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

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

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

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Introduction

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

The rapid onset and brief duration of action of xylazine

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

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

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

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

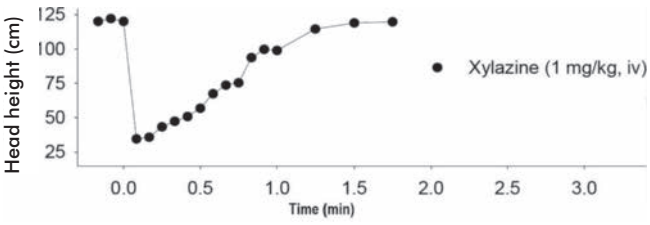

Fig. 1 Head droop following IV injection of xylazine. | *Herabhängen des Kopfes nach intravenöser Injektion von Xylazin.*

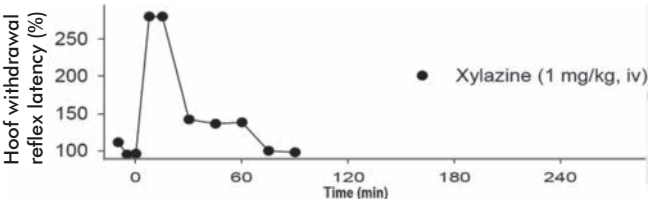

Fig. 2 Hoof withdrawal reflex latency (HWRL) following IV injection of xylazine. | *Latenz des Hufrückzugsreflexes (HWRL) nach intravenöser Injektion von Xylazin.*

The RMTC interim regulatory threshold for xylazine

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

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

Implementation of the xylazine regulatory threshold in Washington State

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

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

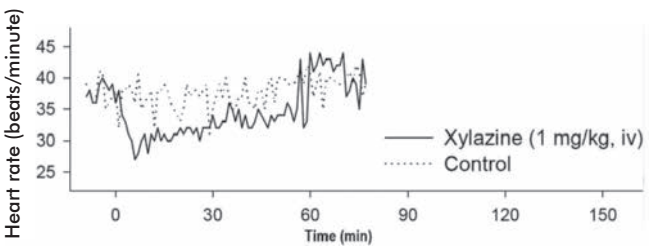

Fig. 3 Heart rates following IV administration of xylazine, showing experimental results as a solid line and control results as dotted line responses. | *Herzfrequenzen nach intravenöser Verabreichung von Xylazin, wobei die experimentellen Ergebnisse als durchgezogene Linie und die Kontrollergebnisse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

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

Review of the RMTC interim regulatory threshold for xylazine

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

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

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

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

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

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

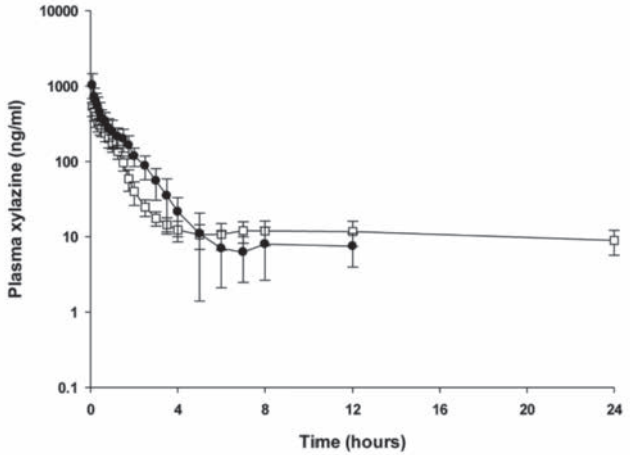

Fig. 4 Mean (± SD) plasma xylazine concentrations for 12 horses administered either 400 mg/horse xylazine I.V. alone (●) or 250 mg/horse xylazine I.V. in combination with 10 mg/horse butorphanol I.V. (□). Reproduced with permission from Noble et al, [2016].[13] | *Mittlere (± SD) Plasma-Xylazin-Konzentrationen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

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

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

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

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

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

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

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

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

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

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

"Phase in" period for new regulatory thresholds

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

The revised RMTC threshold for xylazine

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

Conclusions

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

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Disclosure of interest

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

Animal ethics statement

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

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

References

1 Clarke K, Hall L (1969) "Xylazine" – a new sedative for horses and cattle. Vet Rec 85, 512–517, DOI 10.1136/vr.85.19.512
2 England GCW, Clarke KW (1996) Alpha2 adrenoceptor agonists in the horse – A review. Vet J 152, 641–657, DOI 10.1016/s0007-1935(96)80118-7
3 England GC, Clarke KW, Goosens L (1992) A comparison of the sedative effects of three α2-adrenoceptor agonists (romifidine, detomidine and Xylazine) in the horse. J Vet Pharmacol. Therap 15, 194–201, DOI 10.1111/j.1365-2885.1992.tb01007.x
4 Tobin T (2006) US Patent # 7,074,8346: "Long acting, reversible veterinary sedative and analgesic and method of use. A novel veterinary sedative and tranquilizer."
5 Lehner AF, Dirikolu L, Tobin T (2022) Guanabenz in the horse – a preliminary report on clinical effects and comparison to clonidine and other alpha-2 adrenergic agonists. Pferdeheilk Equine Med 38, 554–565, DOI 10.21836/pem20220607
6 Garcia-Villar R, Toutain PL, Alvinerie M, Ruckebusch Y (1981) The pharmacokinetics of xylazine hydrochloride: An Interspecific Study. J Vet Pharmacol Therap 4, 87–92, DOI 10.1111/j.13652885.1981.tb00715.x
7 Wood T, Weckman T, Woods WE, Tobing T, Dougherty J (1988) Detomidine: A preliminary analysis of its duration of action in the horse by variable interval responding. Equine Vet J 20, 320–322, DOI 10.1111/j.2042-3306.1988.tb01535.x
8 Moens Y, Lanz F, Doherr MG, Schatzmann U (2003) A comparison of the antinociceptive effects of xylazine, detomidine and romifidine on experimental pain in horses. Vet Anaesth Analg 30,183–190, DOI 10.1046/j.1467-2995.2003.00105.x
9 Kullman A, Macarena S, Fosgate G, Montague S, Page P, Rioja E (2014) Effects of xylazine, romifidine, or detomidine on hematology, biochemistry, and splenic thickness in healthy horses. Can Vet J 55, 334–340, PMID 24688132
10 RMTC (2020) RMTC CTS Monograph Series: Xylazine [cited 2023 Oct 31]. Available from: https://rmtcnet.com/wp-content/uploads/2020–10-Xylazine-Monograph-FINAL.pdf
11 Personal Communication (2015) to Mrs. MaryAnn O Connell, Executive Director, Washington State Horsemen's Benevolent and Protection Association.
12 Toutain PL, Lassourd V (2010) Pharmacokinetic/pharmacodynamic approach to assess irrelevant plasma or urine drug concentrations in post-competition samples for drug control in the horse. Equine Vet J 34, 242–249, DOI 10.2746/042516402776185985
13 Noble GK, Sillence MN, Boston RC, Schneiders FI, Zahra P, Fraser LH, Murthi A, Vine JH, Cawdell-Smith AJ, Bryden WL, Dunstan L, Booth J, Salamakha I, Chua AN, Keledjian J, Cawley AT (2016) ETRA update: excretion rates of single and repeated doses of procaine penicillin plus butorphanol, xylazine and a butorphanol-xylazine combination dose in the horse. Proceedings of the 20th International Conference of Racing Analysts and Veterinarians, Mauritius 2014 p 26–33
14 Noble GK, Brewer K, O'Connell MA, Stirling KH, Morales B, Machin J, Hughes C, Tobin T (2015) Revisiting the Racing Medication and Testing Consortium (RMTC) xylazine Guidelines, with Australian Assistance; Presentation to the Summer 2015 Medication Forum of the National Horsemen's Benevolent and Protective Association Meeting. National Horsemen's Benevolent and Protective Association Summer Meeting, Denver, Colorado, Saturday, August 8th, 2015
15 Knych HK, Stanley SD, McKemie DS, Arthur RM, Kass PH (2016) Pharmacokinetic and pharmacodynamics of xylazine administered to exercised thoroughbred horses. Drug Test Analysis 9, 713–720, DOI 10.1002/dta.2047
16 Habershon-Butcher J, Cutler C, Viljanto M, Hincks PR, Biddle S, Paine SW (2019) Re-valuation of the pharmacokinetics of xylazine administered to thoroughbred horses. J Vet Pharmacol Therap 43, 6–12, DOI 10.1111/jvp.12820
17 Tobin T (1981) "Drugs and the Performance Horse". Charles C Thomas Springfield, Illinois, 480pages
18 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.2042-330, DOI 10.1111/j.2042-3306.1995.tb04429.x