Explaining the 95/95 Tolerance Interval

After determining an appropriate withdrawal interval for a specific medication—based on a review of the scientific literature and conventional racetrack applications, investigation of any illicit use, and consideration of a medication's potential to affect performance—the RMTC Scientific Advisory Committee (SAC) reviews the data from the study horses' samples collected at that time point, and applies the 95/95 Tolerance Interval calculation to establish a regulatory threshold.

Doing the math

Figure 1 below displays the raw data from samples collected 24 hours post-medication administration to 20 research horses.

Horse	Concentration,(ng/ml)
1	6.8
2	3.4
3	6.2
4	5.4
5	0.3
6	0.5
7	2.6
8	0.1
9	0.1
10	4.5
11	1.0
12	2.3
13	10.0
14	3.5
15	0.2
16	1.2
17	0.8
18	1.0
19	1.4
20	20.0

Figure 1.

Note that the concentrations range from 0.1 ng/ml to 20.0 ng/ml.

It is not unusual to see this broad of a range of values at a single time point.

All values are considered to be valid; none are discarded.

The Mean (average) of the values at 24 hours is 3.6 ng/ml.

The Standard Deviation* is 4.596

* The Standard Deviation (SD) is a measure of the distribution of values in relation to the mean.

When the values above are plotted on a graph, where the height of each bar on the graph displays how frequently that value occurred in the 20 samples, their distribution looks like this:

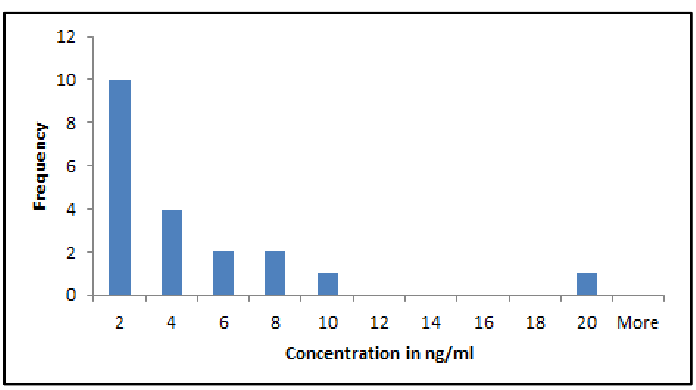

Figure 2.

Most statistical methods include the assumption that the data have a Normal distribution. Data transformation is necessary to make skewed data fit more closely to underlying assumptions of statistical tests of normally distributed data. In order to perform the 95/95 Tolerance interval calculation, it is necessary for the data to be Normally distributed—in a bell-shaped curve, similar to the example below.

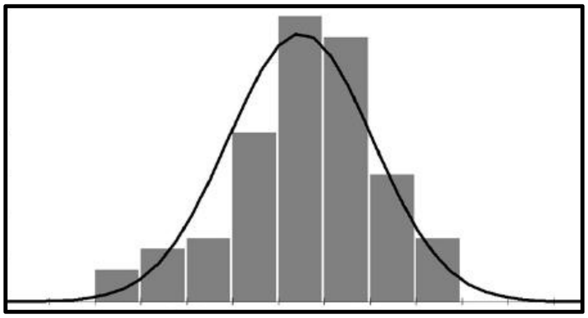

Figure 3.

To normalize the distribution of the data, all values are converted by applying the same formula to each, in this case transforming each to its natural logarithm. The resulting log converted values are displayed below:

Horse	Concentration detected	Log converted concentration
1	6.8	1.916922612
2	3.4	1.223775432
3	6.2	1.824549292
4	5.4	1.686398954
5	0.3	-1.203972804
6	0.5	-0.776528789
7	2.6	0.955511445
8	0.1	-2.302585093
9	0.1	-2.302585093
10	4.5	1.504077397
11	1.0	0
12	2.3	0.832909123
13	10.0	2.302585093
14	3.5	1.252762968
15	0.2	-1.609437912
16	1.2	0.182321557
17	0.8	-0.223143551
18	1.0	0
19	1.4	0.336472237
20	20.0	2.995732274

Figure 4.

Because the same transformation is performed on all data, the relative differences between all the values remain consistent, and a valid analysis can be performed.

The Mean (average) of the log converted values: $\text{Mean}_{\ln}$ = 0.42979

The Standard Deviation of the converted values is: $SD_{\ln}$ = 1.50102

The log transformed numbers are then graphed by frequency, similar to that seen in Figure 2.
The distribution of the log transformed values looks like this:

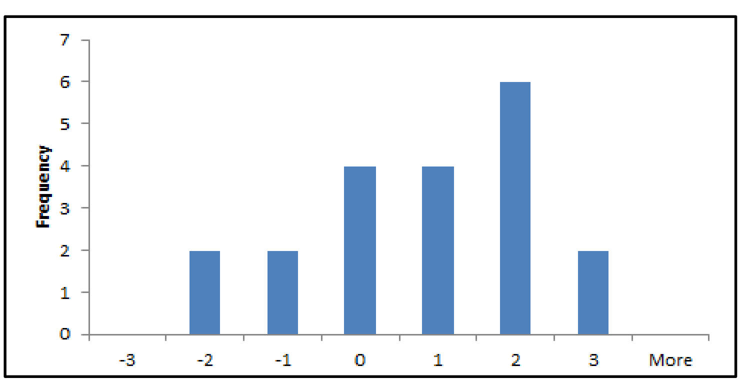

Figure 5.

While not a perfect fit, this distribution still more closely resembles a bell curve, and so the 95/95 TI can be calculated using these transformed values. (If the log transformed values did not approximate a bell curve, another transformation formula could be applied. However, experience indicates that the log transformation produces the most satisfactory distribution of biological data.)

95/95 Tolerance interval calculation equation:

$$\text{Mean}_{ln} + (\text{SD}_{ln} \times \text{K factor}) = 95/95 \text{ TI}$$

The K factor is a pre-determined, statistical value based on the number of study animals. It is intended to provide an added margin of safety as this number increases as the number of study horses decreases. With fewer horses in a study, it is increasingly unlikely that the range of values detected accurately represents the entire population. If only 4 horses were sampled post-administration, those four values alone could misrepresent the range that could be expected to occur if more horses were sampled.

Horse	Concentration,(ng/ml)
1	6.8
2	3.4
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5	0.3
6	0.5
7	2.6
8	0.1
9	0.1
10	4.5
11	1.0
12	2.3
13	10.0
14	3.5
15	0.2
16	1.2
17	0.8
18	1.0
19	1.4
20	20.0

Going back to the concentrations in the 20 samples from the study horses, it is clear that if horses 1, 6, 11, and 16 were the only ones in the study, it would be possible to vastly misunderstand the full normal range of 24-hour concentrations in the whole population.

It is therefore necessary to address the unknown variability that occurs outside of the limited study population.

The equation used to calculate the 95/95 TI uses the K factor to statistically correct for that unknown.

Figure 6.

The purpose then of the K factor, is to prevent the ‘oversimplification’ that can result from attributing too much importance to values from a limited subset of a population. The more horses in the study, the lower the corresponding K factor. Likewise, fewer study horses means a larger K factor. Below is the European Agency for the Evaluation of Medicinal Products’ table of K factors used by the RMTC[i].

n	*k*
2	26.260
3	7.656
4	5.144
5	4.210
6	3.711
7	3.401
8	3.188
9	3.032
10	2.911
11	2.815
12	2.736
13	2.670
14	2.614
15	2.566
16	2.523
17	2.486
18	2.453
19	2.423
20	2.396

← For a 6 horse study, the K factor is 3.711.

← For a 20 horse study, the K factor is 2.396.
Figure 7.

Returning to the 95/95 TI Equation: $\text{Mean}_{\ln} + (\text{SD}_{\ln} \times \text{K factor}) = 95/95\ \text{TI}$

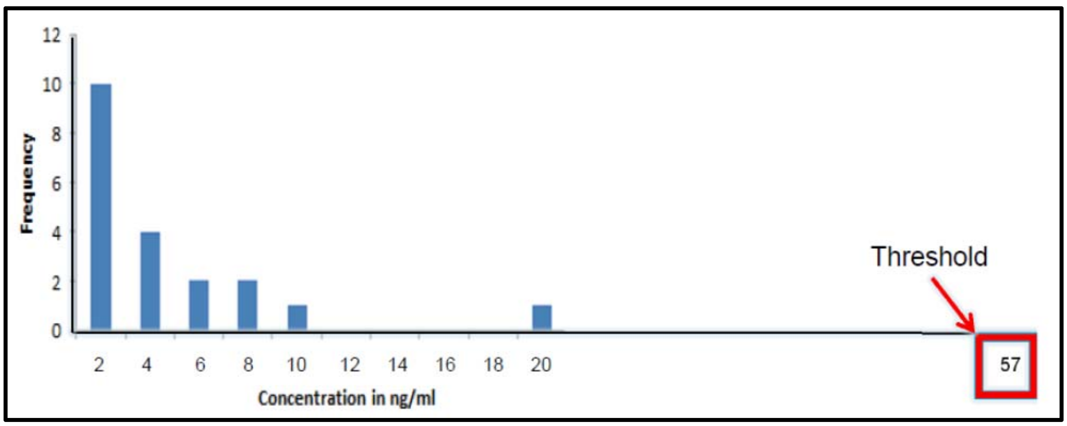
$$0.42979 + (1.50102 \times 2.396) = 4.02623$$

The calculated value, 4.02623 is converted back from a logarithm to a normal number: 56.04983959

Developing a Threshold Recommendation

The RMTC Scientific Advisory Committee considers the calculated value. Their threshold recommendation is always rounded up, not down. So the minimum threshold the RMTC SAC would recommend is 57 ng/ml for a 24 hour withdrawal time.

Comparing that threshold with the 24-hour concentrations in the 20 study horses, recall that the highest detected concentration was 20 ng/ml. Placing the 57 ng/ml threshold on the graph in Figure 2, clearly demonstrates the margin of safety afforded by using the 95/95 TI.



Further, it is likely that after deliberation by the RMTC SAC, the threshold recommendation would be increased to 60 ng/ml in order to accommodate the laboratory's preparation of reference standards that are used when official post-race samples are analyzed.

The Importance of Converting the Data

Some individuals erroneously declare that the RMTC's application of the 95/95 TI results in a 5% risk (or 1 in 20 chance) of a violation when withdrawal guidance is followed. It is true that if the data are skewed and the sample size small, the application of statistics assuming normal distribution will be flawed, resulting in a confidence interval that is wrong.

The calculation below demonstrates the consequence of not performing a log transformation:

$$\text{Mean} + (\text{SD} \times \text{K factor}) = 95/95\ \text{TI}$$

Using the unconverted data from Figure 1: $3.6 + (4.596 \times 2.396) = 14.61$

This number, 14.62 would be rounded up, by the RMTC to a threshold of 15.0 ng/ml. One of the 20 study horses had a concentration greater than 15.0 ng/ml. Using the unconverted data to perform the 95/95 TI constitutes an unacceptable level of risk.   This demonstration emphasizes the importance of the log transformation and the margin of safety it provides to those following withdrawal guidance.

[i] www.ema.europa.eu/docs/en_GB/document_library/Scientific_guideline/2009/10/WC500004496.pdf