Systematic analysis to assess the scientific validity of the international residue limits for caffeine and theophylline in horse-racing

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Abstract

Based on their performance-enhancing potential, caffeine and theophylline are prohibited substances in equine sports. Residues in horses can be caused by wilful application or by unintended uptake of contaminated feed. The International Federation of Horseracing Authorities recently introduced international residue limits (IRLs) to facilitate the discrimination between pharmacological relevant and irrelevant concentrations in doping samples. The objective of this study was to investigate the scientific validity of these IRLs. A systematic analysis was performed to assess the IRLs by different statistical approaches using published pharmacokinetic data. 31 out of 218 potentially relevant publications met the inclusion criteria. Thereby, both IRLs were found to be appropriate for the exclusion of the presence of a relevant pharmacological effect after a wilful application. The IRL of theophylline was also determined to be suitable for the prevention of positive doping tests caused by the ingestion of contaminated feed. In contrast, the IRL of caffeine is not suitable to prevent positive doping test caused by the ingestion of more than 10 mg caffeine per day per horse with contaminated feed. The lack of corresponding regulation for paraxanthine, a major active metabolite of caffeine and theophylline, was recognised as a substantial shortcoming of the current system, rendering both IRLs incomplete.

Introduction

The methylxanthines caffeine and theophylline are considered as substances relevant for doping in horses by major horse sports authorities such as the International Federation for Equestrian Sports (FEI) or the International Federation of Horseracing Authorities (IFHA). Both substances exert a variety of pharmacological effects, such as stimulation of spontaneous locomotion[1] and an increase of the maximum oxygen uptake by bronchodilation.[2] Recently, caffeine and theophylline were detected in several antidoping tests performed by the FEI.[3] Notably, caffeine and theophylline residues in blood or urine can be related to intentional administration or to inadvertent intake as contaminants of feed. Because of the widespread occurrence of methylxanthines in feedstuffs and their dose-dependent effects, a zero tolerance policy is not appropriate for the control of caffeine and theophylline in horse-racing.[4]

Since the 1990s, various threshold levels for caffeine in antidoping samples have been introduced in local jurisdictions.[5] In 2014, the IFHA introduced international residue limits (IRLs) for caffeine, theophylline and other substances in urine.[6] Concentrations in antidoping tests below the IRL are not against antidoping rules, as they do not cause a pharmacological effect relevant for the integrity of horse-racing and could have been caused by the ingestion of contaminated feed. Concentrations above the IRL still constitute a violation of the antidoping rules. The scientific reasoning for the determination of these IRLs has not been published by the IFHA until today.

Therefore, the aim of the present study was to answer the following questions for the IRLs of caffeine

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and theophylline: (1) Does the respective IRL prevent the presence of a pharmacological effect relevant for the integrity of horse-racing after systemic application of the regulated substance? (2) Is the respective IRL suitable to prevent positive doping tests after the ingestion of feed contaminated with unavoidable or legally permissive concentrations of the regulated substance?

Materials and methods

Literature search

Different approaches have been published to determine the thresholds for substances relevant for doping in horses. Lakhani *et al*[7] and Ho *et al*[8] proposed methods based on statistical analysis of population data for substances that are natural components of horse feed, such as salicylic acid and cobalt. Substances that occur in horse feed as contaminants and that can be used as drugs to influence equine performance can be regulated by using the approaches of (1) Toutain and Lassourd,[9] (2) Haywood *et al*[10] and (3) Tobin *et al*.[5] These three methods were used in the present study. The approach of Toutain and Lassourd[9] allows calculation of an effective plasma concentration (EPC) based on a known dose, the dosage interval and published pharmacokinetic data. The dose selected for the calculation should equal the generally recommended dose and dosage interval (eg, the approved dose), or it should represent the lower end of the reported dosage range for the investigated active substance. This dose is then divided by the total plasma clearance of the selected dosage interval. The resulting EPC can now be used to determine the irrelevant plasma concentration (IPC) as a fraction of the EPC by multiplying the EPC with a safety factor like 1/500. The calculated IPC can then be used to calculate an irrelevant urinary concentration (IUC) by multiplying the IPC with the urine–plasma concentration ratio in the steady state or pseudo-steady state (Rss). In a final step, the appropriateness of the calculated IPC and IUC can be checked by calculating the residual amount of the original dose in the body at the moment when the plasma concentration declines to the IPC or by calculating the time required for the concentration of the drug to decline below the IPC. For these calculations, data on the volume of distribution and the terminal half-life are required. The approach of Haywood *et al*[10] measures the concentration of feed contaminants and their metabolites in the plasma and/or urine of horses after the feeding of feedstuff contaminated with small, naturally occurring concentrations of these substances. This allows assessment of the expected levels of these substances after the ingestion of such feed in horses and has been used to determine the internationally recognised urinary threshold for theobromine in equine sports.[4] The procedure proposed by Tobin *et al*[5] experimentally determines the highest dose not able to cause a predefined pharmacological effect and measures the concurrent plasma and urine concentrations of the investigated substance and its metabolites.

Table 1 Search terms used for the literature research

Caffeine	Theophylline
Caffeine horse	Theophylline horse
Caffeine horse urine	Theophylline horse doping
Caffeine horse serum	Theophylline horse urine
Caffeine horse pharmacokinetics	Theophylline horse plasma
Caffeine horse intoxication	Theophylline feed horse
Caffeine horse feed	Theophylline intoxication horse
Caffeine horse doping	Theophylline horse pharmacokinetics
Koffein pferd	Theophyllin pferd

For the assessment of the IRLs of caffeine and theophylline with one or more of the approaches described above, a comprehensive search of the literature to identify studies with relevant information was done in accordance with the principles for systematic meta-analysis stated in the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) statement.[11] Potentially useful studies were identified using the scientific databases PubMed, Web of Science and Centre for Agriculture and Bioscience International (CABI). In addition, the databases of the German-speaking veterinary universities and faculties were searched, and the references given in the review by Budhraja *et al*[4] were considered. Studies published in English or German language and published before July 2018 were searched using the search terms given in table 1.

After the initial collection of studies potentially relevant for this work, all studies were prescreened for their usability in this analysis. Then, all studies not excluded were examined in detail using the following predefined inclusion criteria:

Only studies performed in horses of at least two years of age were considered.

A study was included in the final analysis if it matched the criteria for at least one of the methods given below:

1. For the assessment of IRLs after the method of Toutain and Lassourd[9]: a defined dose of caffeine or theophylline was administered, the route of administration was given, at least one of the pharmacokinetic parameters plasma clearance, volume of distribution, terminal half-life or urine–plasma concentration ratio, either as a distinct value or in the form of plasma and urine concentration values during steady state or pseudo-steady state, were indicated and the pharmacokinetic parameters were not recorded during anaesthesia.
2. For the assessment of IRLs after the method of Haywood *et al*[10]: a defined dose of caffeine or theophylline was administered via feed or via direct oral application, the administered dose corresponds to a dose that could be ingested via contaminated feed and is not obviously intended to influence the performance of a horse, and the highest concentration of the administered substance and/or its metabolites in plasma and/or urine was indicated.
3. For the assessment of IRLs after the method of Tobin *et al*[5]: the investigated critical pharmacological effect was defined, the highest no-effect dose (HNED) was determined, and the

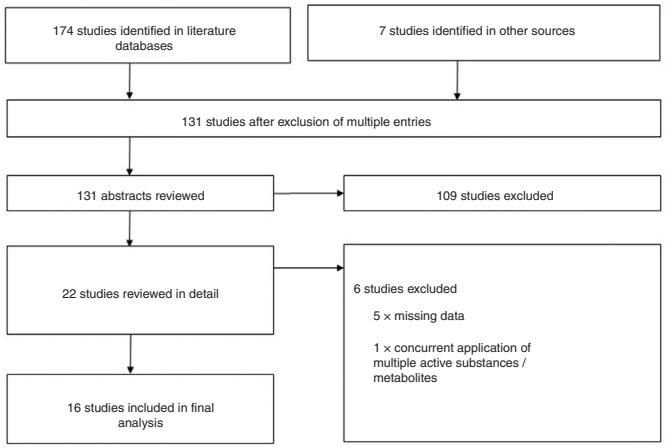

Figure 1 PRISMA flow chart study identification and selection process for caffeine. PRISMA, Preferred Reporting Items for Systematic Reviews and Meta-Analyses.

maximum plasma and/or urine concentrations achieved by the HNED were indicated.

A graphic explanation of the selection process is given in figures 1 and 2, which were designed after the flow chart proposed in the PRISMA statement.[11]

Data collection

Studies included in the final analysis were scanned for relevant data. All parameters of interest were recorded in tables with standardised unions for further analysis. Multiple dosages or modes of application performed in one study were recorded as separate data series. Graphic data, especially about time-concentration curves for urine–plasma concentration ratios, were extracted and transformed into numerical data using the tool WebPlotDigitizer.[12] Since Aramaki *et al*[13] did not report the values for clearance, bioavailability and terminal half-life, but reported data sufficient for the calculation of these parameters, namely the volume of distribution (Vd) and the elimination rate constant (k_e) during pseudo-steady-state conditions and the area under the concentration time curve (AUC), the values for these three parameters were calculated by the known equations: clearance=volume of distribution (Vd) x elimination rate constant (k_e); absolute bioavailability (F)=AUC (orally) / AUC (intravenously); terminal half-life=ln (2) / k_e. The calculated data points are shown in table 2.

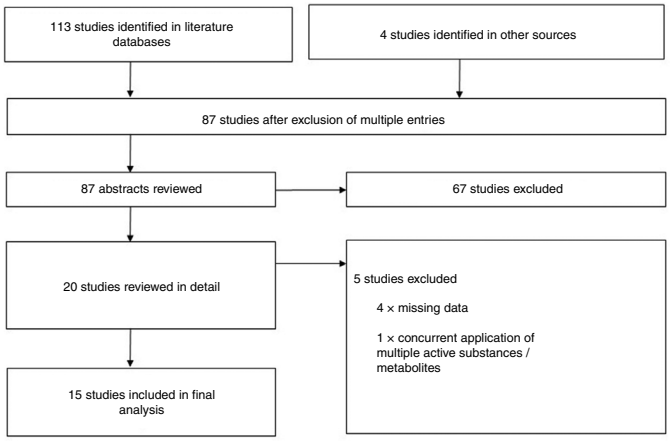

Figure 2 Flow chart study identification and selection process for theophylline.

Statistics

The recorded values for the pharmacokinetic parameters clearance, Vd, half-life and urine–plasma concentration ratio were processed statistically to facilitate a further analysis of the investigated IRLs by the method of Toutain and Lassourd.[9] As outlined by Craigmill *et al*,[14] the calculation of the mean and the respective sd of these parameters is useful and valid for further pharmacokinetic calculations. In a first step, the Grubbs' test for outliers was performed using GraphPad software (http://graphpad.com/quickcalcs/grubbs1), and identified outliers were excluded. Next the data were tested for normality using the Shapiro-Wilk test (SigmaPlot V.11, Systat Software). Data with a P value >0.05 were considered normally distributed. A one-sample *t* test was performed to calculate the mean, the sd and the 95 per cent confidence interval of the mean. Data not normally distributed, as it was the case for the Vd of caffeine, were logarithmically transformed and retested for normality. The mean and the 95 per cent confidence interval for these data were calculated using the one-sample *t* test. The calculated values were retransformed to obtain data with correct units as described by Lacey *et al*.[15] According to Jørgensen and Pedersen,[16] the values of the transformed standard were not retransformed, as this could be misleading. Data for the urine–plasma concentration ratio given as graphic progression data were entered in MS Excel sheets, and the median urine–plasma concentration ratio in the steady or pseudo-steady-state phase was calculated.

Data on the relationship between the ingested dose and the highest corresponding urinary concentration were collected from studies performed according to the method of Haywood *et al*.[10] The data points were entered into a coordinate system and were visually inspected for possible linear correlations. If such a correlation appeared to be possible, a simple linear regression analysis was performed using SigmaPlot V.11 (Systat Software). The total dose per animal and day was considered to be the independent variable, and the highest corresponding urinary concentration was considered to be the dependent variable. To increase the statistical validity of the data, the value x, y=0.0 was added to the data series because both compounds have no endogenous origin.

Results

Literature search

The literature search yielded 131 unique studies of interest for caffeine and 87 for theophylline. After a thorough review of the identified publications, 16 studies matched the inclusion criteria for caffeine and 15 studies for theophylline.

Table 2 Recorded pharmacokinetic data for caffeine

Study	n	D	$Vd_{(p)ss}$	Cl	F	t ½	U/P
Vickroy *et al*[24]	6	3 mg/kg intravenously	760	24	–	11.2	–
Greene *et al*[45]	4	4 mg/kg intravenously	650	24	–	18.26	3
	4	4 mg/kg orally	–	–	0.39	42*	3
Chou *et al*[46]	2	3 mg/kg intravenously	820	39	–	15.3	–
	2	3 mg/kg intravenously	780	37.8	–	14.4	–
Peck *et al*[47]	4	2.5 mg/kg intravenously	1318	51	–	–	–
Aramaki *et al*[13]	4	2.5 mg/kg intravenously	852	42.5†	–	13.88†	–
	4	2.5 mg/kg intramuscularly	834		–		–
	4	2.5 mg/kg orally	809		1.12†		–
Schumacher *et al*[48]	10	500 mg/horse (0.8–1.8 mg/kg) intravenously	694	42.7	–	10.18	–
Aramaki *et al*[49]	4	2.5 mg/kg intravenously	887	40.4	–	15.5	–
	5	2.5 mg/kg intramuscularly	965	36.6	–	18.6	–
	4	2.5 mg/kg orally	881	39	1.04	16.4	–
Todi *et al*[50]	3	2 g/ horse orally	–	–	–	–	3.16†
Kaiser[51]	6	4 mg/kg intravenously	1209	71.6	–	16.1	–
	6	4 mg/kg orally	–	128.2*	0.56	–	–
Machnik *et al*[21]	6	4 mg/kg intravenously	1345	55	–	17.2	2

*Significant outlier.
†Calculated value.
Cl, clearance in ml/hour x kg; D, dose; F, bioavailability;n, number of animals.t ½, terminal half-life in hours; U/P, urine–plasma concentration ratio in the steady-state or pseudo-steady-state; $Vd_{(p)ss}$, volume of distribution in ml/kg in the (pseudo)-steady state;

Caffeine

Table 2 shows the pharmacokinetic values as described in 10 studies which were suitable for the application of the method of Toutain and Lassourd.[9] The calculated means of the pharmacokinetic parameters of these 10 studies (table 3) were used to calculate the EPC, IPC and IUC of caffeine. The dose chosen for this calculation was 2.5 mg/kg intravenously with a dosage interval of 24 hours. This dosage regimen was chosen because it represents the lowest effective dose of caffeine in horses.[1] The urine–plasma concentration ratio required for the calculation of the IUC was assumed to be 2.79, which is the arithmetic mean of the urine–plasma concentration ratios given in the evaluated studies. The generic safety factor proposed by Toutain and Lassourd[9] of 1/500 was used to calculate the IPC from the EPC. The calculated EPC, IPC and IUC for caffeine are given in table 4.

For the analysis by the method of Haywood *et al*,[10] four studies could be used. The data extracted from these studies are given in table 5. The number of identified data points facilitated a linear regression analysis of the relationship between the ingested daily dose of caffeine and the highest corresponding urinary caffeine concentration, which is shown in figure 3. Since caffeine can only appear in the urine of horses after an intake from an external source and in order to achieve a higher statistical power of the regression analysis, the data point 0.0 was added to the data from table 5. The regression line is described by the equation y=3.1131 + (4.7341 x caffeine dose in mg) and the correlation coefficient R^2=0.9616.

For the assessment of the IRL according to the method of Tobin *et al*,[5] only two studies could be identified. The extracted pharmacokinetic data are displayed in table 6.

Table 3 Statistical parameters of pharmacokinetic data for caffeine

Parameter	Mean	sd	95% confidence interval
Terminal half-life (hours)	15.18	±2.66	13.40 to 16.97
Volume of distribution (ml/kg)	893.3	Not calculated	785.2 to 1016.2
Clearance (ml/hours x kg)	41.97	±12.94	33.74 to 50.19

Theophylline

The literature analysis yielded 13 studies that met the inclusion criteria for further analysis by the method of Toutain and Lassourd.[9] The pharmacokinetic data found in these studies are summarised in table 7. The statistical parameters of the pharmacokinetic values needed for further calculations were computed based on these data and are shown in table 8. For the calculation of the EPC, IPC and IUC for theophylline, shown in table 9, a dose of 5 mg/kg orally with a dosage interval of 12 hours was chosen. This dosage regimen equals a dose frequently recommended in the literature for bronchodilation in adult horses.[17 18] A generic safety factor of 1/500 was used to calculate the IPC from the EPC. The urine–plasma ratio used to calculate the IUC was 13.3, which represents the arithmetic mean of the urine–plasma concentration ratios given in table 7.

For the examination of the IRL of theophylline with the method of Haywood *et al*,[10] only two studies were

Table 4 Calculated EPC, IPC and IUC for caffeine

Parameter	EPC	IPC	IUC
Value	2.48 µg/ml	5 ng/ml	14 ng/ml

EPC, effective plasma concentration; IPC, irrelevant plasma concentration; IUC, irrelevant urinary concentration.

Table 5 Data extracted from studies using the method of Haywood *et al*[10] for caffeine

Study	n	Daily dose (mg/horse)	Application scheme	Cmax plasma (ng/ml)	Cmax urine (ng/ml)
Dyke and Sams[52]	3	7.3	Once a day for eight days	–	50
Bonnaire *et al*[36]	2	10	Once a day for two days	–	50
Respondek *et al*[35]	5	5	2.5 mg twice a day for three days	–	30
	5	15	7.5 mg twice a day for three days	–	70
Ohtake *et al*[25]	4	12.5	Once	27	52
	4	25	Once	65	131

cmax, maximum concentration; n, number of horses.

available. The extracted data from these are given in table 10. The small number of recorded data points did not enable a statistical analysis. No study met the inclusion criteria for an assessment of the IRL according to the method of Tobin *et al*.[5] Thus, this method could not be applied for theophylline.

Discussion

To the authors' knowledge, this is the first study that investigates the scientific validity of the IRLs for caffeine and theophylline published by the IFHA by applying more than one method to determine quantitative thresholds for the prevention of the misuse of substances in equine sports. Considering the dual characteristics of the caffeine and theophylline substances as drugs and feed contaminants, an approach that assesses both characteristics to create the scientific basis for IRLs is important.

Methods to determine the source of methylxanthines present in positive antidoping samples would be helpful in order to answer the question if these substances were administered as pharmaceuticals or if they originated from feed. Flenker and Schänzer[19] proposed a method for the discrimination of the origin of caffeine in human beings, which is based on the different $^{12}C/^{13}C$ isotope profile of synthetic caffeine and caffeine produced by plants. This allows an unequivocal statement about the origin of the detected caffeine. However, not only pharmaceuticals with synthetic caffeine but also natural products containing caffeine and other methylxanthines, such as guarana powder,[20] can be used to enhance the performance of horses. Therefore, even the knowledge about the origin of caffeine or other methylxanthines in a positive antidoping sample limits the discrimination between doping and accidental uptake of methylxanthines via contaminated feed.

Figure 3: Linear regression analysis of the dose – urine concentration relation for caffeine

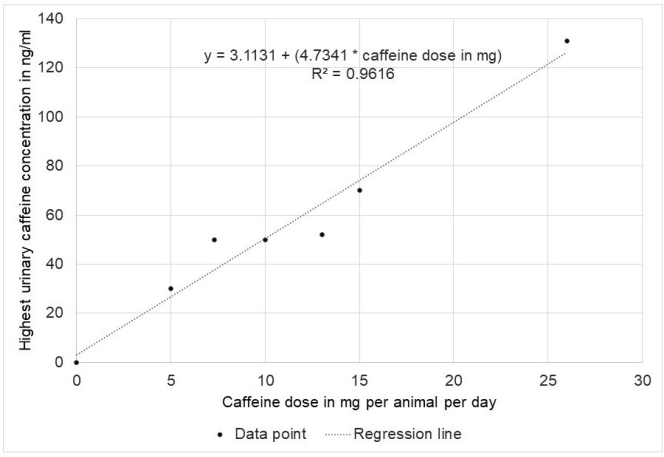

Figure 3 Linear regression analysis of the dose–urine concentration relation for caffeine.

The IRL for caffeine of 50 ng/ml urine is in accordance with the IUC of 14 ng/ml calculated in this study. This IUC is almost equal to the IUC of 12 ng/ml calculated by Machnik *et al*.[21] These concentrations are much lower than those expected after the administration of the HNED (2–2.5 mg/kg) in horses.[1 22] As shown by Fredholm,[23] adenosine receptors in the brain are the main pharmacological targets of caffeine within the therapeutic concentration range in human beings and animals. In vitro studies show that the minimal inhibitory concentration of caffeine at these receptors is about 1 µM (194 ng/ml). As reported by Vickroy *et al*,[24] the concentration of caffeine in the blood and the cerebrospinal fluid highly correlates in horses and shows a concentration ratio of 0.87. In addition, they report a strong correlation between the effect of caffeine on the motor activity of horses and the concentration of caffeine in the cerebrospinal fluid, which can be used as a reasonable indicator of the actual concentration of caffeine in the brain. Based on these facts, it is fair to assume that the concentration of caffeine in the brain of a horse with a caffeine concentration in the plasma at or below the IPC of 5 ng/ml is well below the minimal inhibitory concentration of this substance at its main pharmacological target. Therefore, the presence of a systemic pharmacodynamic effect relevant for doping can almost certainly be excluded at concentrations in the order of magnitude of the IRL.

Studies conducted in accordance with the method of Haywood *et al*[10] demonstrate that a daily oral intake of about 10 mg caffeine per horse is sufficient to cause maximum caffeine concentrations in urine that exceed the IRL of 50 ng/ml. Caffeine is no component of plants and grains used for the production of typical equine feedstuffs.[25] Important sources of feed contamination with caffeine are by-products of the chocolate production, such as cocoa husks, cocoa pods and cocoa powder. These products can be used as ingredients in ruminant feed[26] and can enter equine feed via cross-contamination during the production process. The concentration of caffeine in these products varies widely and can reach levels of up to 9.9 mg/g for cocoa powder[27] and of up to 5.6 mg/g in cocoa husks from mature cocoa beans.[28] Based on these data, a dose of 10 mg per horse per day can be ingested by feed that contains 1 g of cocoa powder or 2 g of cocoa husks. This amount equals a contamination rate of 0.01 per

Table 6 Data extracted from studies using the method of Tobin *et al*[5] for caffeine

Study	n	Investigated effects	Highest dose without effect	Corresponding $C_{plasma/serum}$ (µg/ml)	Corresponding C_{urine} (µg/ml)
Queiroz-Neto *et al*[1]	10	Spontaneous locomotion	2 mg/kg intravenously once	2.3	4.87
Savage *et al*[22]	10	Heart frequency, oxygen consumption, oxygen pulse, carbon dioxide production, time to fatigue	2.5 mg/kg intravenously once	3.3	5.8

C, concentration; n, number of animals.

cent in a standard diet of 10 kg dry matter per day for a horse with a bodyweight of 500 kg. Therefore, feed slightly contaminated with these materials may cause a violation of the IRL. Considering these facts, the IRL for caffeine is too low to prevent positive doping tests after the ingestion of minimally contaminated feed. Today, the feed industry generally observes high safety standards in the production of equine feedstuffs. As outlined in the IFSA Feed Ingredient Standard of the International Feed Safety Alliance (IFSA),[29] an association of major feed producers in Europe, sophisticated principles like hazard analysis and critical control points concepts have been implemented for the prevention of feed contamination. Therefore, the frequency of feed contamination with the discussed substances, especially in compound feed, is probably very low, even though no systematic studies about this topic are available to the authors' knowledge. Nevertheless, several recent cases from Germany,[30] Columbia[31] and the United Arab Emirates[32] show that feed contamination with caffeine still occurs despite these efforts. In the authors' view, this underscores the case for an adaption of the current IRL.

The IUC of 226 ng/ml calculated for theophylline by the method of Toutain and Lassourd[9] is in good agreement with the IRL of 250 ng/ml for this substance. The EPC of 8.55 µg/ml calculated on the basis of the present study's data corresponds well with the plasma concentration effective for bronchodilation in ponies of 10.6 µg/ml.[33] Toxic effects of theophylline have been reported at plasma concentrations of 15 µg/ml.[34] Based on these data, it can be assumed that the EPC calculated in the present study is close to the concentration that produces half of the maximal pharmacodynamic effect (EC_{50}) of theophylline in horses. The calculated IPC of 17 ng/ml represents 1/500 of the EPC. The residual dose of theophylline at a plasma concentration equal to the calculated IPC is about 16.8 µg/kg bodyweight or 0.34 per cent of the dose assumed for the present study's calculations.

Data for the direct evaluation of the IRL of theophylline using the method of Tobin *et al*[5] were not available. Nevertheless, conclusions for theophylline as a major metabolite of caffeine can be derived from relevant studies performed for caffeine. Vickroy *et al*[24] administered 3 mg/kg of caffeine intravenously and

Table 7 Recorded pharmacokinetic data for theophylline

Study	n	D	$Vd_{(p)ss}$	Cl	F	t ½	U/P
Koppe[53]	6	4 mg/kg intravenously	1108	58.4	–	13.6	11.1†
	6	4 mg/kg orally	–	70.5	0.85	–	10.7†
Todi *et al*[50]	2	1.5 g intravenously	–	–	–	–	18.8†
	2	2 g orally once daily for five days	–	–	–	–	10.5†
Perez *et al*[54]	4	3.5 mg/kg intravenously	1100	54	–	14.4	–
Errecalde and Landoni[55]	6	10 mg/kg intravenously	1350	61	–	16.91	–
Stevenson *et al*[56]	4	1.5 g/horse intravenously	–	–	–	–	16.8‡
Goetz *et al*[57]	6	9.94 mg/kg intravenously	787	56.2	–	9.67	–
Ingvast-Larsson *et al*[58]	8	5 mg/kg orally twice daily for 4.5 days	852	36.6	0.87	17.0	–
Short *et al*[59]	6	1 mg/kg intravenously	703	51	–	9.89	–
Ayres *et al*[60]	6	10 mg/kg intravenously	1020	54.6	–	12.4	–
Ingvast-Larsson *et al*[17]	6	3 mg/kg intravenously	1020	51.6	–	14.8	–
	6	3 mg/kg orally	1000	46.8	1.08	15.3	–
	7	6 mg/kg orally	1000	38.4	–	18.7	–
Errecalde *et al*[61]	6	15 mg/kg intravenously	853	40.2	–	15.22	–
	6	15 mg/kg orally	–	39.2	1.08	15.13	–
	3	10 mg/kg intravenously	897	36.1	–	17.2	–
	3	10 mg/kg orally	–	30.2	0.91	20.97	–
Kowalczyk *et al*[62]	6	9.44 mg/kg intravenously	885	51.7	–	11.9	–
Machnik *et al*[21]	6	4 mg/kg intravenously	1268	52.0	–	17.2	12

*Calculated value.
†Graphically determined value.
Cl, clearance in ml/hour x kg; D, dose; F, bioavailability;n, number of animals.t ½, terminal half-life in hours; U/P, urine–plasma concentration ratio in the steady-state or pseudo-steady-state; $Vd_{(p)ss}$, volume of distribution in ml/kg in the (pseudo)-steady state.

Table 8 Statistical parameters of pharmacokinetic data for theophylline

	Mean	sd	95% confidence interval
Terminal half-life (hours)	15.02	±3.06	13.39 to 16.65
Volume of distribution (ml/kg)	988.8	±178.9	885.5 to 1092.1
Clearance (ml/hours x kg)	48.74	±10.59	43.29 to 54.18

detected theophylline concentrations of up to 300 ng/ml plasma four hours after injection. Regardless of a concentration-dependent elimination, a linear extrapolation of these data estimates a maximal plasma concentration of 200 ng/ml four hours after the application of 2 mg/kg caffeine intravenously, which did not exert a significant effect on spontaneous locomotion in horses during eight hours after administration.[1] Therefore, a theophylline concentration of up to 200 ng/ml plasma is probably ineffective in horses. The IRL of theophylline is appropriate for the exclusion of the presence of a relevant pharmacological effect after a wilful systemic application.

Only two studies using the method of Haywood *et al*[10] were available for the evaluation of the IRL of theophylline. The daily oral administration of 6 mg/horse for three days[35] as well as of 15 mg/horse for two days[36] caused respective theophylline concentrations of 300 ng/ml urine or of 502 ng/ml, exceeding the IRL. In contrast, after administration of 2 mg/horse, the detected concentrations were below the limit of detection of 75 ng/ml.[35] Notably, theophylline occurs only in very low concentrations as contaminant in equine feed.[37] Even guarana seed powder with the highest naturally occurring concentration of theophylline contains only about 1.1 mg theophylline per gram of dry matter.[38] It does not constitute a typical source of contamination of equine feed. At least 6 g of guarana powder would have to be consumed by a horse daily for several days before exceeding the IRL. This amount could not be considered as an unavoidable or low level of contamination. All other sources of contamination of feed with methylxanthines possess lower concentrations of theophylline. Therefore, the level of contamination would have to exceed 0.1 per cent of the total dry matter of feed, which is about 10 times the level of contamination required for a violation of the IRL of caffeine. Based on these considerations, it can be concluded that the IRL of theophylline is suitable to prevent positive doping tests after the ingestion of contaminated feed.

Table 9 Calculated EPC, IPC and IUC for theophylline

Parameter	EPC	IPC	IUC
Value	8.55 µg/ml	17 ng/ml	226 ng/ml

EPC, effective plasma concentration; IPC, irrelevant plasma concentration; IUC, irrelevant urinary concentration

Paraxanthine is a major metabolite of both caffeine and theophylline and can be present in the urine of horses at higher concentrations than the original substances.[21] It exhibits stimulatory effects similar or stronger than caffeine in laboratory animals[39] and human beings,[40] and is therefore classified as a prohibited substance in horse-racing and equine sports. Currently, no IRL or analytic cut-off has been published for paraxanthine. Thus, the regulation of the occurrence of methylxanthines in the urine of racehorses may be considered as incomplete.

In addition to the IRLs for caffeine, theophylline and the long-established international threshold for theobromine, the IFHA published IRLs for atropine, bufotenine, dimethyltryptamine (DMT), hordenine, morphine and scopolamine.[6] As shown for atropine and scopolamine in the authors' previous paper,[41] the database for a sound assessment of these IRLs is incomplete, and the IRL concept seems to be of limited use for the control of substances that can be applied locally for illicit purposes without causing a corresponding detectable concentration in plasma or urine. While some pharmacokinetic data for the assessment of the IRLs of morphine[42 43] and hordenine[44] exist, the authors are not aware of comparable data for DMT and bufotenine, although some data about their excretion in urine after ingestion have been published.[35] In summary, the following approaches should be considered to address the shortcomings of the current IRL system. The IRL of caffeine, which the authors assessed as being too low to prevent positive antidoping samples after the ingestion of minimally contaminated feed, should be raised to the order of magnitude of 300 ng/ml urine, as previously proposed by Budhraja *et al*.[4] Based on the discussion above, this limit would still exclude the presence of any relevant pharmacodynamic effect of caffeine on the performance of horses with a reasonable safety margin. In addition, the occurrence of metabolic paraxanthine should be taken into account. This could be achieved by establishing a limit for the sum of the concentrations of caffeine, theophylline and paraxanthine occurring concurrently in the urine. Such an approach would account for the natural variability

Table 10 Data extracted from studies using the method of Haywood *et al*[10] for theophylline

Study	n	Daily dose (mg/horse)	Application scheme	Cmax plasma	Cmax urine
Respondek *et al*[35]	5	2 orally	1 mg twice a day for three days	–	<75 ng/ml
	5	6 orally	3 mg twice a day for three days	–	300 ng/ml
Bonnaire *et al*[36]	2	15 orally	Once daily for two days	–	502 ng/ml

Cmax, maximum concentration; n, number of horses.

of these substances in the origins of feed contamination and the individual differences in the proportion of the different metabolites occurring in the urine of horses as well as their additive pharmacodynamics and doping relevant effects.

Conclusion

The IRLs published by the IFHA for caffeine and theophylline are suitable to prevent relevant pharmacodynamic effects of these substances in racehorses. The IRL for theophylline is appropriate to prevent positive doping tests after the ingestion of minimally contaminated feed, whereas the IRL for caffeine is too low for this purpose. The absence of a corresponding regulation for the metabolite paraxanthine is a major shortcoming of the current system. This could be addressed by developing an aggregated IRL for the three methylxanthines caffeine, theophylline and paraxanthine.

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