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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

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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,

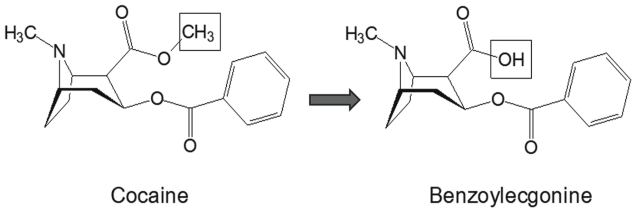

Fig. 1 Chemical structures of cocaine and its major demethylated urinary metabolite BenZoylEcgonine (BZE), the removed methyl group indicated by the box at left. Chemically, BZE is a zwitterion, able to carry a positive charge on the basic bridge nitrogen and a negative charge on its carboxyl group. | *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

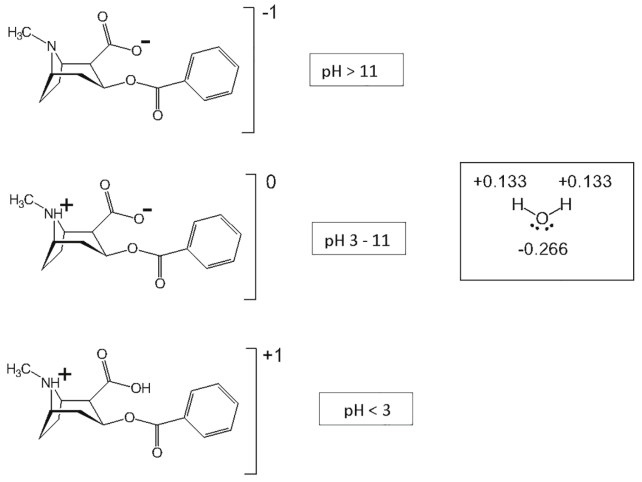

Fig. 2 Left, charges on BZE functional groups depending on environmental pH. The bracket superscript -1, 0 or +1 indicates the net molecular charge depending on whether a proton has been added or subtracted from the tertiary amine or carboxyl functional groups. Right, molecular structure of water. Partial 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.

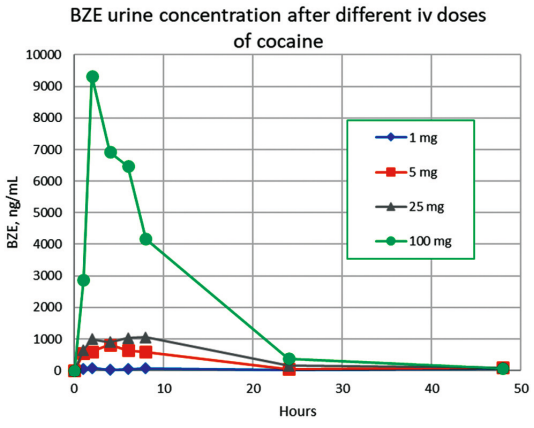

Fig. 3 Urinary concentrations of benzoylecgonine (BZE) following IV administration of the indicated doses of cocaine, replotted from *Lehner* et al.[12]. | *Urinkonzentrationen von Benzoylecgonin (BZE) nach intravenöser Verabreichung der angegebenen Kokaindosen, neu aufgetragen von Lehner et al.*[12].

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.

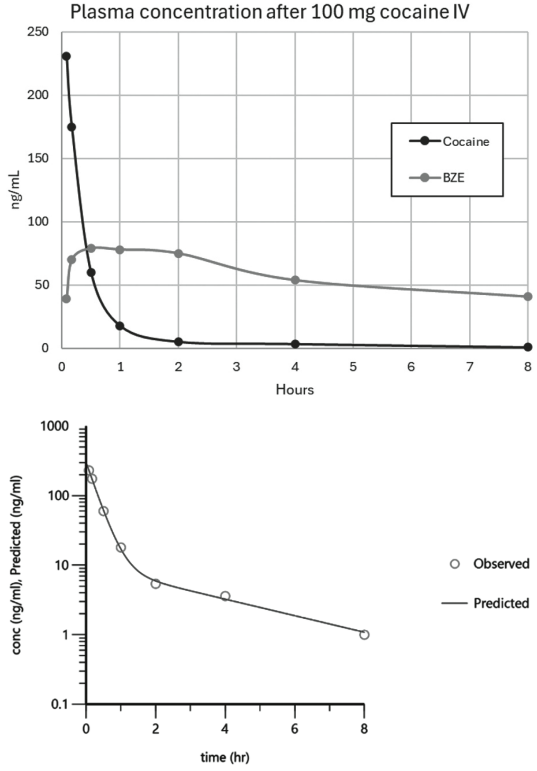

Fig. 4 Figure 4a, above, presents plasma concentration of cocaine and BenZoylEcgonine following IV administration of a 100 mg dose of cocaine, replotted from *Lehner* et al.[12]. Figure 4b, bottom, shows that these plasma cocaine concentration data are well fit by a classic two compartment pharmacokinetic 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]

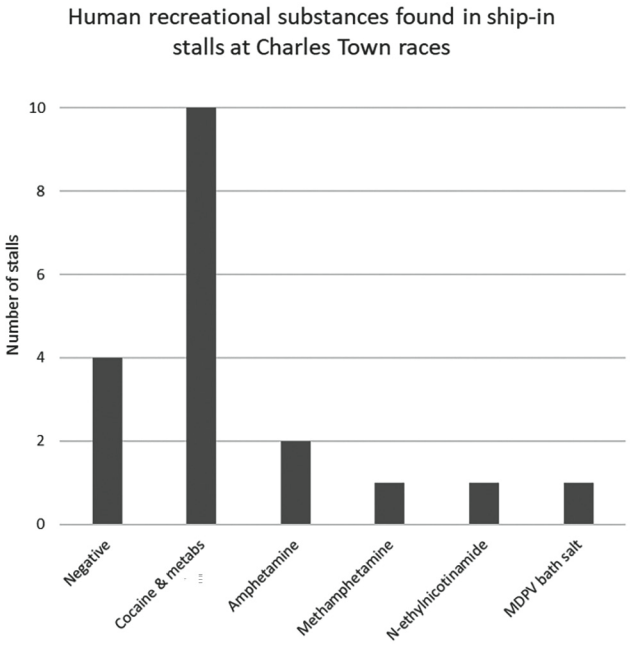

Fig. 5 Human recreational substances identified in "ship-in" stalls at Charles Town Races. Of 21 "ship-in" stalls drug tested at Charles Town Races, 10 stalls tested "positive" for cocaine and metabolites. Reproduced with permission from reference 18. | *Menschliche Freizeitsubstanzen, die in „Ship-in"-Ständen 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-

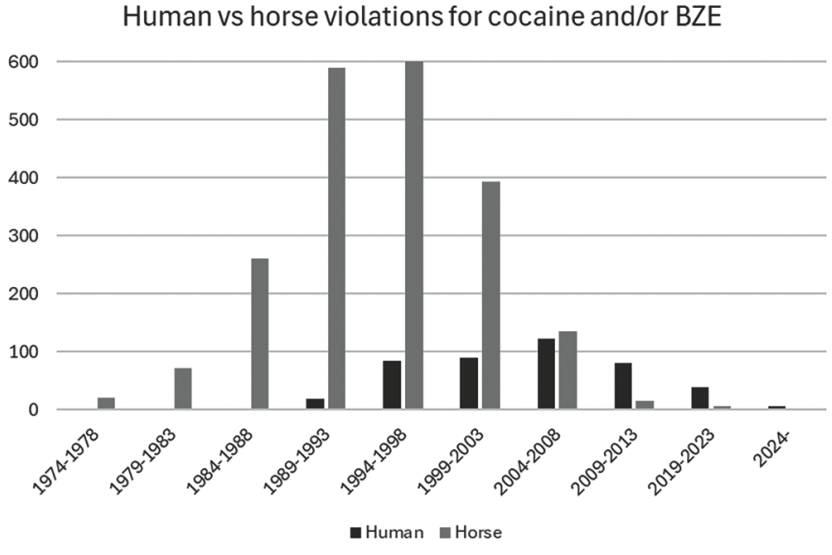

Fig. 6 Number of ARCI-labeled Medication/Drug Violations for humans or animals for cocaine and/or benzoylecgonine in 5-year increments from 1974–2024. These reported violations are about 94% equine and 6% canine and have been labeled as "Horse" in the graph. Only 2024 data are available for what would 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.

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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