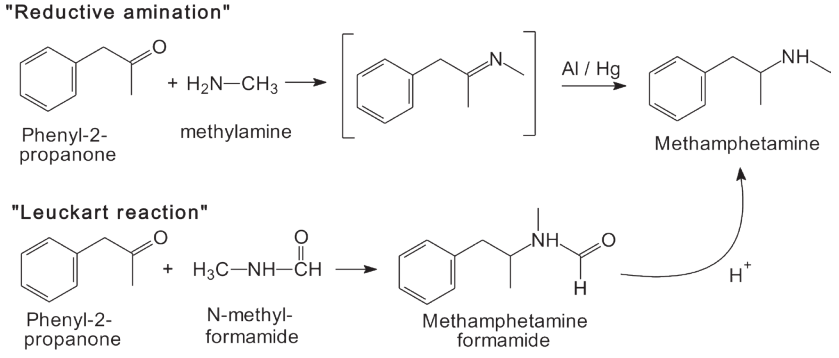

Fig. 7 Synthetic routes to methamphetamine from phenyl-2-propanone, a.k.a. phenylacetone. The "Reductive amination" route combines phenyl-2-propanone and methylamine to create an N-methylimine that undergoes reduction catalyzed by aluminum amalgam. The "Leuckart reaction" relies on nucleophilic attack 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 als Phenylaceton bekannt. 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 IM 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 mucous 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 mucous 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

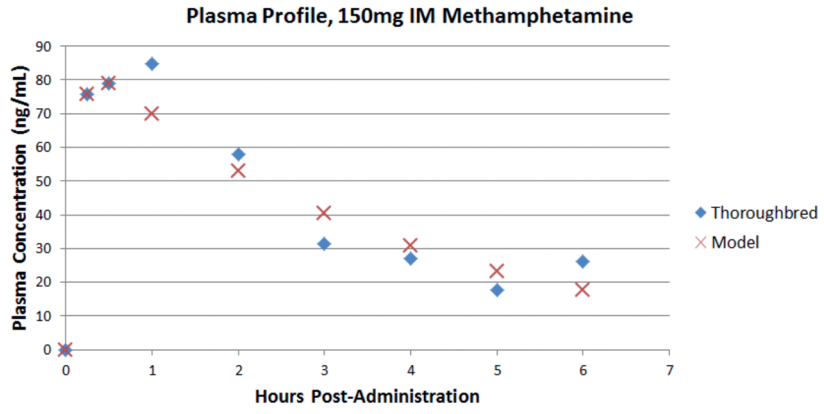

Fig. 8 The blue diamonds show the plasma concentrations of methamphetamine in a Thoroughbred horse administered 150 mg of methamphetamine IM replotted from Ray et al. [28]. The crosses show a first pass best fit kinetic model of these data obtained by back stripping. | *Die blauen Rauten zeigen die Plasmakonzentrationen 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

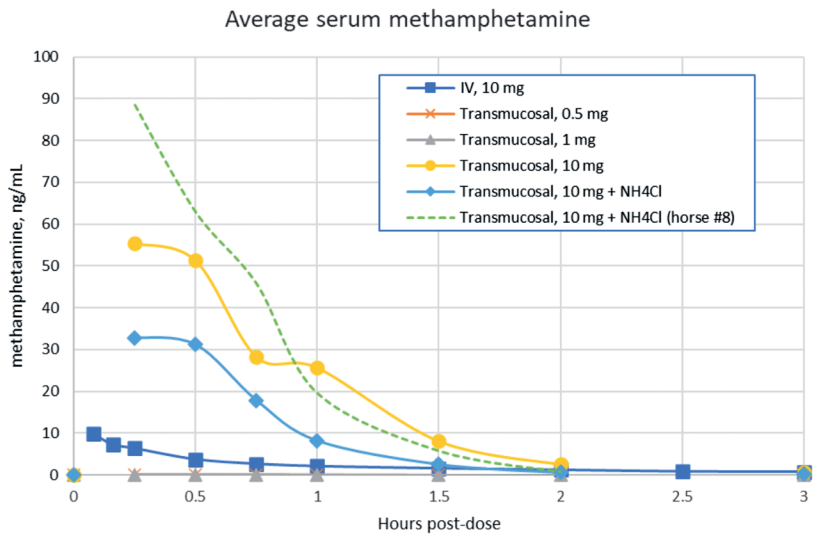

Fig. 9 Comparison of IV and transmucosal administrations of 10 mg doses of d-methamphetamine to six thoroughbreds. The blue squares (■) starting at 0.08-hr present mean serum concentrations of methamphetamine following IV administration (10 mg/horse to six horses). Traces for the remaining symbols (×,Δ,●,♦) 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] | *Vergleich der intravenösen und transmukosalen Verabreichung von 10-mg-Dosen d-Methamphetamin an sechs Vollblüter. 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.*

horse in question should be included in a full investigation of any methamphetamine positive to distinguish between incidental 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 to 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.

References

1 Brewer K, Shults TF, Machin J, Kudrimoti S, Eisenberg S, Hartman P, et al. (2016) A cluster of trace-concentration methamphetamine identifications in racehorses associated with a methamphetamine-contaminated horse trailer: A report and analysis. Can Vet J 57, 860–864

2 Staff PR (2016) Lone Star Park trainers held blameless in methamphetamine positives - horse racing news: Paulick Report [Internet, cited 2023 Dec 23]. Available from: https://paulickreport.com/news/the-biz/lone-star-park-trainers-held-blamelessmethamphetamine-positives/

3 Lyden T Speed (2019) Canterbury Park Race Horses exposed to methamphetamine [Internet]. FOX 9 Minneapolis-St. Paul, [cited 2023 Dec 23]. Available from: https://www.fox9.com/news/speed-canterbury-park-race-horses-exposed-to-methamphetamine

4 Holloway K. (2023) Association of Racing Commissioners International, Personal Communication to Thomas Tobin

5 Fenger C, Catignani M, Machin J, Tobin T (2017) An in-depth look at stall contamination. "An in-depth look at stall contamination." Horsemen's Journal 64(4), 41–44 6 Communications H. (2023) Hisa anti-doping and medication control program will relaunch May 22 - horse racing news: Paulick Report [Internet]. Blenheim Publishing, LLC; 2023 [cited 2023 Dec 23]. Available from: https://paulickreport.com/news/the-biz/hisa-anti-doping-and-medication-control-program-will-relaunch-may-22/

7 Methamphetamine [Internet]. U.S. National Library of Medicine; [cited 2023 Dec 23]. Available from: https://pubchem.ncbi.nlm.nih.gov/compound/methamphetamine

8 Drug fact sheet (2020) Methamphetamine - dea.gov [Internet]. Department of Justice/Drug Enforcement Agency, [cited 2023 Dec 23]. Available from: https://www.dea.gov/sites/default/files/2020–06/Methamphetamine-2020_0.pdf

9 Mendelson JE, McGlothlin D, Harris DS, Foster E, Everhart T, Jacob P, et al. (2008) The Clinical Pharmacology of Intranasal L-Methamphetamine. BMC Clin Pharmacol 8, DOI 10.1186/1472-6904-8-4

10 Puljević C, Zahnow R, Benfer I, Winstock AR, Maier LJ, Barratt MJ, et al. (2020) Patterns of methamphetamine production among an international sample of methamphetamine 'cooks.' Drug Alcohol Rev 40, 1287–1293, DOI 10.1111/dar.13205

11 Internet (2023) Methamphetamine: A Growing Domestic Threat [cited 2023 Dec 23]. Available from: https://irp.fas.org/agency/doj/dea/product/meth/threat.htm

12 Internet (2023) Uniform classification guidelines for foreign substances and ... [Internet]. Association of Racing Commissioners International, [cited 2023 Dec 23]. Available from: https://www.arci.com/docs/Uniform-Classification-Guidelines-Version-17.0.pdf

13 Internet (2023) Hisa Prohibited Substances List - Hiwu , Horseracing Integrity and Safety Authority; [cited 2023 Dec 23]. Available from: https://assets.hiwu.org/a/hisa_bannedprohibitedlist_report_012723a_opt.pdf?updated_at = 2023–02-13T14:38:13.254Z

14 Hegarty M (2023) HIWU creates sub-category for human-abuse drugs [Internet]. DFR; 2023 [cited 2023 Dec 23]. Available from: https://www.drf.com/news/hiwu-creates-sub-category-human-abuse-drugs.

15 Hillstorm K (2015) Methamphetamine. Farmingon Hills, MI: Greenhaven Publishing LLC

16 Skinner HF (1990) Methamphetamine synthesis via hydriodic acid/red phosphorus reduction of ephedrine. Forensic Sci Internat 48, 123–134, DOI 10.1016/0379-0738(90)90104-7

17 Weiss J A, Pertl C, Schmid M (2028) Investigation of two seized Crystal Meth Labs in Austria – parameters and chirality aspects of methamphetamine low scale synthesis. ARC Forensic Sci 3, DOI 10.20431/2456-0049.0301001

18 Internet (2021) How is methamphetamine manufactured. U.S. Department of Health and Human Services, [cited 2023 Dec 23]. Available from: https://nida.nih.gov/publications/research-reports/methamphetamine/how-methamphetamine-manufactured

19 Mendelson J, Uemura N, Harris D, Nath R, Fernandez E, Jacobii P, et al. (2006) Human pharmacology of the methamphetamine stereoisomers. Clin Pharmacol Therap. 80, 403–420, DOI 10.1016/j.clpt.2006.06.013

20 Abbruscato TJ, Trippier PC (2018) Dark classics in chemical neuroscience: Methamphetamine. ACS Che Neurosci 9, 2373–2378, DOI 10.1021/acschemneuro.8b00123

21 Barkholtz HM, Hadzima R, Miles A. (2023) Pharmacology of r- (−)-methamphetamine in humans: A systematic review of the literature. ACS Pharmacol Amp Translat Sci 6, 914–924, DOI 10.1021/acsptsci.3c00019

22 McKetin R, Sutherland R, Bright DA, Norberg MM (2011) A systematic review of methamphetamine precursor regulations. Addiction 106, 1911–1924, DOI 10.1111/j.1360-0443.2011.03582.x

23 Melnikova N, Welles WL, Wilburn RE, Rice N, Wu J, Stanbury M. (2011) Hazards of illicit methamphetamine production and efforts at reduction: Data from the Hazardous Substances Emergency Events Surveillance System. Public Health Rep 126 (Suppl 1), 116–123, DOI 10.1177/00333549111260s115

24 Brzeczko AW, Leech R, Stark JG (2013) The advent of a new pseudoephedrine product to combat methamphetamine abuse. Am J Drug Alcohol Abuse 39, 284–290, DOI 10.3109/00952990.2013.821476

25 Presley B, Bianchi B, Coleman J, Diamond F, McNally G (2018) Efficiency of extraction and conversion of pseudoephedrine to methamphetamine from tamper-resistant and non-tamper-resistant formulations. J Pharmaceut Biomed Analysis 156,16–22; DOI 10.1016/j.jpba.2018.04.016

26 Mayer A, Copp B, Bogun B, Miskelly G. (2020) Identification and characterization of chemically masked derivatives of pseudoephedrine, ephedrine, methamphetamine, and MDMA. Drug Test Analys 12, 524–537, DOI 10.1002/dta.2764

27 Hegarty M. (2016) Kentucky trainer begins suspension for drug violation [Internet]. ESPN; [cited 2023 Dec 23]. Available from: https://www.espn.com.au/horse-racing/story/_/id/18048774/kentucky-trainer-begins-suspension-drug-violation

28 Ray RS, Noonan JS, Tharp PW (1972) Detection of methylphenidate and methamphetamine in equine body fluids by gas chromatographic analysis of an electron-capturing derivative. Am J Vet Res 33, 27–31
29 Knych HK, Arthur RM, Kanarr KL, McKemie DS, Kass PH (2019) Detection, pharmacokinetics, and selected pharmacodynamic effects of methamphetamine following a single transmucosal and intravenous administration to exercised thoroughbred horses. Drug Test Analys 11, 1431–1443, DOI 10.1002/dta.2661
30 Cathey K. (2016) Update to Directive on Commission-Sanctioned Thresholds [Internet]. State of Oklahoma, [cited 2023 Dec 23]. Available from: http://www.oqhra.com/pdfimages/2016/Updated-Directive-Commission-Sanctioned-Thresholds%202–22-16.pdf
31 Beckett AH, Rowland M. (1965) Urinary excretion kinetics of amphetamine in man. J Pharm Pharmacol 17, 628–639, DOI 10.1111/j.2042-7158.1965.tb07575.x
32 Gerkin DD, Sams RA, McKeever KH, Hinchcliff KW, Ashcraft S. (1991) Urinary pH effects on the renal clearance of lidocaine and phenylbutazone in exercising horses. Toxicologist 297
33 Standley SD, Sams RA, Harkins JD, Mundy GD, Boyles J, Woods WE, et al. (1995) Frequency distribution of post-race urine pH from Standardbreds compared with Thoroughbreds: Research and Regulatory Significance. Equine Vet 27, 471–473, DOI 10.1111/j.2042-3306.1995.tb04429.x
34 Tobin T. (1981) Drugs and the performance horse. Charles C. Thomas, Springfield
35 Suwannachom N, Thananchai T, Junkuy A, O'Brien TE, Sribanditmongkol P. (2015) Duration of detection of methamphetamine in hair after abstinence. Forens Sci Internat 254, 80–86, DOI 10.1016/j.forsciint.2015.06.030
36 Kunalan V, Nic Daéid N, Kerr WJ, Buchanan HA, McPherson AR. (2009) Characterization of route specific impurities found in methamphetamine synthesized by the Leuckart and reductive amination methods. Analyt Chem 81, 7342–7348, DOI 10.1021/ac9005588
37 Cunningham JK, Maxwell JC, Campollo O, Liu L-M, Lattyak WJ, Callaghan RC (2013) Mexico's precursor chemical controls: Emergence of less potent types of methamphetamine in the United States. Drug Alcohol Depend 129, 125–136, DOI 10.1016/j.drugalcdep.2012.10.001
38 Hazama S, Ichikawa S, Yonebayashi F. (2008) Convenient method for synthesis of L-Methamphetamine. Jap J Forens Sci Technol 13, 67–72, DOI 10.3408/jafst.13.67
39 Repke DB, Bates DK, Ferguson WJ. (1978) Synthesis of dextroamphetamine sulfate and methamphetamine hydrochloride from D-phenylalanine. Pharmaceut Sci 67, 1167–1168, DOI 10.1002/jps.2600670838

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

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CASE REPORT

WILEY

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

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1 | INTRODUCTION

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

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

2 | THE SOUTH AFRICAN CASE AND RELATED IDENTIFICATIONS WORLDWIDE

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

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

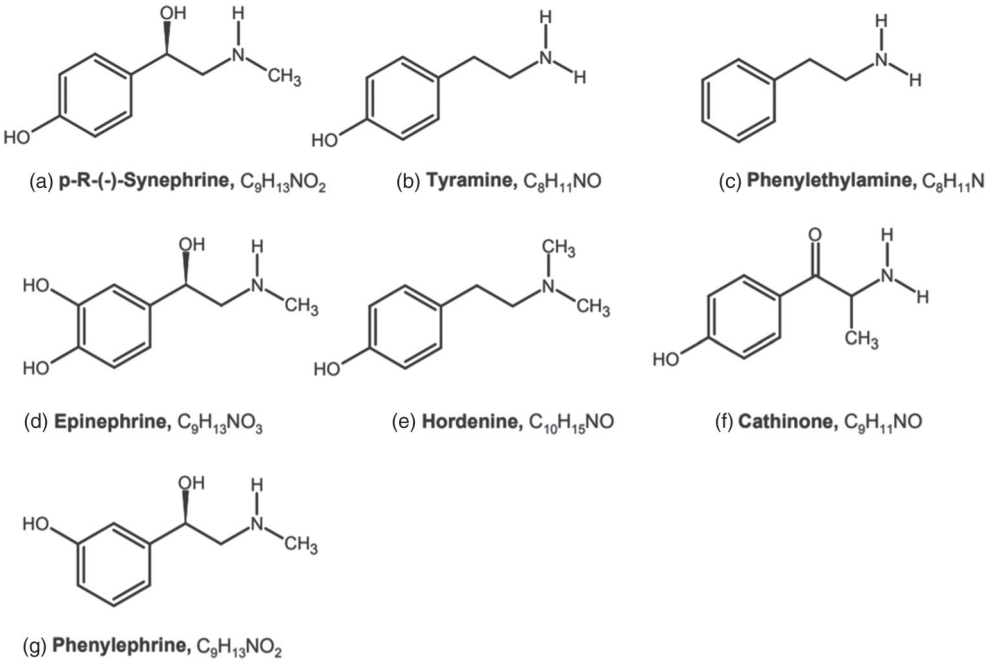

FIGURE 1 (a–g) Synephrine and related substances. (a) *R*-(−)-Synephrine, (b) tyramine, (c) phenylethylamine, (d) epinephrine, (e) hordenine, (f) cathinone, and (g) phenylephrine, a related synthetic pharmaceutical

TABLE 1 Worldwide Synephrine identifications, 2012 → -date

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

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

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

TABLE 2 Synephrine content of Polokwane related South African hay samples

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

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

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

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

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

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

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

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

3 | DISCUSSION

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

4 | CONCLUSIONS

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

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

CONFLICT OF INTEREST

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

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

AUTHOR CONTRIBUTIONS

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

ETHICS STATEMENT

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

DATA AVAILABILITY STATEMENT

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

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REFERENCES

1. Stohs SJ, Shara M, Ray SD. p-Synephrine, ephedrine, p-octopamine and m-synephrine: comparative mechanistic, physiological, and pharmacological properties. *Phytother Res*. 2020;34(8):1838-1846. doi:10.1002/ptr.6649
2. Bader M, Lang T, Lang R, Hofmann T. Synephrine as a specific marker for orange consumption. *J Agric Food Chem*. 2017;65(23):4853-4858.
3. D'Andrea G, Terrazzino S, Fortin D, Farruggio A, Rinaldi L, Leon A. HPLC electrochemical detection of trace amines in human plasma and platelets and expression of mRNA transcripts of trace amine receptors in circulating leukocytes. *Neurosci Lett*. 2003;346(1-2):89-92. doi:10.1016/s0304-3940(03)00573-1
4. De Koch S. FEI Synephrine Positive-Biochemical report. [Personal communication to K. Brewer, November 7th, 2019].
5. Williams L. Synephrine Info. [Personal communication to K. Brewer, March 11th, 2020].
6. Williams L. Synephrine. [Personal communication e-mail to authors K. Brewer and M. M Neidhart, November 6th, 2019].
7. DECISION of the FEI TRIBUNAL dated 30 August 2019. https://inside.fei.org/system/files/Case%202019%20-%20BS11%20-%20FAMORKU%20-%20FEI%20Tribunal%20Decision%20-%20Approval%20of%20Agreement%20between%20Parties%20-%2030%20August%202019.pdf. Accessed May 27, 2021
8. DECISION of the FEI TRIBUNAL dated 30 August 2019. https://inside.fei.org/system/files/Case%202019%20-%20BS10%20-%20FOR%20FUN%20-%20FEI%20Tribunal%20Decision%20-%20Approval%20of%20Agreement%20between%20Parties%20-%2030%20August%202019.pdf. Accessed May 27, 2021.
9. Provisional Suspensions of Eight Horses Lifted. http://www.morgansl.com/en/latest/provisional-suspensions-eight-horses-lifted. Accessed May 20, 2021 [Personal communication to K. Brewer and Morgan Sports Law communication by Lisa Lazarus, and Emma Waters of Morgan Sports Law, https://www.morgansl.com/en].
10. DECISION of the FEI TRIBUNAL dated 6 July 2020. https://inside.fei.org/system/files/Consolidated_Synephrine_cases-Final_Tribunal_Decision-Approval_of_Agreement_between_Parties-6_July_2020.pdf. Accessed November 27, 2020.
11. Brewer K, Dirikolu L, Hughes CG, Tobin T. Scopolamine in racing horses: trace identifications associated with dietary or environmental exposure. *The Veterinary Journal*. 2014;199(3):324-331. doi:10.1016/j.tvjl.2013.12.013
12. Frank M, Weckman TJ, Wood T, et al. Hordenine: pharmacology, pharmacokinetics and behavioural effects in the horse. *Equine Vet J*. 1990;22(6):437-441. doi:10.1111/j.2042-3306.1990.tb04312.x
13. Soring K, Kind AJ, Peterson JD, Brewer K, Hughes C, Tobin T. Cathinone—detection in equine urine and potential environmental origins regulation. In: *Proceedings of the 19th International Conference of Racing Analysts and Veterinarians ICRAV*. Philadelphia, Pennsylvania, USA, September 15–22; 2012.
14. Residue limits—urine and plasma. International Federation of Horseracing Authorities. https://www.ifhaonline.org/Default.asp?section=IABRW. Accessed November 27, 2020.
15. Williams L. (2019, November 6). Synephrine. [Personal communication e-mail to author K. Brewer].
16. Holloway K. ARCI. [Personal communication to T. Tobin, March 5th, 2020].
17. Tiesjema B, Jeurissen S, Mol H, Frank S, Razenburg L. Risk assessment of synephrine. https://www.rivm.nl/bibliotheek/rapporten/. Accessed November 30, 2019.
18. Kusu F, Matsumoto K, Arai K, Takamura K. Determination of synephrine enantiomers in food and conjugated synephrine in urine by high-performance liquid chromatography with electrochemical detection. *Anal Biochem*. 1996;235(2):191-194. doi:10.1006/abio.1996.0191
19. Barker S. The formation of aminorex in racehorses following levamisole administration. A quantitative and chiral analysis following synthetic aminorex or levamisole administration vs. aminorex-positive samples from the field: a preliminary report. *J Vet Pharmacol Ther*. 2009;32(2):160-166. doi:10.1111/j.1365-2885.2008.01015.x
20. Harkins J, Mundy G, Stanley S, et al. Absence of detectable pharmacological effects after oral administration of isoxsuprine. *Equine Vet J*. 1998;30(4):294-298. doi:10.1111/j.2042-3306.1998.tb04100.x
21. Kaats G, Miller H, Preuss H, Stohs S. A 60 day double-blind, placebo-controlled safety study involving *Citrus aurantium* (bitter orange) extract. *Food Chem Toxicol*. 2013;55:358-362. doi:10.1016/j.fct.2013.01.013
22. Combie J, Blake JW, Nugent TE, Tobin T. Morphine glucuronide hydrolysis: superiority of beta-glucuronidase from *Patella vulgata*. *Clin Chem*. 1982;28(1):83-86. doi:10.1093/clinchem/28.1.83
23. Combie J, Blake JW, Nugent TE, Tobin T. Detection of morphine and its analogues using enzymatic hydrolysis: US Patent 4,473,640. 1984. September 28th, 1984.
24. Bosken JM, Lehner AF, Hunsucker A, et al. Direct MS-MS identification of isoxsuprine-glucuronide in post-administration equine urine. *Can J Vet Res*. 2000;64(2):112-116.

25. Gerken DF, Sams RA, McKeever K, Hinchcliff K, Ashcraft S. Urinary pH effects on the renal clearance of lidocaine and phenylbutazone in exercising horses. *The Toxicologist*. 1991;297.
26. Brewer K, Shults TF, Machin J, et al. A cluster of trace-concentration methamphetamine identifications in racehorses associated with a methamphetamine-contaminated horse trailer: a report and analysis. *Can Vet J*. 2016;57(8):860-864.
27. Machin J, Brewer K, Catignani M, et al. An interim screening limit of detection for naproxen in equine plasma: a review and analysis. *Comp Exercise Physiol*. 2020;16(2):153-160. doi:10.3920/cep190044
28. FEI Online General Assembly 2020 Rules Session Inside FEI. https://inside.fei.org/system/files/PPT_Rules_Session_1_for_publication.pdf. Accessed November 27, 2020.

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AGENDA ITEM #10

Classification of Carmoterol

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MEMORANDUM

TO: ARCI Model Rules Committee

FROM: Michael Hardy, DVM, Executive Director, on behalf of the Scientific Advisory Committee (SAC)

DATE: February 24, 2025

RE: ***Carmoterol***

The RMTC has been advised that Carmoterol has been detected in post-race and out-of-competition testing in the US, currently isolated to Quarter Horse racing.

Carmoterol is an experimental long-acting β adrenoreceptor agonist (LABA) with high potency, withdrawn from clinical trials around 2010. The primary focus of the clinical trials were investigations of new once daily dosing of LABA substances or in combination with corticosteroids or long-acting antimuscarinic agents (LAMA) to treat asthma or COPD in humans.

As it is an experimental drug and was removed from clinical trials, Carmoterol has no accepted medical use, no corresponding efficacy or safety data, and does not have FDA approval for use in any species. It has the potential to affect the outcome of a competition.

Carmoterol is not listed in ARCI's Uniform Classification of Foreign Substances, so pursuant to ARCI Model Rules, this substance would be assigned 1A by default.

The FDA advised the RMTC (in response to its inquiry on other substances) that the possession of a non-FDA approved drug is not permitted.

As it lacks FDA approval, it would be classified as a Banned (S0) Substances by HISA's Anti-Doping and Medication Control regulations.

Subsequent to SAC's recommendation, RMTC's Board of Directors, in its February 24, 2025 videoconference, voted unanimously to recommend a 1/A Classification.

Recommendation: 1/A