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How to extrapolate a withdrawal time from an EHSLC published detection time: A Monte Carlo simulation appraisal

P.-L. TOUTAIN*

Ecole Nationale Vétérinaire de Toulouse, UMR 181 de Physiopathologie et Toxicologie Expérimentales INRA, ENVT 23, chemin des Capelles – BP 87614-31076 Toulouse CEDEX, France.

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Summary

Reason for performing study: **For legitimate medications, veterinarians must advise the owners or trainers of horses on appropriate withholding times after a treatment, to avoid the risk of incurring a positive drug test.**

Objective: **To explore the safety span to select that a veterinarian may extrapolate a tailored withdrawal time (WT) from a generic detection time (DT) as published by the European Horserace Scientific Liaison Committee (EHSLC).**

Methods: **Using Monte Carlo simulations, it was shown that for a low variability of pharmacokinetic parameters (CV = 20%), an uncertainty span of about 40% may be selected to transform a mean DT into a WT (i.e. WT = 1.4 DT), which covers 90% of the horse population. In contrast for a highly variable drug (CV = 40%), an uncertainty factor of about 2.1–2.2 needs to be selected, i.e. a WT that is twice the DT.**

Results: **The relative impact of the different factors of variability on the final WT was documented by a so-called sensitivity analysis. It was shown that the parameters that have the greatest influence on the value of a DT are those that control the terminal half-life of the drug disposition. In contrast, parameters controlling the level of urine (or plasma) concentrations (i.e. the actual administered dose, the urine-to-plasma ratio and the bioavailability) collectively have a minimal influence on the DT.**

Conclusions and potential relevance: **In practice, this means that the main sources of uncertainty are of biological origin and cannot be reduced by any managerial options. The influence of the number of experimental horses that are used by EHSLC to establish a DT was shown that with the standard EHLSC protocol of 6 horses, half of the trials lead to a proposed DT that is equal to or higher than the population 90th percentile. Increasing the number of investigated horses to 8 and 10 would increase this last probability to 85 and 90%, respectively.**

Abbreviations

DT:	Detection time
EHSLC:	European Horserace Scientific Liaison Committee
HSL:	Harmonised screening limit
MCSs:	Monte Carlo simulations
WT:	Withdrawal time

Introduction

The European Horserace Scientific Liaison Committee (EHSLC) the committee in charge of harmonising sample testing and policies for racing horses in Europe (Houghton *et al.* 2004; Barragry 2006), have established a general policy that distinguishes the control of any drug exposure for all illicit substances (doping control) and the control of drug effects for therapeutic substances (medication control).

For medication control, the main task of the EHLSC is to establish agreed harmonised screening limits (HSL) for all nations engaged in the EHLSC programme. Technically within the EHSLC, the HSL is known as the recognised limit of detection. The HSL is a confidential instruction to laboratories from racing authorities to screen for the presence of drugs commonly used in equine medication at a plasma or urine level considered not to be a threat to the welfare of the horse, alter its racing performance, or compromise the integrity of racing.

For these legitimate medications, veterinarians must advise owners or trainers on appropriate withholding times after a treatment to avoid the risk of incurring a positive drug test. To help veterinarians to establish their withholding times (called withdrawal time (WT) by the EHSLC), the EHSLC and the International Equestrian Federation (FEI) with which it shares the same results, decided to establish the duration of detection time (DT) of the main medications public when screening is performed with the unavailable HSL.

The DT for urine (or plasma) is defined by the EHLSC as the interval between the time of the last drug administration and the time at which the observed urine (plasma) concentrations of all investigated horses (during an experiment conducted according to EHLSC recommendations) are below the HSL with the routine analytical method. In the context of risk analysis conducted by the EHSLC, this DT is the only information released by the authorities to stakeholders to assist veterinarians in recommending a tailored WT for a given horse. It should be stressed that a DT, as issued by the EHSLC is only a raw experimental observation, whereas a WT is a recommendation and, as such, is a matter for professional judgement of the treating veterinarian. The WT should be longer than the DT because the WT should take into account the impact of all sources of animal variability (age, sex, breed, training, racing, etc.) and those of the medicinal product actually administered

*Author to whom correspondence should be addressed.
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(formulation, route of administration, dosage regimen and duration of treatment). Contrary to a so-called EU withdrawal period (WP) for drug residues in food animals, neither the DT nor the WT are statistically founded and an appropriate safety span needs to be considered when extrapolating a WT from a DT published by the EHLSC.

The goal here is to explore how to extrapolate a WT from a DT, i.e. to propose an order of magnitude of the safety span that must be added by veterinarians to an EHSLC DT to recommend their own WT. This will be done using Monte Carlo simulations (MCSs). A Monte Carlo simulation is a numerical method (Bonate 2001) with a built-in random process that involves combining variability due to different sources. Here, there are 2 main sources of variability (i) intrinsic biological variability between horses for pharmacokinetic (PK) parameters controlling plasma and urine drug disposition (i.e. plasma clearance, volume of distribution, urine-to-plasma ratio, etc.); these sources of variability are explained by factors such as breed, age, sex, etc., (ii) sources of uncertainty associated with the veterinary decision and/or trainer practice as to the actual administered dose, the administration of a dosage form different from that tested by the EHSLC, modalities of administration, trained/untrained conditions. With an MCS, all these sources of variability can be combined simultaneously to generate a large hypothetical population of DTs so that the proportion (percentiles) of horses attaining a given DT value can be determined; and MCSs may replace a large population survey aimed to establish a WT experimentally.

Methods

To determine the variability of a DT by MCSs, an explicit PK model needs to be used that can provide the numerical value of the DT. For medication control in horses, a mono-exponential decay is generally sufficient to describe the elimination phase of interest (*equation 1*):

$$C(t) = Y_z \times Exp(-\lambda_z \times time) \quad (1)$$

Where *C(t)* is the plasma (urine) concentration at time *t* after administration (single dose), Y_z is the intercept (a concentration) of the terminal (elimination) phase and λ_z is the slope of the terminal phase.

The *time* corresponding to any fixed concentration *C(t)* as an HSL is given by *equation 2*.

$$Time = \left[Log_e \frac{C(t)}{Y_z} \right] \Big/ \lambda_z \quad (2)$$

If *C(t)* is the HSL, then *Time* is a DT (*equation 3*)

$$DT = \left[Log_e \frac{HSL}{Y_z} \right] \Big/ \lambda_z \quad (3)$$

In *equation 3*, Y_z and λ_z are hybrid PK parameters. They can be modelled with their physiological determinants. The slope of the terminal (elimination) phase has only 2 physiological determinants, namely, plasma clearance (CL_{plasma}) and volume of distribution (V_z). Thus, the slope of the terminal phase is given by *equation 4*:

$$\lambda_z = CL_{plasma} / V_z \quad (4)$$

Similarly, the intercept Y_z reflects the dose, bioavailability and volume of distribution, and for urine, the urine-to-plasma ratio as given by *equation 5*:

$$Y_z = [F \times BW \times Dose \times R_{ss}] / V_z \quad (5)$$

Where *F* is the bioavailability (from 0 to 1), *BW* is the body weight in kg, *Dose* is the nominal dose expressed by kg of BW, R_{ss} is the urine to plasma ratio and V_z as defined above.

If, in *equation 3*, we replace Y_z and λ_z by their physiological determinants, i.e. by combining *equations 3, 4 and 5*, the DT is given by *equation 6*:

$$DT = \left[Log_e \frac{HSL}{[F \times BW \times Dose \times R_{ss}] / V_z} \right] \Big/ \left[\frac{Cl_{plasma}}{V_z} \right] \quad (6)$$

With *F*, *BW*, R_{ss}, *CL* and V_z as defined above.

Figure 1 shows the 2 different approaches (deterministic vs. stochastic) used to compute a DT. The PK model of DT described by *equation 6* was simulated with the set of parameters given in Table 1. Simulations were performed with Crystal Ball[1], a software that works directly with Excel as an add-in. The following questions have been addressed with this model: (i) What is the overall joint influence of biological variability (inter- and intra-animal variability) and veterinary decisions (selection of a given formulation, actual administered dose) on the DT of a drug? (ii) Which of the aforementioned factors have the most influence on the observed value of a DT (documented by a so-called sensitivity analysis) (iii) Is the EHSLC published DT, which is obtained from a trial involving only 6 horses, robust enough for a future WT and what would the advantage be for veterinarians to increase this number to 8 or 10 horses?

A sensitivity analysis was carried out to assess how the variability in the output of the model (the DT computed with *equation 6*) can be apportioned, qualitatively or quantitatively, to different sources of variation of the model input (in this case: clearance, volume of distribution, urine-to-plasma ratio, bodyweight and bioavailability). If a small change in an input parameter results in relatively large changes in the WT, the WT is said to be sensitive to that parameter. The sensitivity analysis in the Crystal Ball software, uses rank correlation to dynamically calculate the relationships between the different input factors and the predicted output. The results are expressed as the percentage contribution to variance.

According to the EHLSC recommendation, a DT is typically obtained from a group of 6 horses. The question addressed in the present paper is how valuable this information is regarding the actual WT for the target population. To document this question, 20 virtual EHSLC trials involving 6, 8 or 10 horses, were simulated by MCS. The trials were simulated under the conditions given in Table 1 to obtain nominal DTs of 62.15 and 248.6 h. The coefficient of variability was 30% for the normally distributed pharmacokinetic parameters (plasma clearance, volume of distribution, urine-to-plasma ratio and bioavailability). The bodyweight (nominal value of 100) was described by a uniform distribution (from 80 to 120). The DT for each trial was the longest of the 6, 8 or 10 simulated values. The DT was then compared to the population percentiles obtained by simulating 5000 horses with the same simulation assumptions.

Results

Figure 2 shows an example of a probability density function of the DT obtained with MCSs. The PK parameters used for these simulations are given in Table 1. Simulations were carried out at 2 levels of variability for the PK parameters as expressed by

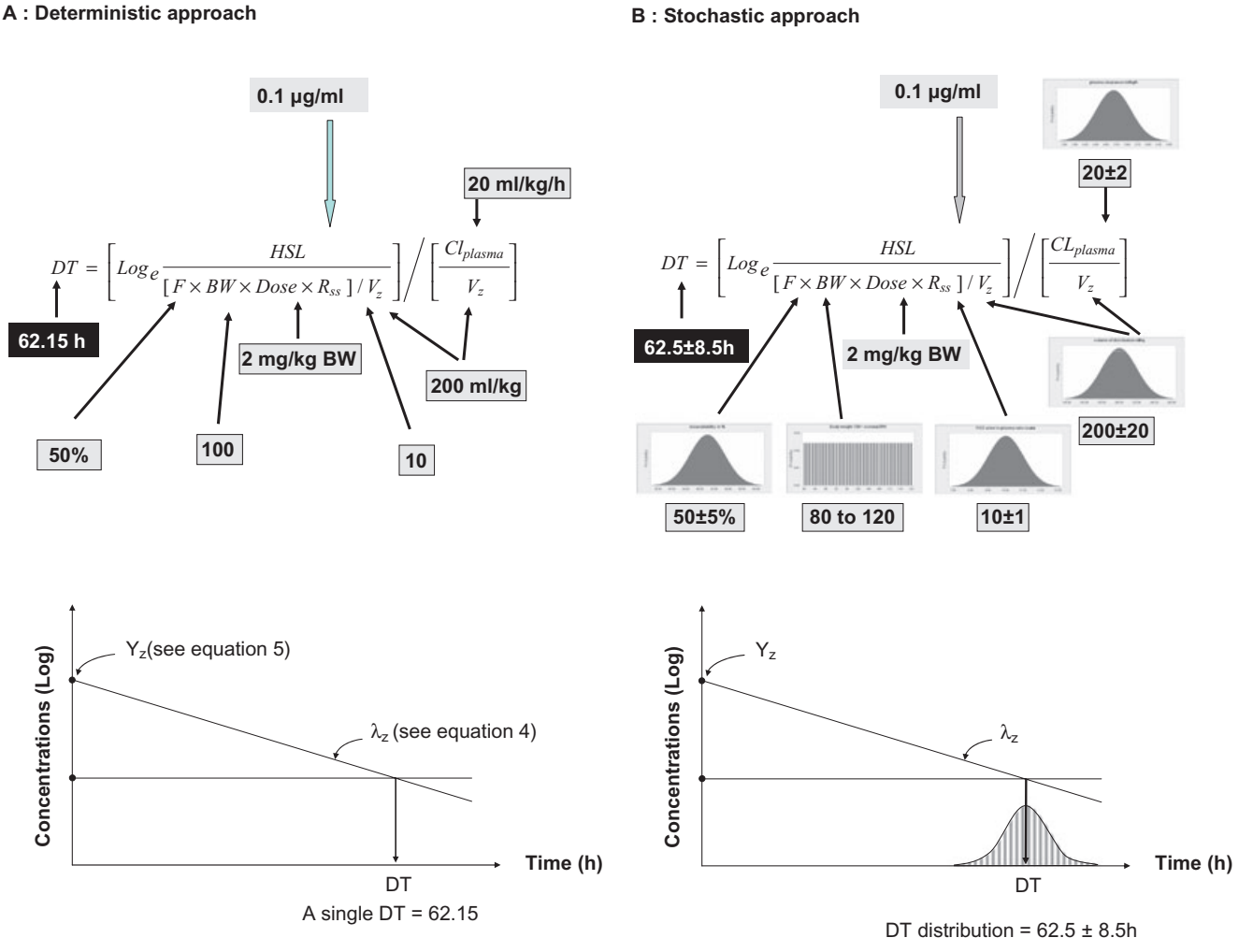

*Fig 1: Deterministic vs. stochastic models for computation of the DT. The DT for a drug may be computed from a simple mono-exponential model (*equation 6*). This model may be used deterministically (a), i.e. be solved with input variables (clearance, volume of distribution, urine-to-plasma ratio, BW, etc. using point estimates (e.g. mean, mode, median, extreme values for a worst case scenario), these point estimates 'determining' the results. In contrast, the same model may be used stochastically, i.e. be solved by replacing point estimates by statistical distributions of clearance, volume of distribution, urine-to-plasma ratio, etc. With a stochastic model (b), different results are obtained every time the model is run, whereas with the deterministic approach the result is always the same and the only way to change the output (DT) is to change one of the single point inputs. For the stochastic approach, plasma clearance (*CL_{plasma}*), volume of distribution (*V_z*), bioavailability (F) and urine-to-plasma ratio were described by a normal distribution (mean ± s.d.). The BW values were described by a discrete uniform distribution (from 80 to 120) for a nominal value of 100.*

TABLE 1: Independent input variables (plasma clearance [CL_{plasma}], volume of distribution [V_z], urine-to-plasma ratio [R_{ss}], bioavailability [F] and body weight [BW]) used to compute the numerical values of the DT according to *equation 6*

Variables	Distribution	Mean parameters	HSL (µg/ml)	DT (h) point estimates mean
F (no units)	Normal	0.5 (50%)		
CL_{plasma} (ml/kg/min)	Normal	20	0.1	62.15
		5	0.1	248.6
		5	0.01	340.7
V_z (ml/kg)	Normal	200		
R_{ss} (no units)	Normal	10		
BW (no units) (relative value)	Discrete uniform Minimum: 80 Maximum: 120	100		

BW = body weight; DT = detection time; HSL = harmonised screening limit; MCSs = Monte Carlo simulations. All simulations were carried out with the same nominal dose (2 mg/kg bwt) and the same mean values for all PK parameters except plasma clearance. Two plasma clearance values were simulated (5 and 20 ml/kg bwt/h). Two screening limits (HSLs) were investigated: 0.1 and 0.01 µg/ml. The model was solved either using single point estimates (mean values of parameters) or distribution of the input variables. The DTs obtained with point estimates are given in the last column. For parameters obeying a normal distribution, MCSs were carried out with different levels of variability around the mean PK parameters, namely variances corresponding to coefficients of variability of 10, 20, 30, 40 and –50%. For BW, the same range of values (from 80–120% of the nominal value) was considered for all MCSs.

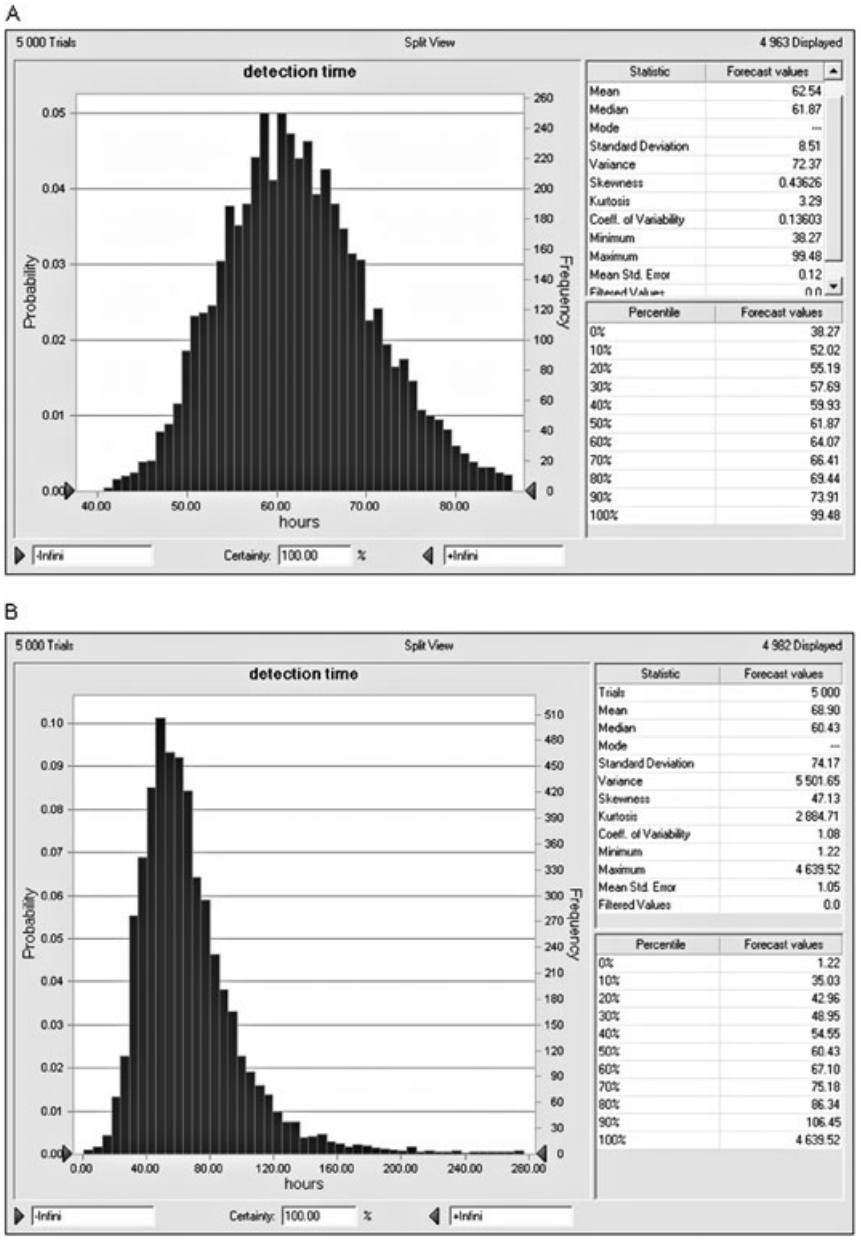

Fig 2: Monte Carlo simulation generated probability density of the DT. (a), DTs were computed for a nominal dose of 2 mg/kg bwt. For plasma clearance, volume of distribution, bioavailability and the urine-to-plasma ratio, a normal distribution with a coefficient of variation of 10% was selected. Plasma clearance was 20 ± 2 ml/kg/h (mean ± s.d.), the volume of distribution of the terminal phase was 200 ± 20 ml/kg bwt (mean ± s.d.), the bioavailability was 50% (0.5 ± 0.05, mean ± s.d.), and the plasma-to-urine ratio was 10 ± 1 (mean ± s.d.). For body weight a uniform distribution (values ranging from 80–120% of the actual bodyweight) was selected. The HSL (i.e. the screening analytical limit) was fixed at 0.1 µg/ml. Equation 6 *in the text was used to solve the model. The histogram shows the probability corresponding to the different forecast values. Percentile values are given in the lower right panel. Monte Carlo simulations show that DTs of 61.87 h and 73.91 are attained in 50 and 90% of the horses, respectively. Using point estimates (no variability), the DT is 62.146 h, a value very close to the mean obtained with the 5000 generated DTs (62.5 ± 8.5 h). (b), DTs were computed with the same mean parameters as for A but the coefficients of variation for plasma clearance, volume of distribution, bioavailability and urine-to-plasma ratio are now 30%.*

coefficients of variation (CV) of 10 and 30%. For a CV of 10%, it can be seen that 50% of the horses in this particular simulation run reach a DT of 61.87 h, i.e. the 50th percentile of the simulated population and is, as expected, very close to the average value obtained using the deterministic approach (here 62.15 h) and that in 90% of the horses the DT is under 73.91 h (Fig 2 panel a). For a coefficient of variation of 30%, 50% of the horses reach a DT of 60.63 h (i.e. the 50th percentile is 60.63 h) and 90% of the horses have a DT under 106.45 h (the 90th percentile is 106.45 h) (Fig 2 panel b).

DTs calculated with different sets of PK parameters, different HSLs and for different levels of variability (from 10–50% for the PK parameters) are given in Table 2.

For the different simulated scenarios, the ratio of percentile 90 to percentile 50 (P_{90}/P_{50}) was then computed to select an uncertainty span to enable the DT obtained by the EHLSC to be extrapolated to a WT (see later). For all simulations, practically the same P_{90}/P_{50} was computed for a given level of PK parameter variability. For example, for a low variability of PK parameters (CV

TABLE 2: Forecast values of detection time (DT) (hours) corresponding to the 10th, 20th, 50th, 70th, 80th and 90th percentiles of the resulting distribution obtained by MCS

Plasma *CL* (20 ml/kg bwt/h) and HSL of 0.1 μg/ml; other parameters as given in Table 1
Deterministic DT = 62.15 h

CV%	10th	20th	50th	80th	90th	P_{90}/P_{50}
10	52.02	55.19	61.87	69.44	73.91	1.19
20	43.33	48.94	61.26	77.42	88.05	1.43
30	35.03	42.98	60.43	86.34	106.45	1.76
*30**	*30.99*	*39.91*	*60.38*	*91.68*	*117.06*	*1.94*
40	28.11	37.13	59.2	96.36	129.45	2.19
50	23.06	32.77	57.87	105.37	151.21	2.61

Plasma *CL* (5 ml/kg bwt h) and HSL of 0.1 μg/ml; other parameters as given in Table 1
Deterministic DT = 248.58 h

CV%	10th	20th	50th	80th	90th	P_{90}/P_{50}
10	208.08	220.76	247.47	277.75	295.65	1.19
20	173.32	195.77	245.03	309.66	352.21	1.44
30	140.1	171.84	241.71	345.37	425.78	1.764
40	112.04	148.21	236.00	380.62	500.96	2.12
50	91.73	129.89	229.00	409.46	564.98	2.47

Plasma *CL* (5 ml/kg bwt h) and HSL of 0.01 μg/ml; other parameters as given in Table 1
Deterministic DT = 340.69 h

CV%	10th	20th	50th	80th	90th	Q_{90}/Q_{50}
10	284.98	302.72	339.64	381.39	405.95	1.23
20	237.87	269.2	337.59	426.76	484.36	1.43
30	192.37	236.2	333.49	477.12	591.07	1.96
40	154.22	203.95	326.09	521.06	687.06	2.11
50	125.4	180.41	318.50	558.55	772.39	2.42

DT = detection time; EHSLC = European Horserace Scientific Liaison Committee; HSL = harmonised screening limit; PK = pharmacokinetic; WT = withdrawal time. *For this simulation, a negative correlation (r^2 = -0.5) between plasma clearance and volume of distribution was included in the statistical model. For all other simulations, it was considered that all the PK parameters were independent of each other. The model described by *equation 6* was simulated with the parameters and conditions given in Table 1. The ratio of the 50th to the 90th percentile (P_{90}/P_{50}) can be tentatively considered for selecting an uncertainty span to allow extrapolation of the DT obtained by the EHLSC to a WT. For example the safety span selected for a DT obtained for a low variable drug (CV = 20%) will be about 40% whereas for a highly variable drug (e.g. CV = 40%), the DT should be doubled to obtain the appropriate WT.

= 20%), a P_{90}/P_{50} of 1.43 was computed meaning that an uncertainty span of about 40% may be selected to transform a mean DT to a WT, which will cover 90% of the horse population. Similarly, for a highly variable drug (e.g. CV = 40%), a P_{90}/P_{50} of 2.1–2.2 was computed meaning that, for that class of drugs, a WT covering 90% of the horse population will be obtained by doubling the mean DT.

A sensitivity analysis was performed to identify the relative impact of the different factors of variability on the final withdrawal time. For DT and for the model assumptions, plasma clearance and volume of distribution are by far the 2 most important factors of variability influencing the population DT and explain almost all 100% of the variance. In contrast, bioavailability, the actual administered dose, and the urine-to-plasma ratio have a minimal effect on the DT. In other words, only those factors affecting the terminal slope (λ_z) of the disposition curve influence DT, whereas factors affecting the initial urine or plasma concentration (Y_z), i.e. the intercept, have no or only a minimal effect. This is explained by the fact that Y_z is subjected to a logarithmic transformation (see *equation 6* and *Discussion*) to give a DT.

TABLE 3: Cumulative frequencies of percentiles (90, 80, 70 or 60) attained from the observed detection times for 20 trials involving 6, 8 or 10 horses

	Number of horses involved in a trial		
Quantile achieved by the observed detection time	6	8	10
Q90	10	11	14
Q80	15	17	18
Q70	18	20	20
Q60	20		
Number of trials	20	20	20

DT = detection time. Trials (n = 20) involving 6, 8 or 10 horses were generated using Monte Carlo simulations with the conditions given in Table 1 to obtain nominal DTs of 62.15 h or 248.6 h. The coefficient of variability was 30% for normally distributed pharmacokinetic parameters (plasma clearance, volume of distribution, urine-to-plasma ratio and bioavailability). The body weight (nominal value of 100) was described by a uniform distribution (between 80 and 120). For each trial, the DT was the longest of the 6, 8 or 10 simulated values. This DT was then compared to the population percentiles obtained by simulating 5000 horses with the same (simulation) assumptions. For example, for a trial involving 6 horses, the DT was equal to or higher than the 90, 80, 70 or 60 quantiles of the population for 10, 15, 18 or 20 of the 20 simulated trials, respectively. The same results were obtained for the 2 DTs tested (62.15 h and 248.4 h).

In order to assess the value of the information provided by EHSLC when establishing a DT with only 6 horses, we simulated 20 virtual EHSLC trials involving 6, 8 or 10 horses under the conditions given in Table 1 to obtain nominal DTs of 62.15 h and 248.6 h. Identical conclusions were drawn from the 2 simulated sets of DT and the results are given in Table 3. Inspection of Table 3 indicates that with the standard EHSLC protocol, half of the trials lead to a proposed DT that is equal to or higher than the population 90th percentile. This means that in a trial involving 6 horses, the probability of observing by chance a horse with a DT equal to or higher than the 90th percentile is about 50%. Similarly, with 6 horses, the probability of selecting a DT equal to or above the 80th percentile of the target population is about 75%. Increasing the number of horses to 8 and 10 would increase this last probability to 85 and 90%, respectively. In addition, with 8 or 10 horses, 100% of horses will be above the 70th percentile, whereas the corresponding figure with 6 horses is 90%.

Discussion

The goal of the present paper was to document how to transform a generic DT from the EHLSC into a WT for a given horse. A WT may differ from a published DT for a number of reasons that include the health status of the horse, horse management, conditions of drug administration and natural interindividual variability associated with age, sex and breed. DT times are obtained in resting, healthy horses, housed in controlled conditions, after the administration of an accurately determined dose, for a given proprietary formulation, etc. In contrast, a WT will apply to drugs that may be used therapeutically in an unhealthy horse, with possible effects of diet, stable management, exercise, etc., and some uncertainty as to the exact dose administered. Thus, it is prudent to fix a WT that is longer than the EHSLC determined DT in order to avoid the risk of a horse being inadvertently positive during a medication control. It is the responsibility of the treating veterinarian to decide the WT. Currently, recommendations to extrapolate a WT from a DT remain qualitative and a veterinarian may be tempted to 'open the umbrella' and deliberately fix an

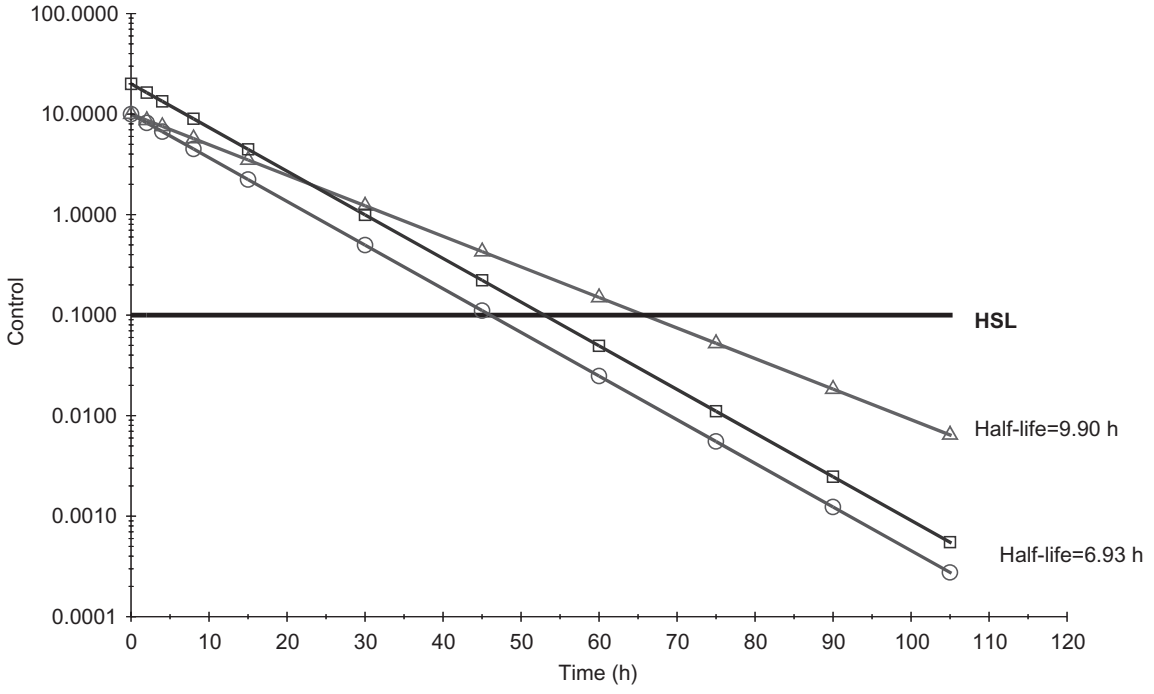

Fig 3: Influence of the administered dose vs. the terminal half-life on a detection time (h) for a given screening limit (HSL) (here 0.1). The DT for control horses (half-time of elimination of 6.93 h) receiving the nominal dose (O) or twice the dose (*) were of 46 and 53 h, respectively, showing that a possible uncertainty on the dose has a rather limited effect on the DT. In contrast, a rather limited reduction (30%) of the rate constant of drug elimination (half-time of elimination of 9.90 h) leads to a prolongation of the detection time to 65 h. This example illustrates that all factors altering the level of drug concentrations throughout a shift on the Y *axis (such as the actual administered dose, bioavailability, urine-to-plasma ratio) have minimal influence on the DT while those influencing the slope of the terminal phase (natural inter-animal variability) may have a major impact on it.*

unnecessarily prolonged WT. An ideal situation for the veterinarian would be to benefit from a statistically founded WP for each commercial formulation as is the case for drug residues in food-producing animals. This would require the EHSLC to release screening limits, i.e. HSL (equivalent to the maximum residue limit for food residues) and drug companies to accept to carry out expensive field investigations to establish a statistically protected WP, because a WP is not a drug property (contrary to a HSL) but a formulation property. In other words, for a single HSL several WP may be computed.

In the present paper, the question has been addressed by applying MCSs, a tool extensively used in risk analysis, to overcome this kind of difficulty. To understand the uncertainty associated with fixing a WT from a DT, it is necessary first to define a structural PK model able to predict an average DT. Here, the selected PK model was a simple mono-exponential model assuming that the basis of the DT resides in the terminal phase of drug plasma (or urine) decay. In most instances, this assumption seems realistic but whenever this assumption is not true, a more advanced model should be used (e.g. a bi-exponential equation). The mono-exponential model that we selected (*equation 1*) has been reparameterised with its physiological determinants (plasma clearance, volume of distribution) as given in *equation 6*. The aim was specifically to explore the joint effect of intrinsic biological variability in horses (factors including breed, age and sex, that in turn influence PK parameters) and the effect of extrinsic factors associated with veterinary decisions (such as dose, the estimated BW and the selection of a given proprietary drug, that in turn affect bioavailability) that can be under the control of the veterinarian or manager. It is then possible, by simply changing the model input variables, to observe the effect of these factors on the DT. This approach provides a range of possible DTs with their respective probabilities, which can be helpful in deciding a future WT. For the sake of simplicity and due to the lack of population information on the actual statistical distributions of PK parameters in horses, we have assumed in this paper that all the PK parameters (plasma clearance, volume of distribution, urine-to-plasma ratio, and systemic bioavailability) were normally distributed. They were defined by their respective mean and s.d. Simulations were carried out for different conditions expressed by a coefficient of variability (CV), i.e. the s.d. over the corresponding mean (CV% = (s.d./mean) × 100). For body weight (BW), a uniform distribution, i.e. all values had the same probability of occurring within the range of observed values (from 80 to 120% of the actual value for all simulations). A uniform distribution is often used when experimental data are limited. In this article, the different scenarios were simulated using the different parameters independently of each other because a correlation between plasma clearance and volume of distribution ($-r^2$ = -0.5) did not have any practical consequence on the DT distribution (results not shown).

Several sets of parameters were selected to simulate short (62 h) and long (248 h) DTs. In addition, for a given set of parameters, 2 different HSL (0.1 and 0.01 µg/ml) were tested. The main result of these simulations is that the parameters that have the greatest influence on the value of a DT are those that control the slope, i.e. the terminal half-life of the drug disposition. In contrast, parameters controlling the level of the urine (or plasma) concentrations (i.e. the actual administered dose as estimated from the BW, the urine-to-plasma ratio and the bioavailability)

collectively had a minimal influence on the DT. In practice, this means that the main sources of uncertainty are of biological origin and cannot be reduced by any management options. Conversely, all factors that can be managed by the treating veterinarian had a minimal impact on the DT. This is the case of bioavailability (associated with a given formulation) and the actual administered dose. This suggests that an inaccuracy in the estimation of the horse's BW, the administration of a formulation different from the one selected for the EHSLC trial or any factor able to influence bioavailability (diet for the oral route of administration, site of administration for IM or SC administration) will probably not have any major influence on the variability of the DT (Fig 3). Similarly, the urine-to-plasma ratio (that is sensitive to the horse's diet) had little influence and all conclusions obtained here for urine also apply to plasma. This is because the influence (very real) of these factors is exerted through a logarithmic operator (see *equation 6*), which reflects the biological fact that drug disposition obeys first-order processes. In other words, MCSs suggests that a veterinarian can do nothing to control a DT through managerial procedures because the main sources of variability are of a biological nature. However, there is a situation in which a veterinarian may strongly influence a DT: this is when a long-acting (depot) formulation is used (e.g. for corticosteroids). In this case, the terminal half-life is no longer controlled by its 2 usual biological determinants (plasma clearance and volume of distribution) but by a slow rate of drug absorption from an injection site (the so-called flip-flop situation). In this situation, the factors with most influence on the DT would be bioavailability factors (see Toutain and Bousquet-Melou 2004 for further explanations).

As veterinary decisions/practices have little influence on a DT, it is the responsibility of EHLSC to release a rather robust DT. In the context of the present article, an EHSLC published DT at least equal to the 70–90th percentile of the target DT population is desirable. Two conditions will be required for this: (i) that the horses investigated by the EHSLC should be representative of the target horse population, and (ii) an appropriate number of horses should be involved in the EHSLC trial to guarantee that at least one of the investigated horses belongs to the upper part (e.g. above the 70th percentile) of the DT distribution. The first condition is difficult to ascertain because horse pharmacogenetics remains largely unexplored. The second assumption is easy to check using MCSs. We compared the merit of trials carried out with three different sample sizes, i.e. 6 (the currently recommended EHLSC figure), 8 or 10 horses. It is apparent that whatever the number of investigated horses, the released DT (corresponding to the horse with the longest observed DT) is always higher than the 50th percentile of the overall target population. With 6 horses, the released DT corresponds to the 90th percentile of the target population for 50% of the trials, the 80th percentile for 75% and the 70th percentile for 90% of the trials. The corresponding target attainment rates for the 90th, 80th and 70th percentiles were 55%, 85% and 100% in simulated trials involving 8 horses and 70%, 90% and 100% in trials with a simulation for 10 representative horses. Therefore, it can be concluded that the current EHLSC recommended sample size (6 horses) has some merit but to increase the predictability of a future WT, at least 8–10 horses would be required (see later).

As quoted above, whatever the sample size, the worst case scenario for an EHSLC DT would be to release a figure corresponding only to the 50th percentile of the population. We therefore propose transforming the released EHSLC DT to a putative WT by multiplying the EHSLC DT by an uncertainty factor (UF), which will guarantee that at least the population 90th percentile is attained. The UF used for this is the ratio of the 90th to the 50th percentile of the target DT population as obtained using MCSs. In the framework of our simulations, this UF was shown to be solely determined by the level of variability of the PK parameters and to be independent of the actual average values of these parameters, and of the selected HSL, thereby conferring this UF with generic value. In other words, the proposed UF may apply to any kind of drug as long as it obeys the above-mentioned assumptions (mono-exponential decay, terminal slope controlled by plasma clearance and volume of distribution, BW estimated with no more than 20% bias).

The UF accounts for interindividual variability. Inspection of Table 2 reveals that a P_{90}/P_{10} ratio of about 3 was observed for an inter-horse variability of 30%. We therefore considered a variability in PK parameters of 30% in horses as a default conservative value and the corresponding Q_{90}/Q_{50} ratio as the default uncertainty factor (UF) for a worst case scenario (here UF = 1.76). Considering that the simulated EHSLC DT obtained with six horses was never lower than the population 60th percentile, a UF equal to Q_{90}/Q_{60} may be also considered (UF = 1.58). If the EHSLC decides to increase the number of horses investigated to eight, the UF could become the Q_{70}/Q_{90} ratio because no DTs are under the 70th percentile and the UF may be lowered to 1.41.

In conclusion, it could be suggested that the EHSLC should increase the number of investigated horses to at least 8 so that a default UF of 1.4 can be applied to transform a DT to a WT. Otherwise, a generic conservative UF of 2 can be selected. If this approach is considered as appealing by the EHSLC, MCSs could also be used to explore other 'what-if' scenarios; for example, what is the effect on the DT of a single vs. a multiple dose drug administration.

Manufacturer's address

[1]www.crystalball.com

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