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

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

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

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Introduction

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

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

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

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

The reported anabolic actions of 20-hydroxyecdysone

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

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

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

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

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

Case 1

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

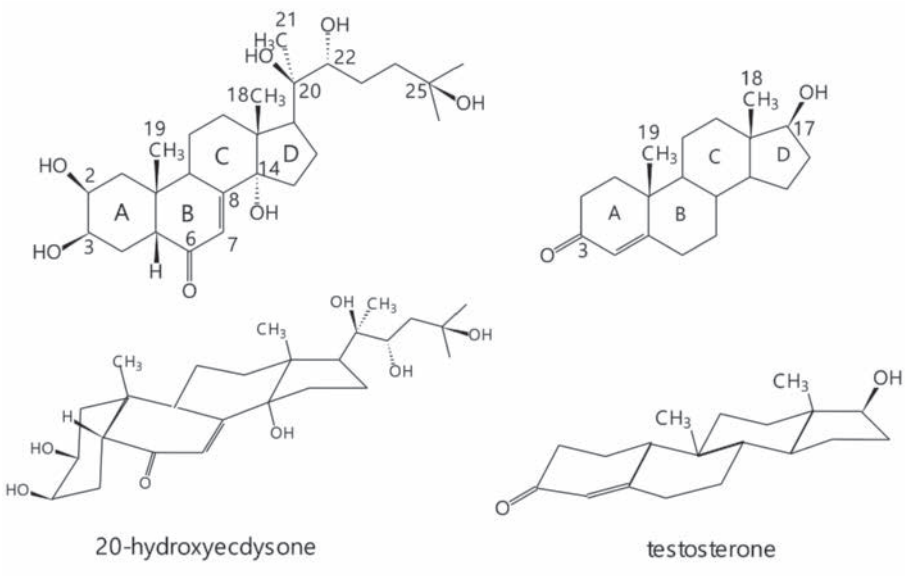

Fig. 1 Left, 20-hydroxyecdysone, (2β, 3β,5β,22R)-2,3,14,20,22,25-Hexa-hydroxychol-est-7-en-6-one, $C_{27}H_{44}O_7$, Molar mass 480.642 g·mol^{-1}, also known as ecdysterone, depicted in structural and configurational diagrams. Right, a typical anabolic hormonal steroid, testosterone, for comparison.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

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

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

Case 2

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

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

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

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

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

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

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

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

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

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

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

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

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

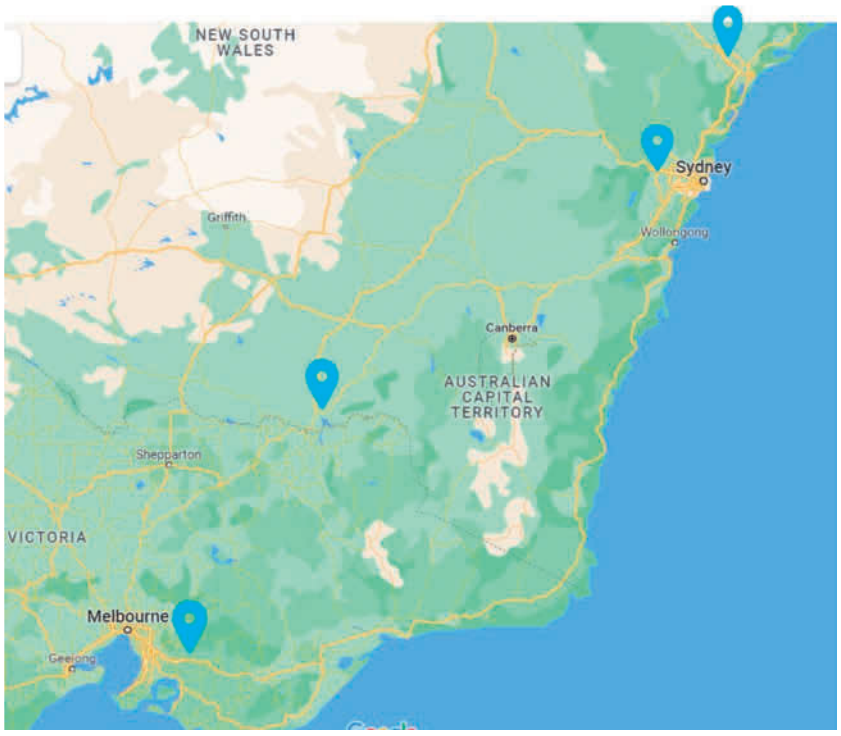

Fig. 3 The home farms and racing locations of the horses involved in these 20-hydroxyecdysone matters. The far north symbol marks the home pasture registered training establishment of the Harness Horse, while the Sydney symbol marks the location of the racetrack where the Harness Horse raced and was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

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

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

Discussion

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

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

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

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

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

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

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

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

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

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

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

Abbreviations

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

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Authors' contributions

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

Availability of data and materials

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

Declarations

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

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