The clinical pharmacology of performance enhancement and doping detection in sports

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FUTILITY OF CURRENT URINE SALBUTAMOL DOPING CONTROL

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ABSTRACT

Salbutamol is used in the management of obstructive bronchospasm, including that of some elite athletes. It is claimed that high salbutamol (oral) doses may also have an anabolic effect. Therefore, inhalation of salbutamol is restricted by the World Anti-Doping Agency (WADA) to a maximal daily dose. Urine is tested for violations, but recent cases have resulted in a debate regarding the validity of this approach. It was our aim to determine whether current approaches are sufficiently able to differentiate approved usage from violations. We extracted pharmacokinetic parameters from literature for salbutamol and its sulphated metabolite. From these parameters, a semi-physiological pharmacokinetic model of inhaled and orally administered salbutamol was synthesised, validated against literature data, and used to perform clinical trial simulations (N=1000) of possible urine concentrations over time resulting from WADA-allowed and oral unacceptable dosages. The synthesised model was able to predict the literature data well. Simulations showed a very large range of salbutamol concentrations, with a significant portion of virtual subjects (15.4%) exceeding the WADA threshold limit of 1000 ng/mL at 1-hour post-dose. The observed large variability in urine concentrations indicates that determining the administered dose from a single untimed urine sample is not feasible. The current threshold inadvertently leads to incorrect assumptions of violation, whereas many violations will go unnoticed, especially when samples are taken long after drug administration. These issues, combined with the dubious assertion of its anabolic effect, leads us to conclude that the large effort involved in testing should be reconsidered.

INTRODUCTION

Recently one of the most successful male cyclists of the last decade, Chris Froome, came into disrepute due to news of a potential doping violation. Doping control revealed a salbutamol (albuterol in the US) concentration exceeding the allowed limit of 1000 ng/mL in a urine sample provided by the British rider during the Vuelta a España of 2017.[1] The WADA prohibited list indicates that salbutamol use is allowed in inhaled dosages up to "1600 micrograms over 24 hours in divided doses not to exceed 800 micrograms over 12 hours starting from any dose", which is considered the maximum therapeutic dose for athletes with a so-called Therapeutic Use Exemption (TUE). Froome was in possession of such a TUE, but "the presence in urine of salbutamol in excess of 1000 ng/mL [...] is presumed not to be an intended therapeutic use of the substance and will be considered as an Adverse Analytical Finding unless the athlete proves, through a controlled pharmacokinetic study, that the abnormal result was the consequence of the use of the therapeutic dose (by inhalation) up to the maximum dose indicated above." Currently, over six months since the concerned urine sample, there is still no news of such a controlled study, which is perhaps not surprising: the burden is with the accused and setting up a robust study requires expertise. Additionally, inter-occasion variability will influence the results of each attempt.

Salbutamol acts on the beta-2-adrenoceptor as a sympathomimetic, commonly prescribed to counteract bronchoconstriction due to allergic and exercise-induced asthma, as well as chronic obstructive pulmonary disease.[2] This mechanism of action has led athletes to believe that there might be a performance enhancing effect of beta-2 agonists both through relaxing smooth muscle cell in the lung, and anabolic effects on skeletal muscles. Two reviews extensively investigated the evidence for these effects and concluded that inhaled beta-2 agonists have no positive effects on muscle strength, sprint or endurance performance, and that only high, systemic dosages can improve muscle strength and sprint performance, but not endurance performance.[3,4] In summary, beta-2 agonists might give an advantage in sports, but only at very high concentrations and for very short (power) disciplines. It is therefore doubtful that multi-stage (endurance) cyclists like Chris Froome would benefit even from high doses of beta-2 agonists apart from when treating asthmatic symptoms. Nevertheless, the WADA currently is of the opinion that all beta-2 agonists are banned substances, necessitating doping control measures. For this purpose, urine analysis is performed, which leads to a problem for the substances that are allowed

with a TUE up to a maximum dose: salbutamol, formoterol and salmeterol. For these substances, urine concentration is used to determine whether the maximum dose was exceeded. It is, however, impossible to determine dose from a single urine concentration due to several pharmacological factors that are at play. Salbutamol plasma pharmacokinetics in particular, are highly variable, mainly due to variability in lung and gut absorption, volume(s) of distribution, metabolism (including first-pass effect) and renal clearance.[5–7] Subsequently, urine pharmacokinetics are even more variable due to the additional factor of urine concentrating abilities of the kidney (urine osmolality between 50 and 1200 mOsm/kg),[8] volumetric production of urine[9] and micturition. On top of all this, the time since last dose in a doping control setting is unknown. Despite these facts, the WADA rules seem to assume that a urine concentration above the set threshold indicates a high chance of having detected use of more than the allowed dose. This chance is apparently considered sufficiently high to warrant sanctioning the athlete but supporting evidence in literature is lacking. The aim of this study is therefore to evaluate the current WADA approach to this problem and determine whether this approach is able to differentiate approved use from violations. As a part of that aim, we evaluate whether WADA approved doses of salbutamol may lead to unacceptable urine concentrations, considering the multiple sources of variability, using a PBPK approach.

METHODS

Several literature data sources were used to synthesise a semi-physiological pharmacokinetic model of plasma and urine salbutamol concentrations.[5–7] In short, a PK model of salbutamol in dogs was used as the basis and extrapolated to humans using allometric scaling.[7] Literature data on clearance of salbutamol and its main metabolite sulphated salbutamol was added to the base model.[5] A separate compartment was constructed to take into account the production of urine based on factors such as cardiac output and concentrating efforts of the kidneys.[9,10] Further adjustment of parameters was performed to align a visual predictive check against literature data of salbutamol plasma and urine concentration from Haase *et al*.[6] All modelling was performed in an environment consisting of Piraña v2.8.1,[11] psn v4.2.0[12] and NONMEM v7.3.[13] The statistical software R® v3.3.0[14] was used for pre and post-processing of data, and statistical and graphical analysis.

Literature model synthesis

Several pharmacokinetic models exist for salbutamol in literature; however, none of these include the urinary pharmacokinetics of salbutamol. A number of articles describe the concentrations of salbutamol in both plasma and urine without describing the parameters involved in the transfer of salbutamol between the two fluids. The only available model that approximates the absorption of salbutamol through both lungs and gut was built on data from dogs.[7] The model was implemented in NONMEM and the parameter values extrapolated to humans using allometric theory.[15] Variability on the fraction of inhalation absorbed through the gut and through the lungs was incorporated to account for inter-patient variability in inhalation efficiency, and differences in inhaler types and formulations.

The allometrically extrapolated model was further expanded by introducing a first-pass effect of gut absorbed salbutamol to its main metabolite sulphated salbutamol (S-SAL). Furthermore, the renal clearances of salbutamol and S-SAL were incorporated from pharmacokinetic parameters reported by Morgan *et al*.[5] Additional compartments for both salbutamol and S-SAL in urine were constructed, assuming that when the compounds are eliminated from plasma at the clearance rates provided by Morgan *et al*,[5] they are directly introduced to urine without delay.

A separate compartment was built to describe urine volume production, to allow voiding this compartment, similar to micturition, thereby better approximating physiology and allowing for the investigation of several micturition scenarios. Urine formation was assumed to occur at a constant rate, calculated using several physiological parameters, such as cardiac output (CO) scaled allometrically to weight ($CO = 0.166 * weight^{0.79}$).[10] The CO for a typical person of 70 kg then is 4.76 L, with a coefficient of variance (CV) of 20%. Of this CO, 21% flows through the kidneys. Typical haematocrit is 0.409 (CV: 7%) in trained cyclists,[16] thus 59.1% of blood is plasma, with 19% of plasma entering the renal capsule. Of the resulting glomerular filtrate, 99.2% is reabsorbed, leaving 0.8% to leave the kidneys as urine. Combining these numbers, we calculate the urine production in litre per hour for a typical adult weighing 70 kg as follows:[9]

$$4.76\ L/min * 60\ min * 21\% * 59.1\% * 19\% * 0.8\% = 0.054\ L/h \qquad \text{EQ. 1}$$

This amounts to 1.2 litres of urine per day, with a 95% prediction interval (PI) of 0.66 – 1.92 L/day, corresponding well with the typical volumes of 1-2 litres per day. This also includes variability due to concentrating by the kidney. Cardiac stroke volume is increased in elite athletes, but due to reduced heart rate at rest the cardiac output is not significantly different from normal healthy controls.[17] And although cardiac output is significantly increased (roughly 3 times) during exercise, renal blood flow is restricted, leading to only a mild to moderate reduction in the urine production rate.[18,19]

Model validation and calibration

We extracted data points of plasma and urine salbutamol concentrations from Haase *et al*[6] using ImageJ v1.50[20] and the Figure_Calibration plugin.[21] Haase *et al* administered 13 subjects with a single salbutamol inhalation of 1600 mcg, followed by regular plasma and urine sample collections. Validation of the synthesised literature model was performed through simulation of salbutamol plasma and urine concentrations in 1000 subjects (weight mean: 84 kg, SD: 17 kg), after a single 1600 mcg dose, and bladder voiding at the indicated time points where a urine sample was collected. The resulting simulated concentrations were compared to the plasma and urine concentrations over time in exercised, dehydrated subject data extracted from Haase *et al* and graphically presented in a visual predictive check plot.[22] Calibration of the parameters derived from the dog model was required, to better correspond to the Haase *et al* data.

Model simulation

Subsequently, we simulated a twice-daily 800 mcg inhaled salbutamol administration at steady-state (maximal allowed dose) in 1000 virtual subjects and determined the percentage of subjects attaining urine salbutamol concentrations above the 1000 ng/mL threshold to determine whether approved doses of salbutamol may lead to unacceptable urine concentrations. Finally, we also investigated a two week regimen of 8 mg oral salbutamol tablets (a dose shown to increase sprint power in elite athletes[23]) in 1000 simulated subjects, to determine the length of washout period associated with non-adverse findings in doping control. Both simulations (inhaled and oral dosing regimens) were used to evaluate whether the current WADA approach is able to differentiate approved use from violations.

RESULTS

Model synthesis

The synthesised model consisted of eight compartments; one each for absorption through the gut and lung, one central and one peripheral distribution compartment for parent salbutamol, one central metabolite compartment, one salbutamol and one metabolite urine compartment, and a single urine volume compartment (Figure 1). When administered through inhalation, 20% of the dose reaches central circulation through the lung, with 80% of the dose ingested and absorbed through the gut. Half of the gut-absorbed amount experiences a first-pass effect and is absorbed as sulphate metabolite, with the other 50% reaching the circulation as unchanged salbutamol. Total apparent clearance of salbutamol parent drug is 13.1 L/h (of which 18.6%, or 2.4 L/h is the rate of conversion to S-SAL), and clearance of S-SAL is 5.9 L/h. The higher apparent S-SAL clearance compared to conversion of salbutamol to metabolite in circulation explains why S-SAL only accumulates after oral administration of salbutamol, and not in the case of IV administration.[5]

Model validation

After several adjustments of pharmacokinetic parameters (Table 1), plasma and urine salbutamol concentrations were adequately predicted, with only a minor bias, and reasonably similar variation bandwidth (Figure 2). The dual absorption peaks, distribution and elimination phases were well-described.

Urine concentrations after appropriate salbutamol use

Simulations of steady-state urine concentrations over time resulting from a bi-daily administration of the approved 800 mcg show a large spread, with a significant portion (15.4%) of the simulated population achieving urine concentrations above the threshold of 1000 ng/mL at the peak concentration at 1 hour post-dose (Figure

3). At 12 hours after the dose administration, or right before the next inhalation, 0.7% of the population still showed urine concentrations above the threshold. It should be pointed out that these numbers do not take into account voiding the bladder before urine testing. In other words, Figure 3 shows the concentrations that would be measured at a certain time after administration when the bladder is voided for the first time since dose administration.

Urine concentrations after ergogenic salbutamol use

Simulations of urine concentrations over time resulting from a daily 8 mg oral dose (unacceptable usage), show that concentrations decline rapidly below the threshold after ceasing the regimen (Figure 4). Within the first 24 hours, the vast majority of subjects is already below the threshold, and after 2-3 days, none of the athletes will produce urine concentrations above the threshold.

DISCUSSION

We synthesised a model based on literature data alone that was able to adequately describe and predict the complex pharmacology of salbutamol in plasma and urine. The developed model was used to simulate possible outcomes of the maximum allowed dose of salbutamol in elite athletes, to show how current (WADA) standards for urine collection and analysis do not adequately take into account the large number of factors contributing to variability (dose amount and timing and physiological variability). This large variability leads to large uncertainty in determining the dose that was used, showing the implemented approach is not fit for purpose. It will lead to incorrect accusations of violation, whereas many violations will go unnoticed.

Applied correction factors by WADA

The general WADA rules for salbutamol use and control depicted in the introduction apply in all cases, but WADA describes two additional relevant specifics for the procedure. First, when collecting the urine sample from the athlete, the doping control officer will check the specific gravity of the urine produced by the athlete. If this is smaller than 1.005 when measured with a refractometer or smaller than 1.010 when measured with lab sticks, the athlete is required to provide further samples, until a suitable sample is collected.[24] The testing authority together with the laboratory decide which samples shall be analysed, although it is not clear on which criteria this decision is based. This procedure is presumably designed to prevent false negative findings due to diluted urine. However, it should be noted that due to bladder voiding for the first sample, the urine concentration of any subsequent sample will be driven by the plasma concentration at that moment and therefore potentially be a substantial underestimation, as plasma concentrations have fallen since dose administration. Correcting urine concentrations for urine osmolality (more accurate than specific gravity) of a sample would therefore be a much more rational way to normalise urine concentrations, and in addition avoid the need for multiple samples and their drawbacks.

Secondly, WADA corrects a measured urine concentration for assay variability (measurement uncertainty) by adding to the threshold concentration a guard band. The guard band corresponds to the expanded measurement uncertainty of the assay giving > 95% coverage interval for a result at the threshold concentration based on a 1-tailed normal distribution. It is calculated as 1.645 * ucMax (the maximum acceptable combined standard uncertainty of the assay, being 100 ng/mL for salbutamol), rounded up to 2 significant figures. A sample is determined to contain an adverse analytical finding only if the concentration is above the threshold plus the guard band, which is called the decision limit (i.e. 1200 ng/mL for salbutamol).[25] When this decision limit is applied, 9.95% of subjects were above the limit at 1-hour post-dose in our simulated scenario. Throughout the rest of this article the limit of 1000 ng/mL is used for clarity, as this is termed the threshold level by the WADA, also in the Prohibited List.

WADA deems it unnecessary to account for instability of salbutamol or its metabolite in urine, which potentially could impact measured salbutamol urine concentrations, as previous research showed that both salbutamol and its conjugate metabolites seem to be stable in urine.[26]

Involving pharmacology

So, the procedure involving specific gravity is rather dubious, and although the correction for assay variability seems appropriate, variability due to pharmacological processes are not discussed by WADA. Our developed pharmacokinetic model visualises the underlying pharmacokinetic theory linking urine concentration and dose, namely, the administered dose being absorbed into the circulation and appearing in urine as a fixed proportion of the amount in blood (renal clearance). This concentration in urine varies with dose amount and time after dose, bioavailability and absorption rate from the lung and gut (for inhalation), distribution over the body, renal clearance, urine volume and voiding. From a clinical perspective, this is very similar to creatinine clearance, and as clinicians might know: it is impossible to calculate the 'dose of creatinine' using only a single urine concentration. Therefore, to be able to make an informed estimate of the dose, one would need to know factors such as the time of dose (which in the doping control setting they do not), the physiological variability of the athlete (not known), volume of urine over a timed period (not known) and the plasma concentration (not known). And because these factors are unknown in the doping control setting, dose cannot be determined from the urine concentration. This is exemplified by simulations from the model in Figure 3, right panel, showing that even from a single dosing scenario, an extremely wide range of urine concentrations can be found. This would perhaps not be as problematic if a certain dose (the maximum allowed dose) would never lead to urine concentrations above the threshold for any subject, but our simulations show that this is not the case either. When collecting urine at 1 hour (close to t_{max}), 15.4% of our simulated subjects are above the threshold if they did not void since dosing. Conversely, due to the large variability and the unknown frequency of voiding and time since dosing, there is also a high chance of finding urine concentrations below the threshold with doses above the maximum allowed dose. Given the relatively short half-life of salbutamol, anabolic use (i.e. large oral doses) could even be halted shortly before a race, with no urine concentrations above threshold to be found by urine doping tests. Using our model, simulations of daily oral administration of 8 mg salbutamol over two weeks, with a washout period of 2 days resulted in 0% urine concentrations above the WADA threshold (Figure 4) when applying a normal micturition pattern (3 times a day). Moreover, within 24 hours, the majority of subjects would already produce a urine concentration below the threshold. This indicates that even high doses that have been shown to improve peak sprint power in elite endurance athletes,[23] will not lead to adverse analytical findings in the majority of cases if the athlete ceases dosing at least the day before an event.

Burden of proof

In the current situation, the WADA does seem to acknowledge the problem of variability to some extent as an athlete that produced a urine sample with an unacceptably high salbutamol concentration, is given the possibility to prove this was a result of a dosing scheme within WADA limits by means of a controlled pharmacokinetic study. Hereby the WADA transfers the responsibility of resolving the flaws in the rules designed by WADA itself to the athlete. Setting up such a study and getting the desired result will take months at least. And even if an athlete does prove his innocence, this could already do major damage to a reputation (see the Froome case). This is to say, if showing innocence will be successful at all, as this might not prove simple. Although intra-subject variability will be smaller than the previously described inter-subject variability, substantial variability will still be present within a subject. It is therefore not unlikely that many trials will be needed to produce another urine sample that exceeds the threshold with allowed dosages, even more so because it will be difficult to reproduce the circumstances leading to the original finding. Aside from this being a very expensive and time-consuming venture for the athlete, the fact that the foundation on which these WADA rules are based in the first place are flawed as we have shown, makes placing the burden of proof with the athlete completely unacceptable.

Alternative solutions

All these arguments make one wonder why the current procedure is being used by WADA. Speculation about a used dose of salbutamol from a urinary concentration (even when it would be adjusted for osmolality) is open to serious criticism and cannot be used to affect the career of an athlete. A better approach would be to collect timed urine samples over a specified period and possibly take a midpoint blood sample. In addition, S-SAL concentrations in urine could be incorporated, as

this metabolite would accumulate more, especially after oral dosing, due to slower clearance and the first-pass effect. One may in fact use an approach using Bayesian hierarchical modelling, as proposed by Mu & Ludden, to determine the most probable dose and dose-administration time.[27] Such an approach would require the development of an accurate population pharmacokinetic model of salbutamol (and S-SAL) in cyclists, including salbutamol in urine, with samples corrected for osmolality. This approach could help approximate the dose but would require large amounts of samples to be taken to achieve sufficient accuracy and precision. Without such dense data, uncertainty in estimating the dose would remain due to individual pharmacokinetic variability and the unknown factor of time since dosing. Seen how the performance enhancing activity of beta-2 agonists is dubious, especially in endurance sports, the question arises whether it is worth the effort of screening for these compounds.

Limitations

In this paper we reviewed the procedure implemented by the WADA in the control for salbutamol doping and indicated that these are fundamentally flawed. Many of these problems are no different from those encountered in clinical practice and clinical pharmacology, and so the theory and knowledge from these disciplines were applied in this study to the issue of doping control. We developed a population pharmacokinetic model based on literature data to substantiate and quantify that theory, but there are some limitations to the model. No individual data on both plasma and urine concentrations was available for model development, such as that of Haase *et al*.[6] Such data would allow proper estimation of inter-individual variability, leading to better prediction of concentration bandwidths. However, given the good performance of the visual predictive check in Figure 2, there does not seem to be over-prediction of the variability in urine concentration. The results of this visual predictive check also make it unlikely that inter-laboratory or inter-assay differences would impact extrapolation of our model to the WADA doping control laboratory setting: the pharmacokinetic analysis in the study used for the plot was performed by the WADA-accredited doping control laboratory in Norway.[6] Furthermore, variability on physiological parameters could only partially be based on actual data. For example, data on additional variability due to the extreme circumstances during professional cycling is not available and thus could not be accounted for. Two variabilities were fixed to 23% (omega=0.05), which is typically referred to when discussing physiology, and deemed reasonable. In addition, several parameters originally derived from dogs required manual adjustment to properly align with the Haase *et al*[6] data. Furthermore, the salbutamol concentration in the doping control assay used by the WADA is based on the sum of the glucuronide conjugate (expressed as the free drug) and free salbutamol concentrations. For the purpose of this study, we did not explicitly take into account the glucuronide conjugate of salbutamol, as its contribution to the concentration measured in urine is only very limited, with concentrations below the LLOD of 2 ng/mL in 100% of subjects after inhalation of 800 mcg of salbutamol and 70% of subjects after oral administration of 8 mg (and <3% compared to the unconjugated salbutamol for the remaining subjects).[28] However, if there would be an impact, this would only add to the variability in observed urine concentrations. Similarly, this variability might increase if we would incorporate variable urine production (due to increased fluid intake or dehydration) rather than a constant urine production rate that was applied in the model.

Finally, our model is supported by data from a clinical study,[28] reporting that out of 28 subjects (including 8 asthmatic elite athletes) inhaling a single dose of 800 mcg of salbutamol, there was one subject with a urine concentration above the 1000 ng/mL threshold when analysing the urine sample taken 4 hours post-dose (urine collection between 0-4 hours). This means in this study, 3.6% of subjects exceeded the urine concentration threshold with allowed use, which is similar to the observed 3.0% when simulating 1000 subjects with our model in this scenario, supporting the validity of our model.

Our approach therefore shows the feasibility of modelling exercises, including integration of pre-clinical and clinical data, with the possibility of clinical trial simulations / not-in-trial simulations and optimal design. Above all, we show that this approach, originating from traditional clinical pharmacology, can also be applied in this setting of doping control.

CONCLUSION

Pharmacokinetic theory dictates that it is not possible to derive the administered dose with any certainty from a single random urine concentration when there is no information about timing of the dose, urine volume or osmolality and individual physiological variability. Using a pharmacokinetic model based on literature data we substantiated this notion. We demonstrate that the current approach to detect excessive salbutamol use is fundamentally flawed and cannot differentiate between illegal and allowed use and inadvertently leads to incorrect assumptions of violation. If the community is determined to control for excessive salbutamol use, these procedures should be changed. The expertise present in the field of clinical pharmacology is clearly relevant in doping control, and we therefore advocate a closer collaboration between the two disciplines to work towards a sport that is as clean and fair as possible.

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FIGURE 1 DIAGRAM REPRESENTING FINAL MODEL STRUCTURE Arrows represent the flow of drug amounts. Bioavailability for lung and gut absorption after inhalation is represented as percentages. Lung-absorbed amount is directly introduced into the central compartment and consists of 100% parent salbutamol. Of the amount absorbed from the gut, 50% is parent salbutamol and 50% is directly converted to sulphated salbutamol metabolite (first-pass effect).

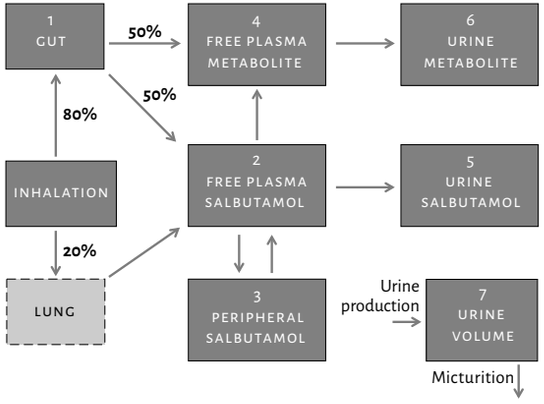

FIGURE 2 VISUAL PREDICTIVE CHECK OF SIMULATED CONCENTRATIONS AFTER AN INHALATION OF 1600 MCG SALBUTAMOL Black solid line: median predicted concentrations. Grey dashed lines: 95% concentration prediction interval. Grey points: observations extracted from Haase *et al.*[6] Left panel: plasma concentrations. Right panel: urine concentrations

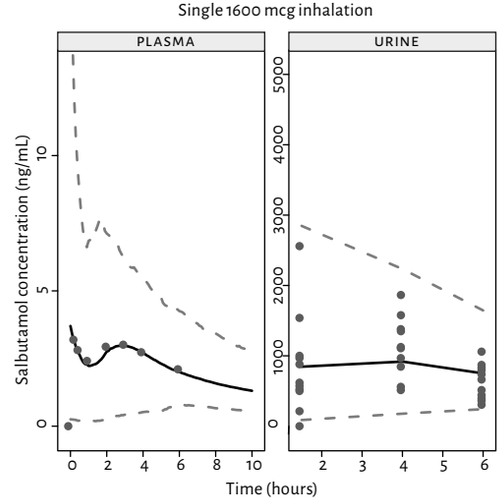

FIGURE 3 SIMULATED URINE CONCENTRATIONS OVER TIME, AFTER ADMINISTRATION OF THE ACCEPTED INHALATION OF 800 MCG SALBUTAMOL BI-DAILY AT STEADY-STATE (*LEFT PANEL*) AND THE RESULTING SPREAD IN MEASURED CONCENTRATIONS WHEN TIME IS NOT TAKEN INTO ACCOUNT IN DOPING CONTROL (*RIGHT PANEL*) Note: the left panel shows the concentrations that would be measured at a certain time after administration when the bladder is voided for the first time since dose administration, with a constant urine production rate. Black solid line: median predicted concentrations. Grey dashed lines: 99.9% concentration prediction interval. Bar plot (right panel): median (black point) and 99.9% prediction interval (bar). Note that the upper limit of this bar is lower than the upper limit peak concentrations in the left panel due to depicting a 99.9% prediction interval of an untimed sample in the right panel.

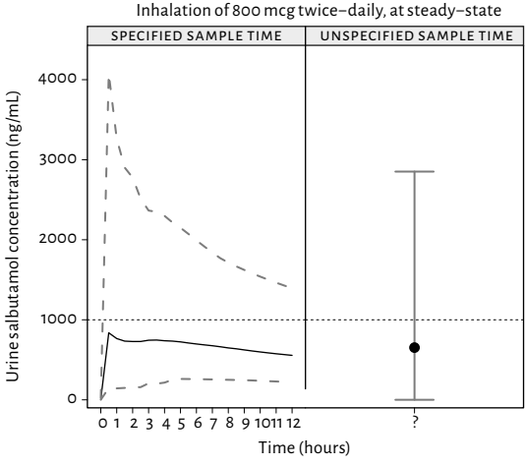

FIGURE 4 SIMULATED URINE SALBUTAMOL CONCENTRATIONS AFTER THE LAST DOSE OF A TWO-WEEK TREATMENT WITH 8 MG ORAL SALBUTAMOL TABLETS, AT REGULAR MICTURITION INTERVALS OF 8 H WITH A CONSTANT URINE PRODUCTION RATE The last dose of steady-state dosing is shown at 0 h, illustrating that levels decline to below WADA levels of 1000 ng/mL (horizontal dashed black line) well within 48 h of the last dose taken for 99.9% of the simulated study subjects. Black solid line: median predicted concentrations. Grey dashed lines: 99.9% concentration prediction interval.

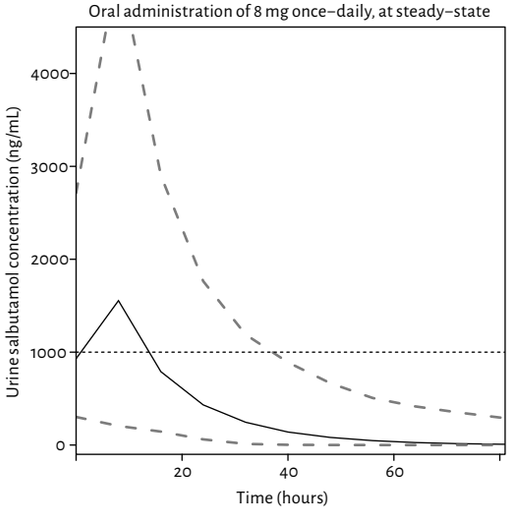

TABLE 1 MODEL PARAMETERS USED IN THE SIMULATIONS, WITH THEIR SOURCE

Parameter	Typical value	cv%	Source
Cardiac output (x weight$^{0.79}$) (L/min)	0.166	23%[b]	Holt[10]
Haematocrit	0.409	7%	Mørkeberg[16]
Bio-availability Gut (%)	80	23%[b]	Auclair[7]
Absorption constant gut (h^{-1})	0.5[a]	57%	Auclair[7]
Absorption lag gut (h)	1.5	83%	Auclair[7]
Renal clearance salbutamol (L/h)	17.5	25%	Morgan[5]
Renal clearance S-SAL (L/h)	5.91	25%	Morgan[5]
Central volume salbutamol (L/kg)	1.12[a]	63%	Auclair[7]
Peripheral volume salbutamol (L/kg)	1.92[a]	50%	Auclair[7]
Intercompartmental clearance (L/h)	0.56[a]	37%	Auclair[7]
Proportional error salbutamol (%)	23		
Proportional error S-SAL (%)	23		

a. Adjusted to better correspond to data from Haase et al.[6]

b. Fixed to 23% (omega = 0.05 in NONMEM*) due to limited data.*