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PHARMACOKINETIC/PHARMACODYNAMIC ASSESSMENT OF IRRELEVANT DRUG CONCENTRATIONS IN HORSE PLASMA OR URINE FOR A SELECTION OF DRUGS

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ABSTRACT

The lower limits of detection of the analytical techniques currently used for drug testing in horses result in the dilemma of whether or not to report trace levels of drugs legitimately used for therapeutic medication. A non-experimental pharmacokinetic/pharmacodynamic approach for the determination of irrelevant drug plasma concentrations (IPC) and irrelevant urine concentrations (IUC) has been put forward by Toutain and Lassourd (2002). The published plasma clearance is used to transform an effective dose into an effective concentration (EPC). EPC is then transformed into an IPC by applying a safety factor (SF). This method is based on several assumptions (eg drug effects reversibly driven by plasma concentration, linearity of drug disposition). To explore the feasibility and attractiveness of this approach, this article reports EPC, IPC and IUC for a selection of drugs.

INTRODUCTION

A doping control policy can be motivated by 2 forms of logic: checking for drug exposure or checking for the absence of any relevant effect. In the latter, concentration becomes a surrogate for effect. If the purpose is to control the possible use of a prohibited drug, any analytes (drug, active or inactive metabolites, etc) can be used for monitoring and the highest performance analytical technique should be used. On the other hand, using analytical techniques with a reduced limit of detection (LOD) can lead to the detection of very low concentrations of legitimate drugs used for therapeutic horse medication, leading the regulatory authorities to decide whether or not concentrations near the limit of detection should be ignored (ie considered as biologically irrelevant and of no regulatory concern; see Houghton 1995; Tobin *et al.* 1999, 2000, for reviews). To assist the authorities in their decision, Toutain and Lassourd (2002) recently proposed a general method to predict irrelevant drug plasma concentration (IPC) and irrelevant urine concentrations (IUC). Briefly, this non-experimental method consists of retrieving published pharmacokinetic (PK) parameters to calculate IPC and IUC. The method can be applied to any drugs with a systemic effect and for which plasma concentration is the driving force, directly and reversibly controlling effects in question. In contrast, this approach is not recommended for locally administered drugs or drugs with an effect not directly linked to plasma concentration, as drugs which result in a cascade of biological events make the relationship between plasma concentration and the ultimate effects of interest too hard to distinguish (eg some steroids).

The objective of this report is to review the approach and to provide a first series of IPC and IUC for a selection of drugs in order to trigger a discussion on its feasibility, value and limits.

THE PK/PD FRAMEWORK OF THE METHOD

The most important relationship in PK is the one which links an effective dose to an effective plasma concentration (Equation 1).

$$\textit{Effective dose rate} = \frac{\textit{Plasma clearance} \times \textit{effective plasma concentration}}{\textit{Bioavailability}}$$

Equation 1 shows that an effective dose rate is a PK/PD hybrid variable determined by 2 PK parameters (plasma clearance and bioavailability) and one pharmacodynamic (PD) parameter, ie the effective plasma concentration. Plasma clearance is the genuine PK parameter that expresses the ability to eliminate a drug. Bioavailability (from 0 to 1) expresses the extent of systemic availability for a given formulation and/or a particular type or route of administration. In the present approach, only iv

routes are considered and bioavailability is fixed at 1. Effective plasma concentration is the average plasma concentration over the dosing interval following administration of an effective dose.

Plasma clearance and effective dose rate are known for many equine drugs and the proposed method simply consists of calculating the EPC from published clearances and recommended effective dose rates.

Calculation of Irrelevant plasma concentration (IPC) and irrelevant urine concentration (IUC)

The IPC and IUC are defined as drug plasma (serum) or urine concentrations that guarantee the absence of any relevant drug effect. IPC calculation first requires calculation of an effective plasma concentration (EPC).

For any drug, calculation of the average EPC over the dosing interval used in a standard dosage regimen can be obtained by rearranging Equation 1 into Equation 2:

$$EPC = \frac{\textit{Standard dose (per dosing interval)}}{\textit{Plasma clearance (per dosing interval)}}$$

In Equation 2, the standard dose is the generally recommended effective dose by iv injection.

In a second step the IPC can be deduced from the EPC by applying a safety factor (SF) to EPC (Equation 3):

$$IPC = EPC/SF$$

The selection of an SF is mainly a regulatory decision. A default value of 500 has been offered.

Subsequently, the IUC can be derived from the IPC using Equation 4:

$$IUC = IPC \times Rss$$

In Equation 4, Rss is the steady-state urine to plasma concentration ratio.

The relevance of the IPC can be checked by calculating the residual amount (RA) of a drug in the body when the plasma concentration is equal to IPC; it is given by Equation 5:

$$RA = IPC \times Varea$$

where Varea is the volume of distribution calculated by the area method. This RA can then be compared to the recommended dosage regimen and should be lower than a given percentage of the recommended dose (eg 1%).

In addition, the suitability of the IPC can be checked by calculating the shortest possible withdrawal time (WT) for the drug using Equation 6:

$$WT = \left(1.44 \times \textit{selected half-life} \right) \times Log \left(\frac{\textit{intercept of the selected half-life}}{IPC} \right)$$

TABLE 1: Dose (mg/kg), dosing interval (h) and plasma clearance (ml/kg/h) considered to compute the effective plasma concentrations (EPC) (ng/ml) for a selection of drugs used in horse

Drugs	Dose mg/kg	Dosing interval (h)	Clearance (ml/kg/h)	EPC (ng/ml)
Acepromazine	0.100	24	2600	1.6
Bromhexine	0.200	24	3200	2.6
Butorphanol	0.100	5	450	44
Caffeine	5.000	24	35	5952
Carprofen	0.700	24	8.7	3352
Clenbuterol	0.0008	12	350	0.19
Codeine	0.600	24	800	31
Detomidine	0.020	24	400	2.1
Dexamethasone	0.020	24	480	1.7
Dipyrone	5.000	24	300	694
DMSO	1000	24	80	520833
Eltenac	0.500	24	74	282
Flunixine meglumine	1.100	24	60	764
Furosemide	1.000	8	500	250
Glycopyrrolate	0.004	24	1000	0.17
Guaiphenesin	100	24	300	13889
Heptaminol	10	24	1245	335
Hordenine	2.000	24	4300	19
Ibuprofen	10	24	164	2541
Isoxsuprine	0.600	24	2600	9.6
Ketamine	2.200	24	1500	61
Ketoprofen	2.200	24	300	306
Meclofenamic	2.200	24	120	764
Meperidine	1.0	5	1100	182
Methadone	0.100	5	680	29
Methylprednisolone	0.400	24	1000	17
Morphine	0.100	5	500	40
Naproxen	10	24	32	13021
Omeprazole	0.250	24	600	17
Pentazocine	0.300	5	1700	35
Phenylbutazone	4.400	24	41.3	4439
Quinidine	5	24	330	631
Salicylate	20	24	120	6944
Triamcinolone	0.020	24	486	1.7
Vedaprofen	1.000	24	70	595
Xylazine	1.100	24	1200	38

where the selected half-life is the half-life that encompasses the calculated IPC (generally the terminal half-life) and the intercept of the selected half-life, the plasma concentration at time zero when the (terminal) phase of interest begins to decay (see Fig 2 in Toutain and Lassourd 2002 for selection of a terminal half-life).

Selection of a dose rate and plasma clearance for Equation 2, selection of a safety factor for Equation 3, selection of an Rss for Equation 4 and how to compute a WT using Equation 6, the issue of active and inactive metabolites for an IPC and IUC calculation were extensively discussed by Toutain and Lassourd (2002). To illustrate the method, the example of phenylbutazone was explained step by step.

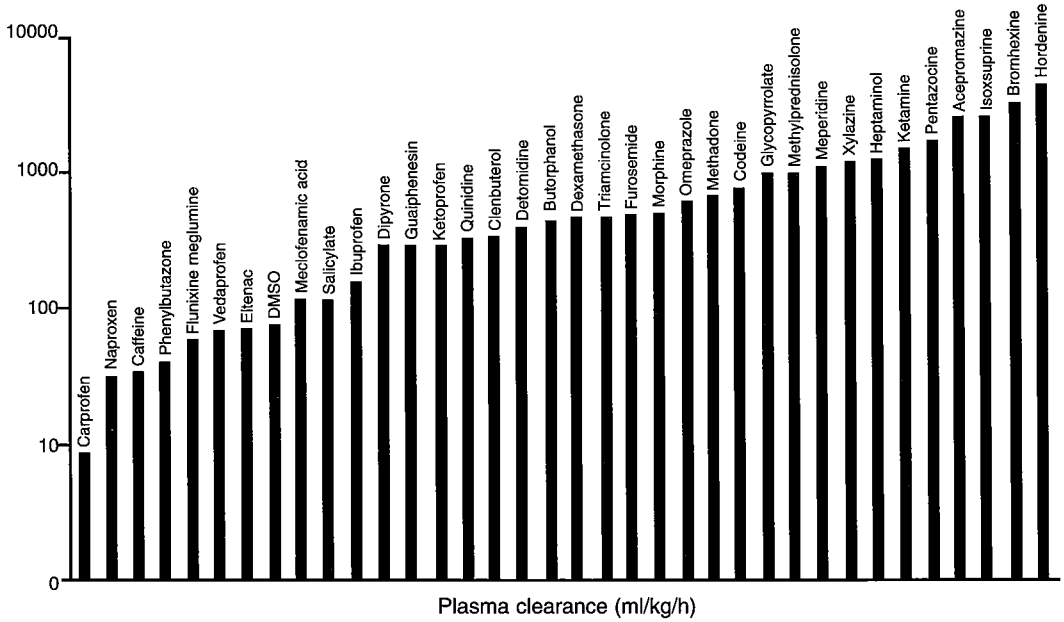

Fig 1: Plasma clearance (ml/kg/h) for a selection of equine drugs. Clearances lower than 130 ml/kg/h should be considered as low, and clearances higher than 1,500 ml/kg/h should be considered as high in a horse.

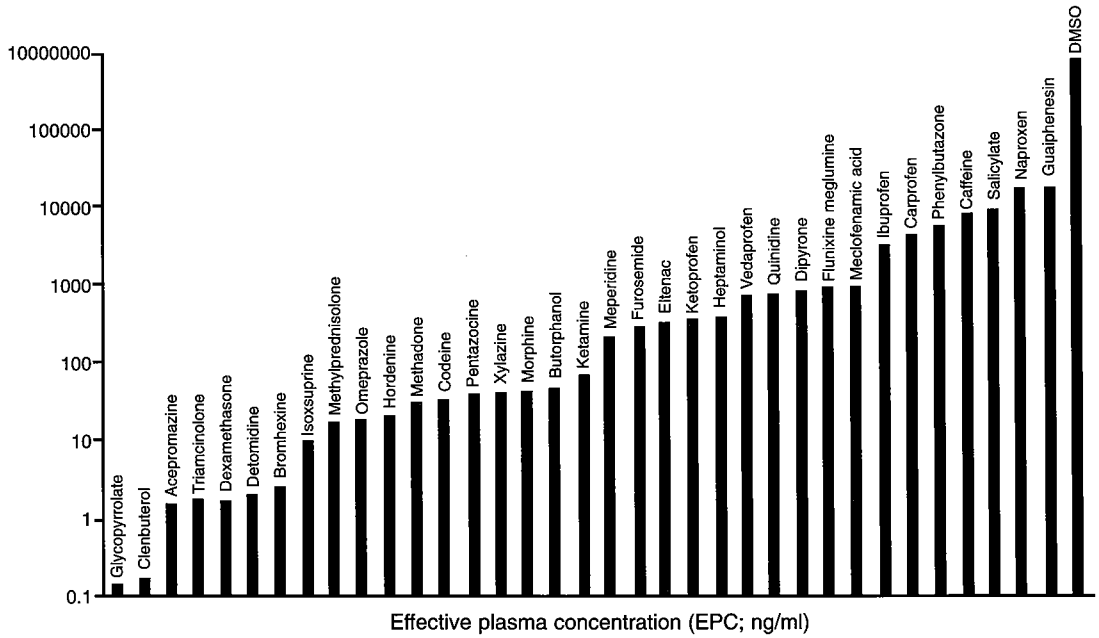

Fig 2: Effective plasma concentration (EPC) in ng/ml for a selection of drugs. EPC is a measure of drug potency.

Application of the method to a selection of drugs

Selection of drugs and retrieval of pharmacokinetic parameters

To explore the method, a set of 36 drugs (or substances of dietary origin) has been selected, irrespective of their possible policy status (exposure vs effect control). Drugs have been selected because enough pharmacokinetic data were available in the published literature. These drugs, including non steroidal anti-inflammatory drugs (NSAID) (phenylbutazone, eltenac, ketoprofen, vedaprofen, dipyrone, flunixine meglumine, meclofenamic acid, ibuprofen, carprofen, salicylic acid and naproxen),

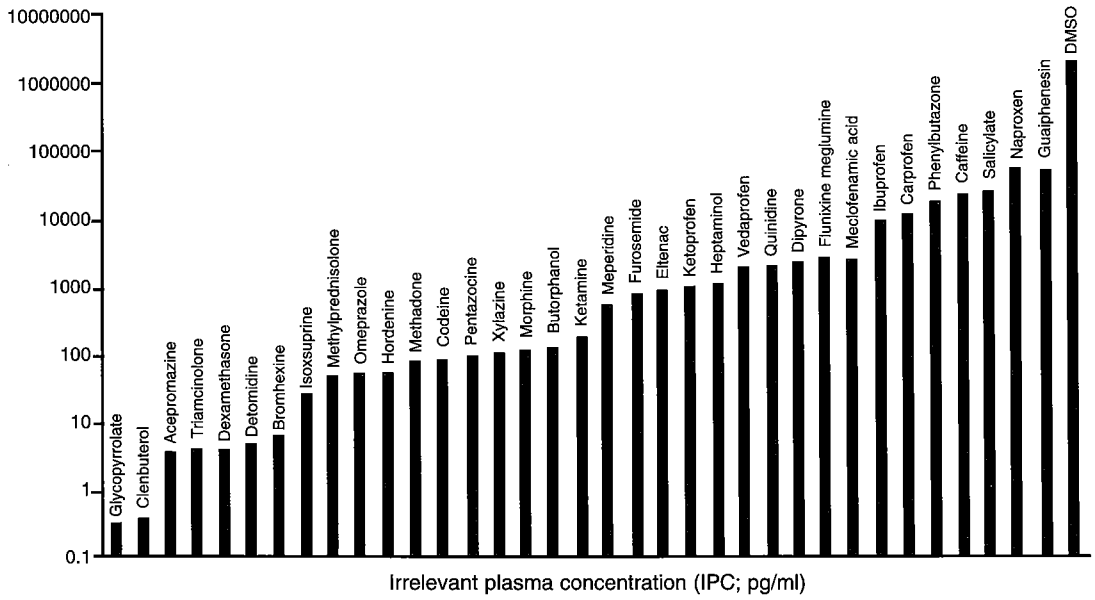

Fig 3: Irrelevant plasma concentration (IPC) for a selection of drugs. IPC was calculated from EPC using a safety factor (SF) of 500.

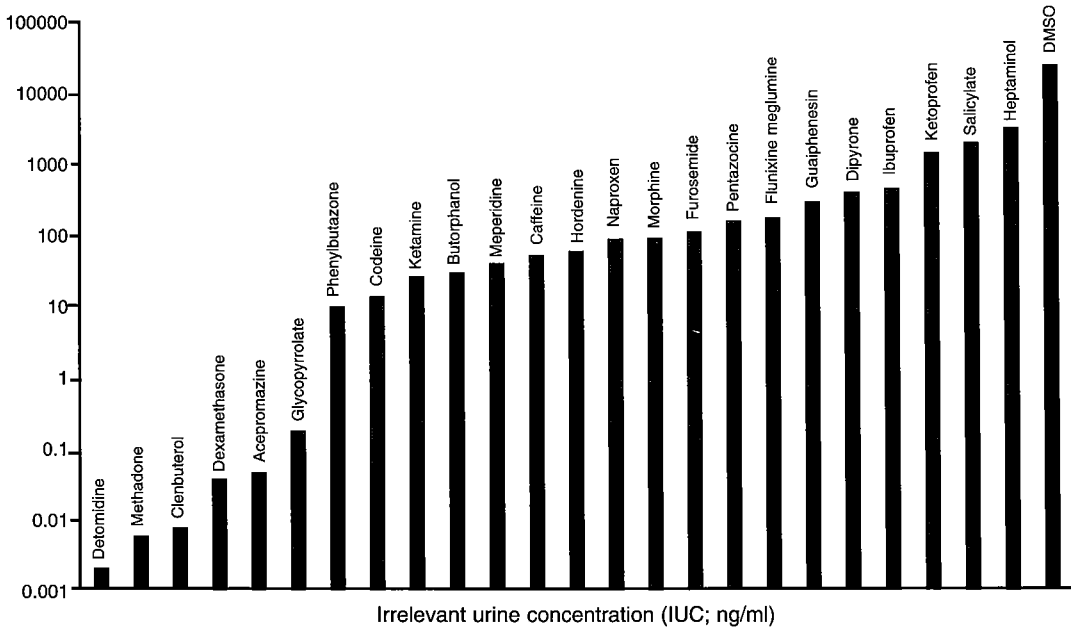

Fig 4: Irrelevant urine concentration (ng/ml) for a selection of drugs. IUC was calculated from IPC scaled by the steady-state plasma to urine ratio.

glucocorticoids (dexamethasone, triamcinolone and methylprednisolone), opioids (methadone, codeine, pentazocine, meperidine, morphine, butorphanol), tranquillisers (acepromazine), anaesthetics and related drugs (ketamine, xylazine, detomidine, guaifenesin), diuretic (furosemide) a muscarinic blocking agent (glycopyrrolate), drugs acting on the cardiovascular and/or the respiratory system (quinidine, heptaminol, clenbuterol, isoxsuprine, caffeine, bromhexine) proton pump inhibitors (omeprazole) and miscellaneous agents (dimethyl sulphoxide and hordenine).

For these analytes, pharmacokinetic parameters (plasma clearance (ml/kg/h), volume of distribution (Varea) (l/kg) and the terminal half-life (h)) for plasma and urine were collected using a

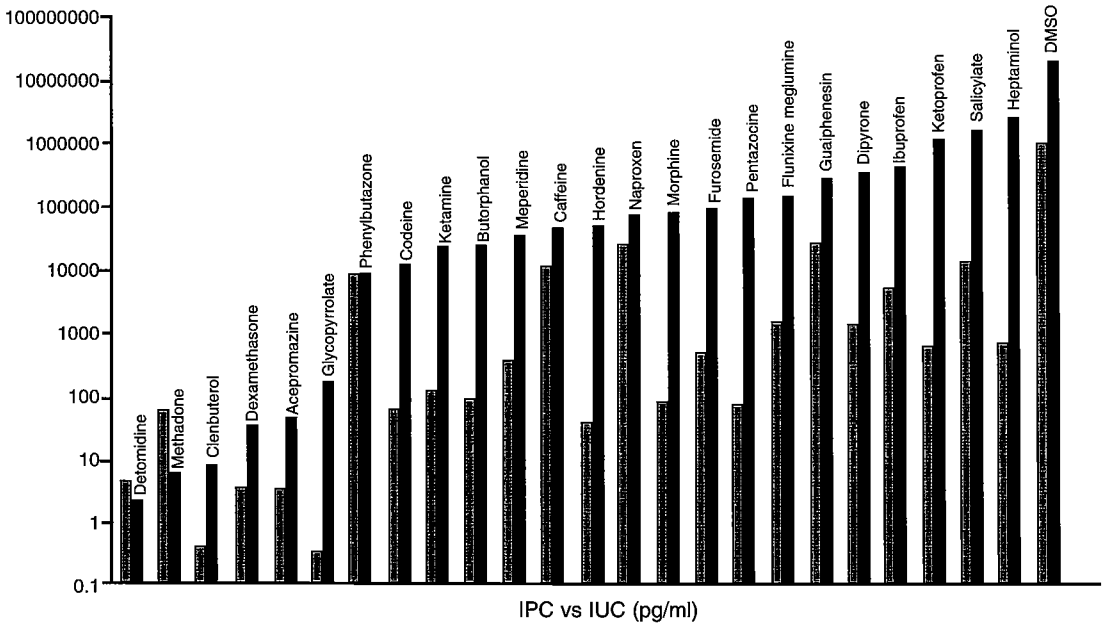

Fig 5: Comparison of irrelevant plasma concentrations (IPC) (left bars) and irrelevant urine concentrations (IUC) (right bars) for a selection of drugs. Concentrations are given in pg/ml. For all drugs but methadone and detomidine, IUC is higher than IPC giving urine an advantage in terms of analytical detectability.

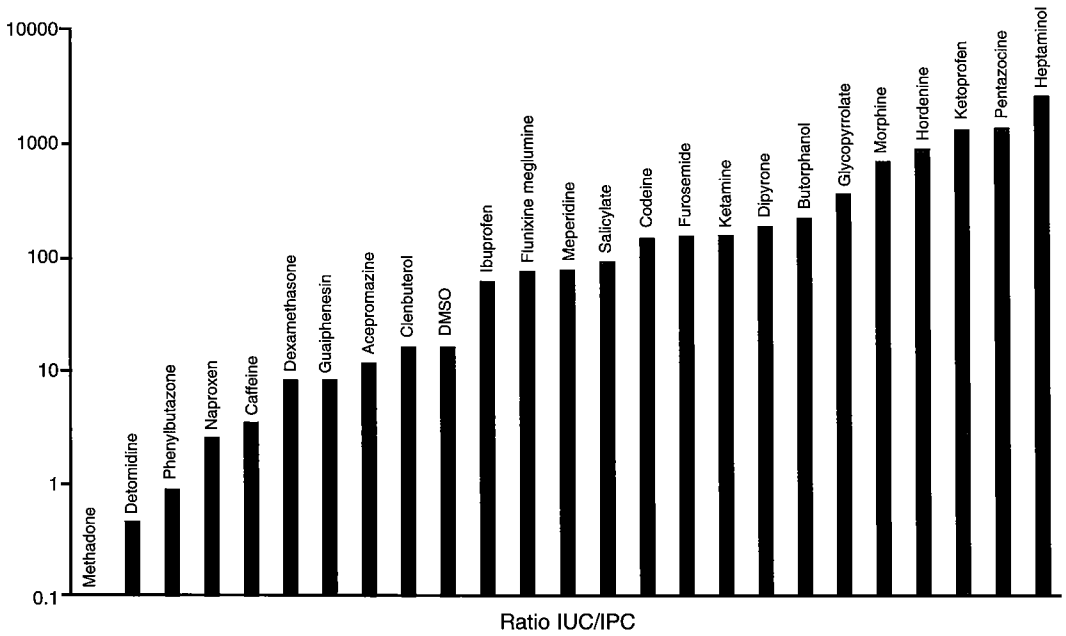

Fig 6: Ratio of irrelevant urine concentrations (IUC) to irrelevant plasma concentration (IPC). For most drugs IUC/IPC is higher than 10.

systematic approach (V. Lassourd and P. L. Toutain, unpublished data).

EPC calculation

The EPC was calculated using Equation 2, considering a dosing interval of 5, 8, 12 or 24 h (see Table 1). Figure 1 gives typical plasma clearance and Figure 2 gives corresponding EPC, glycopyrrolate having the highest potency (170 pg/ml) and dimethyl sulphoxide the lowest (0.5 mg/ml).

IPC calculation

The IPC was derived from the EPC, using a safety factor (SF) of 500 (Equation 3; Fig 3).

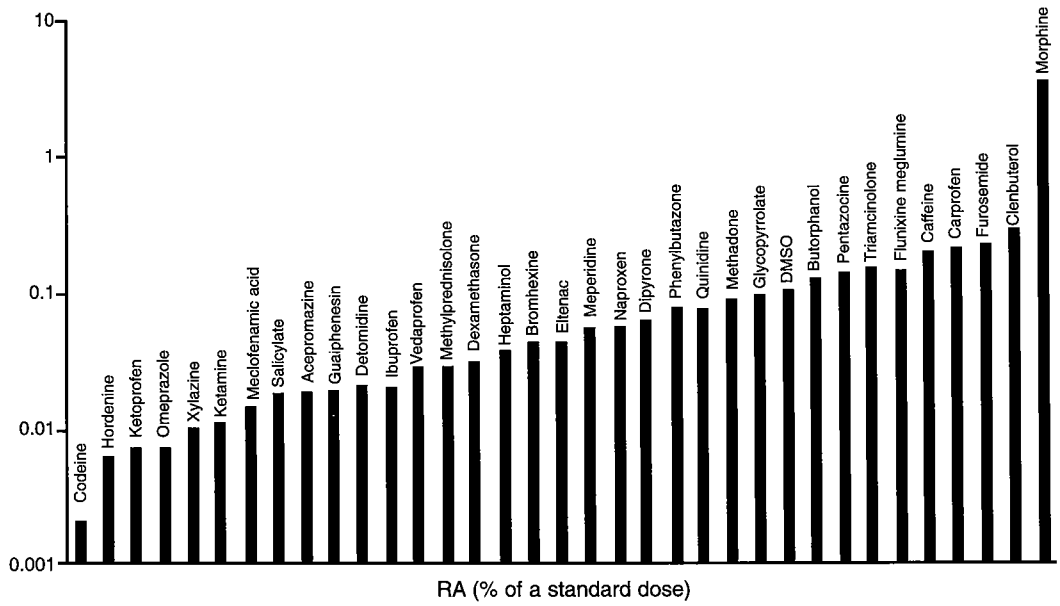

Fig 7: Residual amount (RA) of drug for a plasma concentration equal to IPC. RA was calculated with equation 4. RA was lower than 1% of the standard dose for all drugs but morphine.

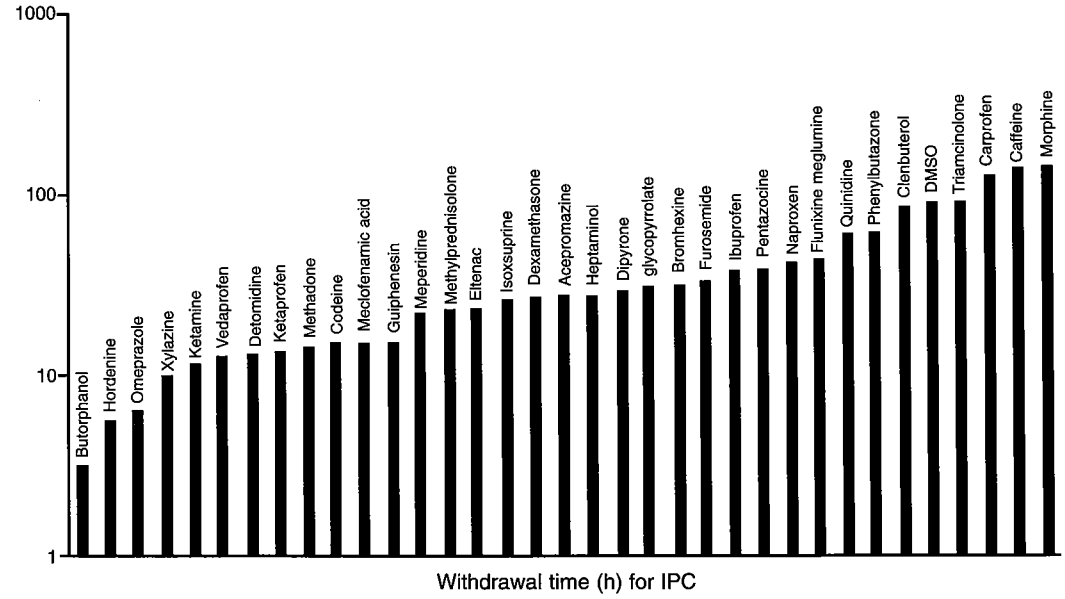

Fig 8: Withdrawal time (WT) (h) is the time necessary to reach IPC when administering a standard single dose by iv injection.

IUC calculation

Equation 4 was used to compute IUC from IPC. Equation 4 requires knowledge of the Rss, the steady-state urine to plasma concentration ratio. Rss is seldom reported and was approximated from raw data or/and published figures. Figure 4 gives the IUC for 25 of the 36 investigated drugs and Figure 5 compares the IPC and IUC. Inspection of Figure 5 shows that for all drugs except detomidine and methadone, the IUC is higher than the IPC. Figure 6 gives the ratio between the IUC and IPC and shows that the IUC, for most of the drugs, is at least 10 times higher than the IPC.

Checking IPC and IUC

To check whether the calculated IPC and IUC were realistic or not, the amount of the drug remaining in the body was calculated when plasma

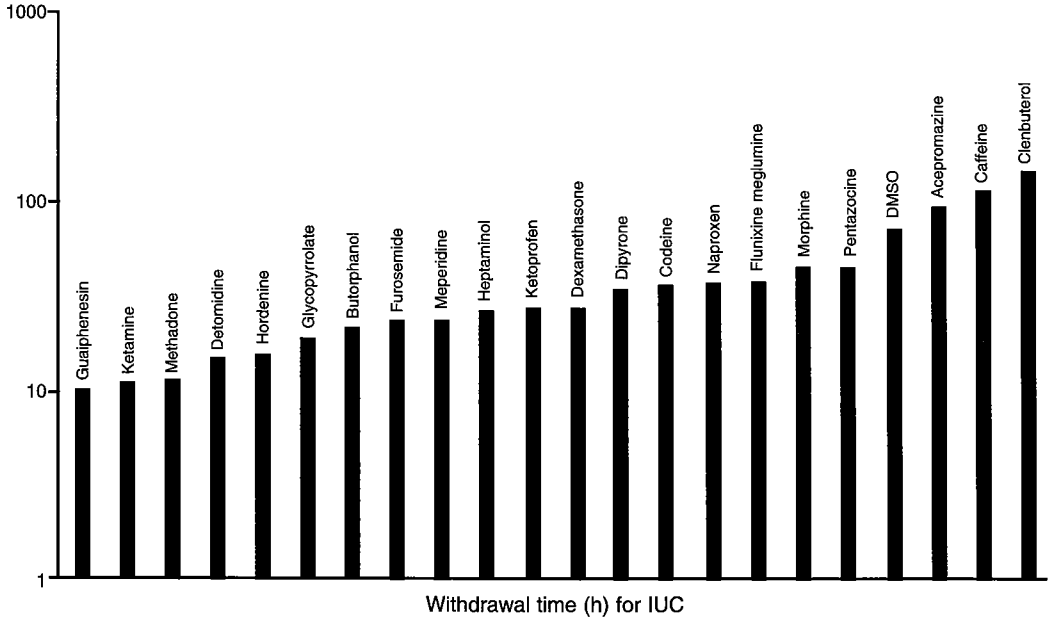

Fig 9: withdrawal time (WT) (h) is the time to reach an IUC when administering a standard single dose by iv injection.

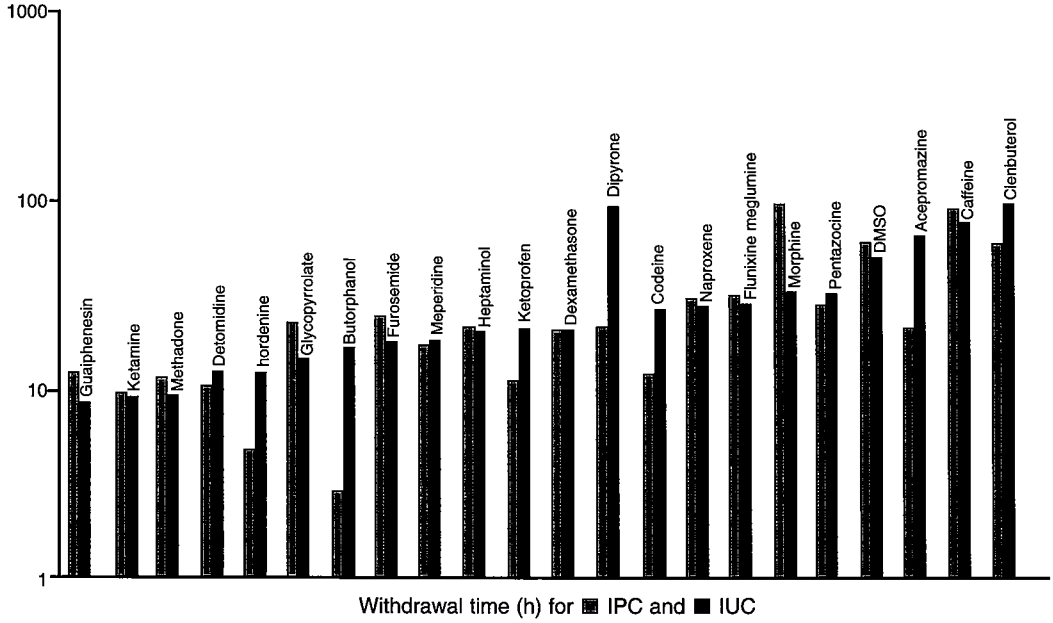

Fig 10: Comparison between withdrawal time (WT) to reach IPC (left bars) and IUC (right bars). Theoretically, the terminal half-life is the same for plasma and urine.

concentration was equal to IPC (or urine concentration equal to IUC). The residual amount (RA) of the drug at IPC was calculated using Equation 5. For all the investigated drugs but morphine, the RA was less than 1% of the standard dose (Fig 7). It is also desirable to have a rough estimate of the withdrawal time (WT) required to reach IPC (or IUC) after administering a standard dose. WT was calculated using Equation 6 with plasma (Fig 8) and urine (Fig 9) data. Figure 10 compares urine and plasma WT and Figure 11 gives the ratio of urine to plasma WT.

Discussion

The proposed method aims at determining the scale of the required sensitivity of the analytical technique, to enable doping control for legitimately

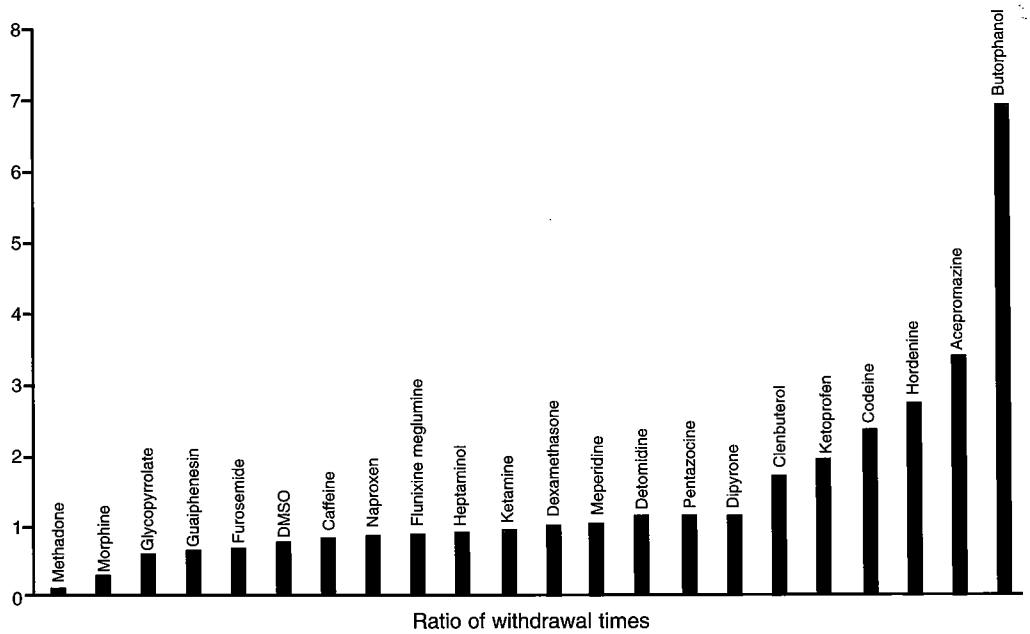

Fig 11: Ratio of withdrawal time (WT) to reach IPC and IUC. For most drugs, WT for IPC and IUC can be considered to be in reasonable agreement.

used drugs for equine therapy without impairing treatment by detecting insignificant concentrations of drugs long after a horse has received medication.

From a scientific point of view, the key parameter to be calculated in order to apply the proposed approach is EPC, which is a measure of the *in vivo* drug potency.

The concept of drug potency in the framework of PK/PD analysis has been reviewed by Toutain (2002). EPC is a PD parameter, independent of the route by which the drug is administered, and in the present approach, iv experimentation was only considered to guarantee that the dose selected for computation is totally available.

Computation of EPC requires knowledge of the effective dose rate and plasma clearance. The question of effective dose rate was discussed in our original paper (Toutain and Lassourd 2002). Plasma clearance is considered as the most 'robust' pharmacokinetic parameter because its determination only requires knowledge of the area under the plasma vs time curve (AUC), a measurement of overall drug exposure. The determination of AUC is relatively insensitive to the limit of quantification (LOQ) of the analytical method used to perform the PK investigation. In addition, plasma clearance is often calculated using a non-compartmental approach (ie as Clearance = Dose/AUC) which avoids difficulties linked to data modelling.

It is likely that the currently calculated EPC are reasonable evaluations of the drug potency, with the possible exception of drugs for which a very high plasma clearance has been reported, such as hordenine, bromhexine, acepromazine and isoxsuprime. Indeed, for a drug undergoing both a 100% hepatic and a 100% renal first pass effect, the equine body clearance should not be higher than about 1,700 ml/kg/h ie half the cardiac output because hepatic and renal blood flows represent about half the cardiac output (see Toutain 2002 for further explanations). For these drugs, it would be interesting to know the blood (rather than plasma) clearance, to check whether or not the reported plasma clearances are overestimated. On the other hand, for some drugs (eg detomidine), a dosing interval of 24 h can be considered as rather conservative, because the duration of sedative action of a single dose iv is less than 24 h but this class of drugs also has other effects (eg growth hormone secretion). Conversely, taking a dosing interval of 5 h for opioids may be not conservative enough because opioids may have effects other than proved analgesic properties.

From these EPC, IPC were calculated using a standard SF of 500. Actually, it is the responsibility of the authorities to determine this factor, ie to determine what is acceptable or unacceptable in terms of residual drug effect. The magnitude of SF also involves several scientific considerations, which include the quality and extent of the available experimental data, the reliability of such data, the inter-individual variability, the existence or not of different effects for which a drug has different potencies and also the shape of the dose-effect relationship as explained by Toutain and

Lassourd (2002). Whatever the SF, it seems imperative to fix SF *a priori* before any computation, to avoid the risk of adapting the SF to what it is secretly anticipated. A default SF of 500 (ie 10 x 50) was proposed because a factor of 10 is necessary to take into account the inter-individual variability and a factor of 50 to transform an effective concentration into an ineffective one.

To assess the usefulness of the proposed method, the calculated IPC should be compared to the LOQ or LOD of the different analytical techniques in use in the different control laboratories. The ultimate goal of the method is to make a qualitative judgement on the sensitivity of the analytical techniques either to avoid oversensitive methods (for drugs legitimately used in equine therapy) or to improve the current analytical technique for prohibited substances or for substances administered locally, because that class of drugs (eg opioids, glucocorticoids) requires an LOD at least lower than IPC.

Urine is the most convenient biological matrix to enforce doping control and computation of IUC is desirable. However, urine is a less than ideal matrix to control the drug effects because, in the framework of the present approach, which consists of controlling drug effects and not drug exposure, urine concentration is only a surrogate for the corresponding plasma concentration. The drugs for which the IUC/IPC ratio is not too high (eg less than 10) are candidates for plasma rather than urine monitoring (see Fig 6). The main difficulty with IUC is the uncertainty of the retrieved Rss which is seldom reported and difficult to evaluate. It is influenced by different biological factors (eg urine pH, diuresis) and a given snapshot urine concentration may correspond to very different plasma concentrations (and effects) because nothing guarantees that the horse at the time of sampling is under steady-state conditions. For all these reasons, urine is not an attractive matrix to control drug effects, despite higher drug concentration than in plasma.

To check the possibly unrealistic value for IPC and IUC we suggested computing the residual amount (RA) of a drug and obtaining a rough estimate of the withdrawal time (WT) required to reach IPC (or IUC) after administration of a standard dose. For all the investigated drugs but morphine, RA was less than 1% which is satisfactory. The calculated WTs correspond to those derived only from the iv data because these WTs reflect only overall drug elimination. Indeed, a terminal half-life (and thus WT) can also reflect slow drug absorption. Theoretically, terminal half-life is the same in plasma and urine (Gibaldi and Perrier 1982) which is not the case for several of the investigated drugs (see Fig 10); for the largest discrepancy, this is probably due to the fact that the true terminal phase is often missed from plasma data for analytical reasons. Actually, inconsistency between plasma and urine terminal half-life evaluation is not an issue for the proposed method which only aims at fixing IPC and IUC (ie to determine the range of performance for an analytical technique), not recommending WT. It is the responsibility of drug companies to estimate the WT for their selected dosage regimen, method of administration, drug formulation etc.

In conclusion, this paper proposes and illustrates a non-experimental approach for estimating the domain of analytical performances, for drug control effects, on the day of racing. Its purpose is to avoid detecting and reporting irrelevant concentrations, which can be detected using some high performance analytical methods. We applied the proposed method on a set of 36 drugs and the resulting IPC and IUC now need to be assessed in the real world of doping control to determine its potential usefulness. Some of the proposed values can be refined by reconsidering the dose and/or the dosing interval selected for the EPC computation. But it should be kept in mind that the overall goal of this non-experimental approach is only to determine the order of magnitude of what is an acceptable negligible concentration, not to determine a statistically founded threshold value for therapeutic substances.

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