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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].

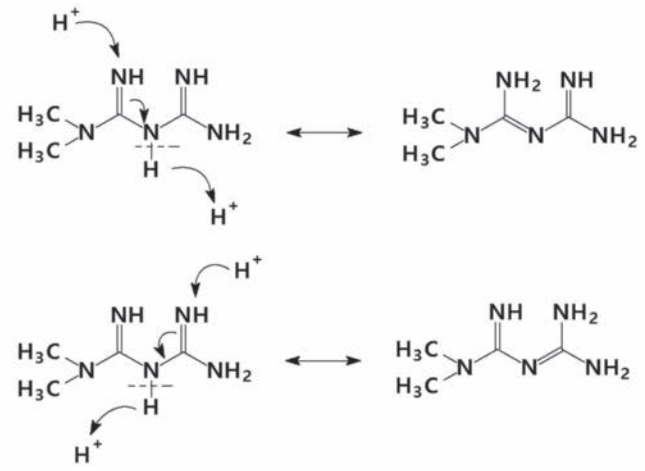

**Fig. 1** Equilibrium structures of metformin, *N,N*-dimethylbiguanide, $C_4H_{11}N_5$, molar mass 129.167 g/mol. In acid, protonation of the C-2 imine induces release of a proton from the central nitrogen resulting in a 2-3 double bond (top). Protonation of the C-4 imine induces release of a proton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

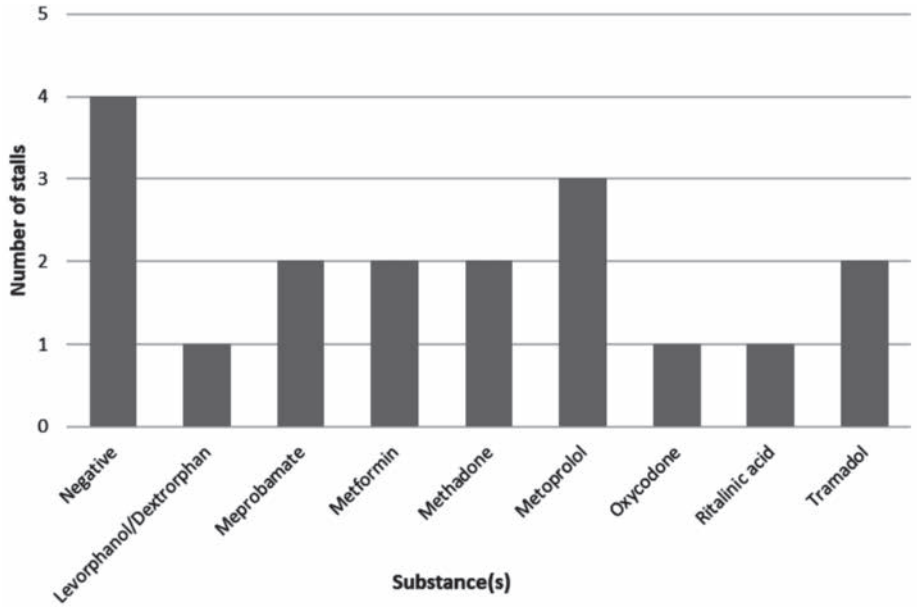


**Fig. 2** Human therapeutic substances found in the "ship-in" stalls at Charles Town races. | *Humantherapeutische Substanzen, die in den „Ship-in"-Ständen bei den Rennen in Charles Town gefunden wurden.*

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 242.5 ng/ml urine | $25000 | Suspended 2 years; forfeit $21600 purse | Pay $8000 HIWU arbitration costs |
| 6/11/2023 | Guadalupe Munoz Elizondo | Quinton's Charmer | NM | Plasma/ serum | 162 pg/ml Plasma | | | Work, not race |
| 6/24/2023 | Javier Morzan | Lady Liv | Delaware Park | Plasma/ serum | 253 pg/ml; Corrected 222 pg/ml | | Dismissed | Work, not race |
| 8/3/2023 | Angel J Castillo | Pylon | Delaware Park | | | | Provisonally suspended | |
| 8/5/2023 | Michael Lauer | Mowins | Horseshoe Indianapolis | Urine | 40 ng/mL; Plasma data unavailable | | | |

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

## Reported Metformin identifications

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

## Closing summary

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

## Abbreviations

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

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## Author's' contributions

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

## Availability of data and materials

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

## Declarations

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

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