Betamethasone Analysis

1. Betamethasone (Intra-articular injection) :
   a. Corticosteroid (anti-inflammatory) for intra-articular use
   b. Current ARCI Threshold: 10 pg/mL serum/plasma
   c. Current ARCI Withdrawal Recommendations: 7 days after Intra-articular administration of 9 mg Betamethasone as BetaVet
   d. Therapeutic Uses:
      i. For direct articular therapy of both acute synovitis and chronic osteoarthritis in all species of animals. It has an intermediate duration of effect.
      ii. Has also been used for local anti-inflammatory effect in inflammation of attachments of ligaments (similar to tennis elbow), applications no longer available with the current thresholds
   e. Threat to Racing Integrity:
      i. Immediately pre-race: No ability to directly affect racing performance
      ii. Over 24 hours pre-race: regulatory concerns are about overuse negatively impacting the welfare of the horse, by eventually degrading joints. There is actually no evidence that this occurs with betamethasone.
   f. Validity of the Threshold and Withdrawal: **none**
      i. Threshold of metabolites in plasma with a 7 day withdrawal is unsupported by published research.
      ii. At the time at which the threshold was established, there were no published reports. There has been a subsequent publication in 2017,[1] which studied 12 exercised Thoroughbred research horses after injection of a single joint with 9 mg Betamethasone. The Limit of Quantitation for betamethasone in this study was 25 pg/mL, well above the threshold set by the RMTC. No conclusions can be drawn about how the horses may have processed the injected betamethasone at levels in the range of 10 pg/mL when the study could not accurately quantify betamethasone at that level. No indication of when samples were collected in relation to exercise is included in the paper. Exercise may increase the blood supply to the joint resulting in an increase on the drug level in plasma, so the time of blood collection in relation to an exercise event is critical. Further, there is a significant difference between the group of horses that had joint taps for the purpose of drug measurement, and those that did not. Despite this difference, the two groups were combined to determine pharmacokinetic parameters and drug clearance. The exercise regime of the horses that did not have their joints tapped appears to be more strenuous than those that had their joints tapped. The exercise regimes could have been responsible for the difference in plasma concentration of the drug, making any conclusions from this study inapplicable to the racing population at large. In all post-racing samples, the collection of the blood occurs shortly after a strenuous exercise bout, which is an

[1] Knych HK, Stanley SD, Harrison LM, McKemie DS. 2017. Pharmacokinetics of betamethasone in plasma, urine, and synovial fluid following intraarticular administration to exercised thoroughbred horses. *Drug Testing and Analysis* DOI 10.1002/dta.2170.

event likely to change the blood supply to the joint and the mechanical forces on corticosteroid particles within the joint, both factors that affect the plasma level.

iii. In January 2019, the RMTC posted a monograph that included a summary of the betamethasone research that served as the basis for the threshold on their website.[2] The research has never been published in a peer-reviewed scientific journal. In this monograph, the RMTC authors indicate that all of the data in the 20 horse experiment were below the lower limit of quantitation (LLQ). They indicate that the data between the LLQ and the limit of detection (LOD) were estimated, but it presented no indication of how the plasma levels below the LOD were handled.

iv. In the actual spreadsheet[9] containing the raw data from that experimental study, there were only 19 samples from the 7 d (168 h) time point, not 20 as indicated by the RMTC monograph. This makes the online monograph erroneous at best, and intentionally misleading at its worst, as the 95/95 tolerance level was determined using a k-factor associated with 20 horses, when there were actually only 19 horses sampled on day 7 of the study.

v. In the spreadsheet containing Dr. Sams' data, at 7 days after the administration of 9 mg of the FDA-approved betamethasone sodium phosphate/acetate into a joint, these data show that one horse, "Annie" was over the threshold at 7 days, at 11.5 pg/mL. Further, another horse, "Jr" was below the threshold from 3 to 8 days, and then above the threshold (11.5 pg/mL) at 9 days post administration.

vi. The fluctuation of drug concentrations up and down after about 3 days is not unexpected for this type of drug. The betamethasone ester deposited into the joint space is likely to be released into the blood inconsistently because of motion, exercise and blood supply to the joint, all of which may differ depending upon the horse's activity level prior to blood sampling. No controls for movement or proximity to exercise appear to have been in place in the experimental studies performed by the RMTC, and these betamethasone data reflect that the RMTC researchers should have identified that some such factor was resulting in variability in the data, and adjusted their threshold accordingly.

vii. The variation of the level going up and down throughout the withdrawal period reflects an underlying process that is neither explained nor addressed in the setting of this threshold.

viii. The threshold derived from the Sams' database at 9 days provides a threshold of 75 pg/mL, even higher than the threshold at 7 days. This is because the plasma levels consistently decline over the first 48 hours after joint injection, and then subsequently go up and down in the same horses over the low pg/mL levels. At 9 days, JR has an unexplained high reading of 11.5 pg/mL, and in combination with the remaining horses being at much lower plasma concentrations, this high reading makes the variability of the data very wide, resulting in the considerably higher threshold of 75 pg/mL.

[2] Available upon request.

ix. There were 11 samples that were below the ability of the Liquid chromatography-tandem mass spectrometer (LC/MS-MS) to estimate a concentration.[9] In both the monograph and the Knych et al study, there are synovial fluid concentrations up to at least 14 days, which means that there are actually plasma levels at the 7 day (168 h) time point below the ability of the LC/MS-MS to detect or estimate their concentration. These levels are known as censored data or non-detects.

x. The RMTC does not indicate how these censored data are handled in their calculation of the mean and standard deviation. They must have included them (and one horse for which no sample was collected at the 7 day point) in the calculation, because they claim to have used the appropriate k-value for a 20 horse sample. In order to get the mean and standard deviation that is presented in their monograph, they had to have assigned a number above zero and below the LOD. This is a commonly misapplied method of handling non-detects.[3]

xi. The assignment of a constant value for non-detects erroneously skews the measurement of the variability of the data. Having, in this case, 11 datapoints that do not vary because they are all assigned the same value results in a lower standard deviation than the true standard deviation. The standard deviation is a key input into the 95/95 tolerance method of determining a threshold. If one of the key inputs into the tolerance determination is erroneous, then the threshold is erroneous.

xii. The most appropriate means of estimating censored values in a dataset is to use an imputational method that does not skew the measurements of variability of the data.[12] Using the imputational method of Robust Regression on Order,[7] and applying non-parametric statistics to the actual data produced by the RMTC, the betamethasone threshold should have been 41 pg/mL rather than 10 pg/mL, as promulgated by the RMTC.

xiii. Betamethasone is the substance which was the subject of a positive test in Delaware, which was appropriately dropped by regulatory authorities because the scientific basis for this threshold and withdrawal could not be produced[4].

[3] Baccarelli A, Pfeiffer R, Consonni D, Pesatori AC, Bonzini M, Patterson DG Jr., Bartazzi PA, Landi MT. 2005. Handling of dioxin measurement data in the presence of non-detectable values: Overview of available methods and their application in the Sevesco chloracne study. Chemosphere 60:898-906.

[4] http://www.theracingbiz.com/2015/07/24/pletcher-overage-that-wasnt-has-major-implications-for-racing/