Veterinary Medicines and Competition Animals: The Question of Medication Versus Doping Control

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Abstract In racing and other equine sports, it is possible to increase artificially both the physical capability and the presence of a competitive instinct, using drugs, such as anabolic steroids and agents stimulating the central nervous system. The word doping describes this illegitimate use of drugs and the primary motivation of an equine anti-doping policy is to prevent the use of these substances. However, an anti-doping policy must not impede the use of legitimate veterinary medications

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and most regulatory bodies in the world now distinguish the control of illicit substances (doping control) from the control of therapeutic substances (medication control). For doping drugs, the objective is to detect any trace of drug exposure (parent drug or metabolites) using the most powerful analytical methods (generally chromatographic/mass spectrometric techniques). This so-called "zero tolerance rule" is not suitable for medication control, because the high level of sensitivity of current screening methods allows the detection of totally irrelevant plasma or urine concentrations of legitimate drugs for long periods after their administration. Therefore, a new approach for these legitimate compounds, based upon pharmacokinetic/pharmacodynamic (PK/PD) principles, has been developed. It involves estimating the order of magnitude of the irrelevant plasma concentration (IPC) and of the irrelevant urine concentration (IUC) in order to limit the impact of the high sensitivity of analytical techniques used for medication control. The European Horserace Scientific Liaison Committee (EHSLC), which is the European scientific committee in charge of harmonising sample testing and policies for racehorses in Europe, is responsible for estimating the IPCs and IUCs in the framework of a Risk Analysis. A Risk Analysis approach for doping/medication control involves three sequential steps, namely risk assessment, risk management, and risk communication. For medication control, the main task of EHLSC in the risk management procedure is the establishment of harmonised screening limits (HSL).The HSL is a confidential instruction to laboratories from racing authorities to screen in plasma or urine for the presence of drugs commonly used in equine medication. The HSL is derived from the IPC (for plasma) or from the IUC (for urine), established during the risk assessment step. The EHSLC decided to keep HSL confidential and to inform stakeholders of the duration of the detection time (DT) of the main medications when screening is performed with the HSL. A DT is the time at which the urinary (or plasma) concentration of a drug, in all horses involved in a trial conducted according to the EHSLC guidance rules, is shown to be lower than the HSL when controls are performed using routine screening methods. These DTs, as issued by the EHSLC (and adopted by the Fédération Equestre Internationale or FEI) provide guidance to veterinarians enabling them to determine a withdrawal time (WT) for a given horse under treatment. A WT should always be longer than a DT because the WT takes into account the impact of all sources of animal variability as well as the variability associated with the medicinal product actually administered in order to avoid a positive test. The major current scientific challenges faced in horse doping control are those instances of the administration of recombinant biological substances (EPO, GH, growth factors etc.) having putative long-lasting effects while being difficult or impossible to detect for more than a few days. Innovative bioanalytical approaches are now addressing these challenges. Using molecular tools, it is expected in the near future that transcriptional profiling analysis will be able to identify some molecular "signatures" of exposure to doping substances. The application of proteomic (i.e. the large scale investigation of protein biomarkers) and metabolomic (i.e. the study of metabolite profiling in biological samples) techniques also deserve attention for establishing possible unique fingerprints of drug abuse.

Keywords Detection time · Doping · Horse · Irrelevant urine/plasma concentration · Medication control · Risk analysis · Threshold · Withdrawal time

1 Introduction

Even though there is a debate about what exactly constitutes an animal sport, it is accepted that the three most common sporting animals are horses (racing, jumping, eventing, polo), dogs (greyhound racing, sled dog racing, coursing, hunting) and camels (racing). However, many other mammalian species, including cattle (bull-fighting, American rodeo), and birds (pigeon racing) may compete or participate in events.

In all animals participating in sports, there are requirements for high physical capability and the presence of a competitive instinct. These traits are normally acquired through training programmes and selective breeding. It is also possible to strive to reach these objectives using certain ergogenic drugs, such as anabolic steroids, and to promote stamina by administering drugs acting on the central nervous system. Thus, two major issues relating to drugs and animals in sport arise and these are sometimes difficult to delineate: the "good", that is treatment given for the best health and welfare interests of the animal (legitimate medication) and the "ugly", that is the use of drugs primarily to alter or restore athletic performance. The word "doping" is reserved for this latter illegitimate use of drugs.

The aim of this chapter is to provide an overview on doping/medication control and to summarise recent advances in terms of scientific assessments and managerial options implemented by the International Federation of Horseracing Authorities (IFHA), a body which represents the main racing authorities in the world, and by the International Equestrian Federation (FEI), which is the world governing body of equestrian sports. For a recent overview on doping control see Higgins (2006) and the earlier seminal book of Tobin (1981).

2 Rationale for Anti-doping Versus Medication Control

An anti-doping programme is characterised by a set of values, some being common to man and animals, such as ethics, fair play and honesty, chosen to ensure competition based on true merit. Other values are specific to animals and used to protect the species or breed. "A level playing field" is considered to be pivotal for both the credibility and image of the racing industry, because this sport relies on betting and the confidence of the punter is therefore essential; this explains why, for racing horses, most racing authorities in the world which operate under the medication rules of the IFHA, excluding USA, have signed the so-called Article six of the International Agreement on Breeding and Racing. This article prohibits the

presence of any substances in a horse during a race which could give a horse an advantage or a disadvantage in that race.

Whilst the primary motivation of equine anti-doping control rules has been to prevent any attempt to alter a horse's performance i.e. to actually protect a business model, it is now accepted that a goal of this policy must be, to not indirectly impede the *bona fide* use of veterinary medications. Anti-doping rules should also protect the animal and guarantee its welfare. The European Convention for the Protection of Pet Animals expresses similar values when stating that "*no substances shall be given to, treatments applied to, or devices used on a pet animal for the purpose of increasing or decreasing its natural level of performance: during competition or at any other time when this would put at risk the health and welfare of the animal*". Even in bullfighting, which is not generally regarded as a sport, but rather as a cruel activity in many countries, drug tests are performed to detect the presence of substances such as tranquillisers that are considered as "unfair" for the bull. This latter example shows how an anti-doping policy may rely on a very different set of values and is contextual.

3 Medication Versus Doping Control: Progress Towards a General Policy Giving Priority to the Welfare and Safety of the Horse

The FEI and the European Horserace Scientific Liaison Committee (EHSLC), which is the European scientific committee in charge of harmonising sample testing and policies for racehorses in Europe (Barragry 2006; Houghton et al. 2004), have established a general policy that distinguishes the control of any drug *exposure* for all illicit substances (doping control) and the control of a drug *effect* for therapeutic substances (medication control). For sport horses, the FEI qualifies in its code that a doping agent is a substance with no generally accepted medical use in competition horses but which is able either to alter a horse's performance or to mask an underlying health problem. A list of these prohibited substances is given in the FEI medication code. This list includes many drugs acting on the central nervous system (stimulants, tranquillisers), anabolic steroids and growth promoters, genetically recombinant substances (erythropoietin, growth hormone), hormonal products (natural or synthesised) etc.

In the USA, the situation differs and, until recently, the use of anabolic steroids in horse racing was largely unregulated. In 2002, to address public concerns and the lack of uniformity between American states regulations, a Racing Medication and Testing Consortium (RMTC) was formed to represent most US industry stakeholder groups. The RMTC proposed a ban on exogenous anabolic steroids and testing for endogenous anabolic steroids (testosterone, nandrolone, boldenone); these proposals will be progressively enforced in the different American states by 2009. This new US approach is based on a model rule that now recommends no race

day medication. Currently, the main differences in opinions and practises between the RMTC and countries that have signed article 6 of the IFHA are the permitted use in the USA of the loop diuretic furosemide as an "anti-bleeder" medication (*vide infra*) and the permitted plasma levels of three non-steroidal anti-inflammatory drugs (NSAIDs), namely phenylbutazone (5 μg/mL), ketoprofen (10 ng/mL) and flunixin (20 ng/mL). For these three drugs, an IV administration is permitted at least 24 h before the "post" time for the race4.

3.1 Doping Agents and Doping Control Issues

The use of furosemide, a "high ceiling" diuretic, is currently the main obstacle towards international harmonisation. It is an exemplar to show how the same drug may be classified either as a doping agent or a beneficial drug for horse welfare by different jurisdictions. Furosemide is extensively and legally used in the USA prior to racing for its putative role in the prophylaxis of exercise-induced pulmonary haemorrhage (EIPH). It is proposed that it is in the horse's best interests to race using furosemide; if so, the horse is placed on the official furosemide list and can then be treated with furosemide no less than 4 h prior to "post-time" for the race in which the horse is entered. Furosemide should be administered by the IV route, the dose should be between 150 and 500 mg per animal and plasma concentrations may not exceed 100 ng/mL (For further information see section, "RMTC: Equine Veterinary Practises, Health and Medication" in chapter, "Veterinary Medicines and the Environment").

Such use is totally forbidden by Article 6 of IFHA and FEI. In the USA, furosemide is viewed as the "modern version" of blood-letting, because a dose of 1 mg/kg produces a rapid reduction in blood volume of approximately 8–9% of total volume. Furosemide modifies the haemodynamic response to exercise (see review of Hinchcliff and Muir (1991)). It was hypothesised that furosemide could reduce the lung-fluid volume by reducing arterial wedge pressure during exercise and could thereby mitigate the risk of EIPH. While the pharmacological cardiovascular effects of furosemide are well established, their actual protective role in EIPH is more controversial. A poor repeatability of an endoscopic score after furosemide treatment was shown (Pascoe et al. 1985) and a significant difference between untreated and furosemide-treated EIPH-positive horses (Sweeney and Soma 1984) could not be detected. However, a recent investigation showed that furosemide was able to decrease the incidence and severity of EIPH in thoroughbreds (Hinchcliff et al. 2009). It should be stressed that epidemiological surveys have provided evidence that furosemide may improve racing performance (Soma and Uboh 1998). In horses, furosemide decreased the oxygen debt and the rate of blood lactate accumulation. This effect can be reversed by adding to the horse a weight compensating for the loss of body weight due to the diuresis produced by furosemide (approximately 16 kg), suggesting that changes in performance observed in bleeder horses after a furosemide treatment is due to a small reduction in body

weight and not to a selective pharmacological action on bleeding mechanisms (Soma and Uboh 1998; Zawadzkas et al. 2006). In addition, furosemide is disapproved of because it causes diluted urine, i.e. its consumption is seen as an attempt to mask other illicit substances. For all these reasons, furosemide is considered as a doping agent by the FEI and most racing authorities in the world.

Anabolic steroids with androgenic properties (testosterone, stanozolol, nandrolone, boldenone) have been used routinely in the US as performance-enhancing substances in the horse. They possess behavioural effects and are credited with increasing the competitive instinct. Testosterone, boldenone (1,2-dehydrotestosterone) and nandrolone (19-nortestosterone) are endogenous to horses and their control requires the establishment of a threshold (Table 1). In horses, 19-nortestosterone is naturally produced by the testes as well as by the ovaries. This steroid can easily be detected in mares and geldings, because its major metabolite (estranediol which is the 5-alpha-estrane-3beta,17-alpha diol) is found only in the urine of treated horses. In contrast, in colts, estranediol is found in normal urine and it was shown that the ratio of estranediol (the metabolite) over the 5-estrene-3beta,17alpha diol, (a natural related steroid which is not a metabolite of nandrolone) may be considered as evidence of the possible abuse of nandrolone (Houghton and Crone 2000), because the probability of having a ratio higher than 1 in normal post-race urine was 1 in 10,000. In the USA, a threshold of 1 ng/mL is proposed for nandrolone. The logic, advantages and drawbacks of selecting a ratio rather than a simple cut-off value to establish a threshold are discussed in Sect. 7.

Genetically recombinant substances, such as recombinant growth hormone (reGH) and recombinant erythropoietin (reEPO) as doping agents are particularly difficult to control using available analytical approaches, because their effects last much longer than their presence in detectable concentrations in body fluids. An equine recombinant growth hormone (reGH) has been marketed for horses in Australia. It has been used illegally in racing horses. It is a methionyl equine somatotrophin produced by DNA technology. There is no controlled study to demonstrate any beneficial effect of reGh administration in supra-physiological amounts on trained horses. Chronic reGH administration does not alter aerobic capacity and indices of exercise performance in unfit aged mares, so that reGH was not an ergogenic substance in a subpopulation of unfit horses (McKeever et al. 1998). GH exerts its anabolic effect in part via secretion of Insulin-like Growth Factors (IGFs) by the liver. In horses the plasma concentration of IGF is increased by GH treatment but the duration of the response is too short to be an effective approach to control GH abuse (Popot et al. 2000). Current strategies for screening GH abuse in horses rely on the long-term detection (up to 200 days) of specific anti-reGH antibodies, produced as a consequence of repeated reGH administrations (Bailly-Chouriberry et al. 2008a). A confirmatory method for reGH detection in plasma/urine is required for regulatory purposes. An analytical strategy based on LC-MS/MS through the identification of the reGH N-terminal characteristic peptide was developed but the detection time (DT) is very short (48 h) reflecting the possible delayed effects of this class of compound (Bailly-Chouriberry et al. 2008b).

Table 1 Substances for which a threshold has been adopted or proposed by different jurisdictions or organisations

Substance/jurisdiction	Threshold
Arsenic/IFHA	• 0.3 μg total arsenic per mL in urine
Boldenone/IFHA, RMTC, FEI	• 0.015 μg free and conjugated boldenone per mL in urine from entire male horses (not geldings) • No boldenone shall be permitted in geldings or female horses
Carbon dioxide/IFHA	• 36 mM available carbon dioxide per litre in plasma
Dimethyl sulphoxide/IFHA, FEI	• 15 μg/mL in urine, or • 1 μg/mL in plasma
Estranediol in male horses (other than geldings) as a biomarker of nandrolone abuse/IFHA, FEI	• 0.045 μg free and glucuroconjugated 5α-estrane-3β,17α -diol per mL in urine
Estranediol in male horses (other than geldings) as a biomarker of nandrolone abuse/Hong Kong Jockey Club,Emirates Racing Authorities, Fédération Nationale des courses françaises and some other juridictions	• The mass of free and conjugated 5*a*-estrane-3β, 17*a*-diol to the mass of (other than geldings) free and conjugated 5(10)-estrene -3β, 17*a*-idol in urine from entire male horses (not geldings) at a ratio of 1
Nandrolone/RMTC	In geldings, mare and fillies: 1 ng/mL in urine
Hydrocortisone/IFHA	• 1 μg/mL in urine
Methoxytyramine/IFHA	• 4 μg free and conjugated 3-methoxytyramine per mL in urine
Salicylic acid/IFHA	• 750 μg/mL in urine, or • 6.5 μg/mL in plasma
Salicylic acid/FEI	• 625 μg/mL in urine, or • 5.4 μg/mL in plasma
Testosterone/IFHA, RMTC	• 0.02 μg free and conjugated testosterone per mL in urine from geldings, or • 0.055 μg free and conjugated testosterone per mL in urine from fillies and mares (unless in foal)
16β-hydroxystanozolol (metabolite of stanozolol)/RMTC	• 1 ng/mL in urine for all horses regardless of sex; • Forbidden by IFHA and FEI
Theobromine/IFHA	• 2 μg/mL in urine
Caffeine/RMTC	• 100 ng/mL of serum or plasma

IFHA: International Federation of Horseracing Authorities
RMTC: Racing Medication and Testing Consortium
FEI: Federation Equestre Internationale

To overcome the limitations of traditional methods, new and sensitive methods based on fingerprint strategies are currently being considered (see Sect. 5).

Erythropoietin is a natural glycoprotein hormone, produced mainly by the kidneys. It regulates mammalian erythrocyte and haemoglobin production. There is evidence that recombinant human EPO (rhEPO), Darbepoietin, (a synthetic long-acting rhEPO) and many biosimilar (generic) rhEPOs are used in horses. The expected effect of EPO in horses is an increase in the red blood cell mass providing

improvement in oxygen-carrying blood capacity and enhancing the horse's aerobic exercise performance. The administration of rhEPO (Eprex, Janssen-Cilag at a dosage of 50 μg/kg BW, IV three times weekly for 3 weeks) increased haemoglobin concentration, haematocrit and red blood cell count by 25% in horses. Peak values were reached 1 week after the last treatment and the increased values persisted for 3–4 weeks (Lilliehook et al. 2004). In unfit horses it was shown that rhEPO enhanced aerobic capacity without either altering anaerobic power or improving exercise performance (McKeever 1996). The effects of EPO on the performance of a fit horse are unclear. Horses, in contrast to man, have an erythrocyte storage type of spleen, exerting the role of a reservoir, which can, in resting conditions, store up to 30% of the total red blood cells, and a spleenic contraction can mobilise up to 12 L of extra blood. During exercise, this reserve may be liberated immediately into the circulation by splenic contraction, thereby increasing the blood oxygen-carrying capacity. Horses may be described as "natural blood dopers". In this context, the actual effect of EPO on performance in horses remains unclear. Whatever the actual EPO effect, the prolonged half-life of RBCs (140 days in the horse) allows a putative benefit of the EPO to develop over several weeks without the risk of being detected as positive.

Using an ELISA test, the excretion profile after EPO administration to horses indicates that rhEPO may be easily detectable during the first 10 h after an IV administration but, after a delay of 48 h, EPO concentrations were indistinguishable from background levels (Tay et al. 1996). rhEPO may also be directly detectable for a few days only in horses by detecting the peptides of EPO using sensitive LC/MS/MS technology (Guan et al. 2007). Long-term use of rhEPO can be detected by screening horse plasma for EPO antibodies but no change in the level of rhEPO antibodies was observed after 3 weeks of rhEPO administration (Lilliehook et al. 2004) This immunological response to rhEPO has been responsible for an adverse response in the form of an immune-mediated anaemia and the deaths of treated horses (Piercy et al. 1998). From a mechanistic point of view, a recent study showed that rhEPO binds to the surface of the EPO receptor (EPOr) and that the rhEPO–EPOr complex is subsequently internalised into EPOr containing cells, where the rhEPO is degraded by lysosomal enzymes. RBCs possess EPOr but no lysosomal degradation system and it was shown in horses that rhEPO may accumulate in RBCs and remain elevated for up to 13 days (Singh et al. 2007). It was suggested that analysis of rhEPO in RBCs may be a better indicator of rhEPO abuse in horses. Another option to control rhEPO and all other analogues and biosimilar substances is to perform unforeseen regular controls on horses out of competition and to develop, as for eGH, new approaches to assess the imprinting of EPO using genomic resources (see Sect. 5).

3.2 Medication Issues and Medication Control

In contrast to anti-doping control, equine medication control rules seek to prevent medication violations, while protecting the welfare of the horse. In the FEI

medication code, these substances are classified in the equine Prohibited List either as Class A Medications (drugs attracting moderate sanctions and penalties) or Class B Medications (drugs attracting minor sanctions and penalties). Examples of class A medications are substances which could influence performance by relieving pain (NSAIDs, local anaesthetics, etc). Examples of class B medications include substances that have either limited performance enhancing potential (e.g. mucolytics and cough suppressants) or to which horses may have been accidentally exposed, including certain dietary contaminants (e.g. bufotenine, hordenine etc.).

The FEI acknowledges that the use of medication in a horse close to an event may be required but is inherently risky in term of medication control if insufficient time has elapsed for elimination of the drug from the horse. To support good veterinary practises, the FEI selected some twenty essential drugs that are collectively known as the FEI "Medicine Box". These are all legitimate treatments that might be used in routine clinical practise during the time closely preceding an event and for which the FEI decided to provide the information (detection times) needed for appropriate use.

Certain medications are permitted under FEI Rules. These currently include rehydration fluids, antibiotics (with the exception of procaine benzylpenicillin) and anti-parasitic drugs, with the exception of levamisole. In addition, some drugs to treat or prevent gastric ulcers may be given (i.e. ranitidine, cimetidine and omeprazole). The use of altrenogest is currently permitted for mares with estrus-related behavioural problems because altrenogest suppresses behavioural estrus in the mare within the 2–3 days following the beginning of the dosing schedule and, at the recommended dose, has no effect on dominance; hierarchy; body mass and condition score (Hodgson et al. 2005).

4 Analytical Method and Doping Testing

A sample (plasma, urine or any other matrix) that has been collected under a secure chain of custody (Dunnett 1994) must be tested by means of validated, state-of-the art drug-testing assays. Due to legal implications, all aspects of the testing procedures should be traceable and all *ad hoc* documents should be available for possible court testimony. Laboratories involved in doping control programmes should comply to a set of minimal standard as described by the AORC *Guidelines for the Minimum Criteria for Identification by Chromatography and Mass Spectrometry* to ensure that the quality and integrity of the data are defensible and fit for purpose. In addition, to conduct a referee analysis i.e. to perform a confirmatory analysis on the split (or so-called B) sample, referee laboratories should be accredited to ISO/IEC 17025 (Hall 2004), and must be member laboratories of either the association of official racing chemists (AORC) or the World anti-doping agency (WADA).

Drugs are commonly analysed and identified using chromatographic/mass spectrometric techniques, which allow for the determination of approximately 95% of

all target analytes (Thevis et al. 2008). Gas chromatography–mass spectrometry (GC–MS) and liquid chromatography–mass spectrometry (LC–MS) are techniques that can provide unequivocal evidence of the presence of a prohibited substance (Thevis and Schanzer 2007; Van Eeno and Delbeke 2003). They are considered as the sole techniques that are suitable on their own for confirmatory methods.

One of the analytical challenges for horse doping control is to distinguish hormones of endogenous vs. exogenous origin (e.g. cortisol, testosterone). Gas chromatography/combustion/isotope ratio mass spectrometry (GC/C/IRMS) is an isotopic method able to measure accurately small differences in the $^{13}C/^{12}C$ ratio of endogenous vs. synthetic steroids. In horses this technique has been explored for cortisol (Aguilera et al. 1997) and nandrolone (Yamada et al. 2007). However, this approach has a low sensitivity and requires concentrations of about 10–20 ng/mL to reliably measure the $^{13}C/^{12}C$ ratio of a molecule. In addition, it is a labour intensive and costly method to perform and is used only to provide supportive evidence of the exogenous administration of hormones.

The major scientific challenge faced today for horse doping control is the case of recombinant biological substances (EPO, GH, growth factors) having putative long-lasting effects while being difficult or impossible to detect over a few days (see Sect. 4). Innovative bioanalytical approaches are now progressing for solving these relevant emerging problems in horse anti-doping control. A promising approach is based on the analysis of gene expression in peripheral blood cells (leucocytes). There is evidence that white blood cells respond to many of these anabolic factors and this is observable for a long time after the disappearance of the substance itself. Using molecular tools, it is expected in the next future that transcriptional profiling analysis would be able to identify some molecular "signatures" of exposure to these doping substances. Resources of proteomic (i.e. the large scale investigation of protein biomarkers) and metabolomic (i.e. the study of metabolite profiling in biological samples) also deserve attention in establishing possible unique fingerprints of drug abuse.

5 Blood Versus Urine Testing and the Rationale for Selecting a Matrix for Doping and Medication Control

Currently, most controls are performed using urine but blood (plasma) should be seriously considered as a better matrix for medication control. From a pharmacokinetic/pharmacodynamic (PK/PD) point of view, the drug (free) plasma concentration is considered as the best surrogate of the drug biophase concentration. Thus, the plasma concentration is the best predictor of the drug's effect. Exceptions are diuretics for which the urine concentration is a better predictor of the drug's effect because all diuretics gain access to their target receptor directly from renal tubular fluid and not from the blood. Plasma concentrations control the amount of drug (or metabolites) excreted in urine. As such, urine drug concentrations may be viewed as

a surrogate of plasma concentrations. However, urine concentrations may also be influenced by many other factors such as urine volume and pH (for ionisable drugs) rendering the relationship between plasma and urine concentrations imprecise. The urine-to-plasma concentration ratio (Rss) varied very considerably between drugs and is also a time dependent variable. It is equal to zero just after an IV drug administration (i.e. when drug effect may be near maximal as for an anaesthetic drug) and it becomes only "invariant" i.e. a useful "parameter" after some delay i.e. when an equilibrium between plasma and urine concentrations is achieved. For a multiple dose administration regimen (and whatever the route of drug administration), the relationship between the plasma and urine concentration may be confounded by a hysteresis (lag-time between plasma and urine concentrations) and it is possible to have plasma and urine concentrations out of phase. In this situation, a peak effect may correspond to the trough urine concentrations. For some drugs, there is no (or very low) renal clearance and for that class of drugs urine is not an appropriate matrix for testing. For proteins, the renal clearance of the intact molecule is generally negligible due to the high protease activity in the proximal tubule of the nephron (some exceptions exist such as for GH and EPO) rendering urine unsuitable for monitoring many peptides or proteins of potential abuse. In addition, in man, proteases may be added fraudulently to the urethra rendering it difficult to detect protein in the urine (Thevis et al. 2008; Thevis and Schanzer 2007). Conversely, metabolic reactions of bacterial origin may occur in urine samples (for example for some corticosteroids) spuriously increasing the concentration of the analyte of interest after the sampling. For all these reasons, urine is a less robust matrix than plasma and the parent plasma drug concentration is generally the best analyte to select and to assess the systemic drug effect. The main consideration for changing from urine to plasma to enforce a medication control policy is an analytical issue, because for most drugs urine drug concentrations are higher or even much higher than plasma concentrations.

Other matrices are usable for doping control such as hair and faeces. Thanks to the major advances in analytical methodology, hair analysis may provide additional analytical evidence to that obtained from blood or urine analyses (Dunnett and Lees 2003; Popot et al. 2002). Hair is a very stable medium, in which drugs and their metabolites can be detected over prolonged periods. Hair analysis can thus provide a historical record of drug exposure for some critical drugs such as anabolic steroids. Hair seems more suitable for population surveys and investigation surveillance than for routine individual doping control. The limitation of hair as a matrix is a possible contamination of the sample from external sources such as urine, sweat from another horse etc.

It is known that endogenous steroids and different xenobiotics are eliminated by faeces and faeces may be an attractive alternative matrix to collect in yearlings for safety reasons. The presence of boldenone in horse faeces was confirmed after an oral administration of 1,4-androstadiène-3,17-dione and meclofenamic acid was detected for 6 days post-administration (Popot et al. 2004). For pigeon racing, taking blood for routine drug testing is too invasive to be acceptable for pre-race testing and faeces (actually a mixture of faeces and urine) is the appropriate matrix (de Kock et al. 2004).

6 Substances Requiring a Threshold

Horses may be regularly exposed to prohibited substances that are natural components of their feed. Salicylic acid (SA) is a stress plant substance found in many plants including alfalfa (lucerne) which explains the natural occurrence of SA in horse urine and the possible detection of SA in all post-race urine samples. As SA is the active metabolite of aspirin, a NSAID, SA is a prohibited substance and without a threshold, it would be necessary to report all these innocent positive cases. Dimethyl sulfoxide is another example of an ubiquitous natural product. Horses may also inadvertently be exposed to substances that are contaminants of manufactured feeds (e.g. theobromine due to presence of cocoa husks in feed) or by contaminants coming from the environment (e.g. arsenic). The concept of threshold was introduced to solve these unavoidable exposures of alimentary origin (Houghton 1994) i.e. when it was considered there was no other management option to solve the problem of innocent positive samples. For SA a threshold was fixed at 750 μg/mL (see Table 1) because natural exposure cannot result in a urine SA concentration above this cut-off value with a risk of about 1 in 10,000. The threshold was recently re-investigated and it was shown that a threshold of, 614 μg/mL, in urine was more suitable (Lakhani et al. 2004). For some other substances contaminating equine feed, no threshold has been fixed, because it was considered as undesirable in terms of communications for the industry to release such a threshold. This is the case for morphine (contamination by poppy seed) and for benzoylecgonine which is a metabolite of cocaine.

In addition to these exogenous substances, some endogenous hormonal substances can be administered, either to rest or a "natural" hormonal profile as is the case for testosterone in a gelding or to obtain an overexposure to achieve some pharmacological effects as is the case for cortisol which is a psychostimulant. Two approaches are used to fix a threshold: either to fix a single cut-off value as for cortisol in urine (1.0 μg/mL) or rather to use a concentration ratio between a marker of the administered compound (the substance itself or one of its metabolites) and another endogenous substance that plays the role of an "internal standard", i.e. an analyte structurally related but that is not metabolically related to the administered substance of concern. The logic of selecting a ratio rather a single cut-off value is the assumption that a ratio will be less variable regarding inter-subject differences and to possibly benefit from some negative feedback which may amplify the shift of the ratio in the case of exogenous administration. This is the case for the ratio testosterone/epitestosterone in man, used for the control of testosterone administration or for the ratio estranediol over the 5-estrene-3beta,17alpha diol for the control of nandrolone in colts. In the case of exogenous testosterone administration, the numerator (testosterone) is increased as expected, whereas the denominator (epitestosterone a substance that is produced only locally by the transformation of endogenous testosterone in the testis) is reduced by the negative feedback on the natural testosterone production in the testis. This possible advantage of a ratio should be balanced against the ability to manipulate a ratio by also administering

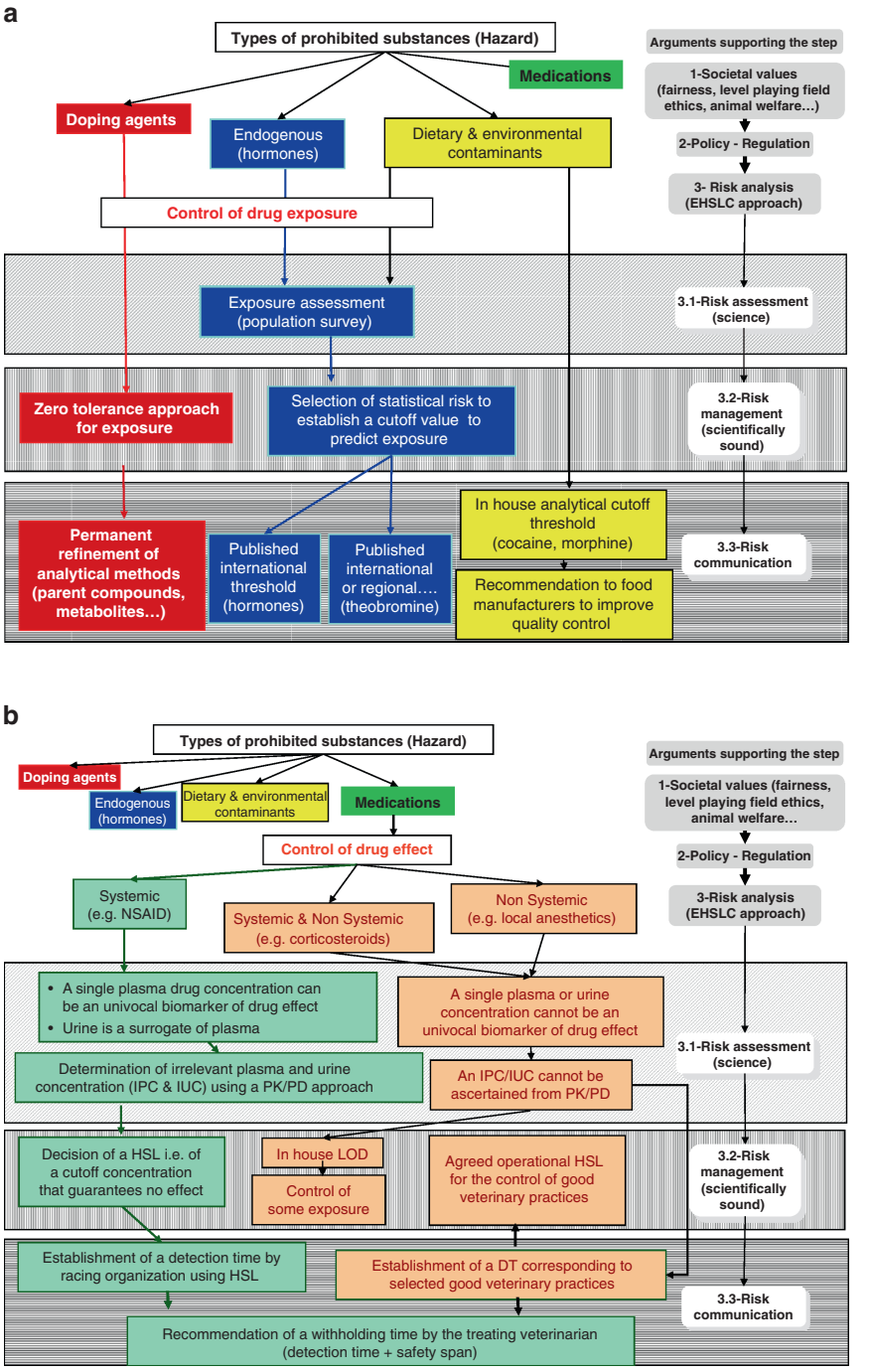
the "internal standard" to maintain the ratio value in its physiological range. In addition, the ratio approach is more challenging and time consuming from an analytical perspective, especially if one of the analytes is suppressed by negative feedback. For that reason, the principle of a single testosterone cut-off was selected in horses.

The establishment of a threshold requires the analysis of a large number of representative (international) samples (e.g. post-competition samples) collected from the future targeted population(s) and some administration/food trials. The data set is then statistically analysed with the aim of determining a critical value corresponding to a given population quantile. As generally the number of samples is too low to select directly a quantile (e.g. 1/10,000), the critical value is calculated from the observed or assumed distribution. Very often, the data are not normally distributed but positively skewed as for example for the log-normal distribution. The selection of an appropriate transformation is critical because the threshold that is subsequently calculated, for a given nominal risk, may be very different depending on the selected distribution. For example, both a log-normal and a cube root transformation were able to normalise the observed urine cortisol distribution but the cut-off value for a 1/10,000 quantile was 1,025 ng/mL (rounded to 1,000 ng/mL) with the log-normal distribution against 410 ng/mL for the cube root transformation; finally the most conservative cut-off (from the horse's perspective) was selected (Popot et al. 1997). There is no single accepted critical quantile but the case of SA likely created a precedent and quantiles lying between 1/1,000 and 1/32,000 are generally selected (Houghton and Crone 2000).

Due to regional differences in food ingested (e.g. Lucerne hay in the USA versus grass hay in Europe) and feed contamination, it may be difficult and/or unsatisfactory to fix a single international threshold covering with the same statistic routinely at risk to all horses in the world. It may be more meaningful to develop regional thresholds reflecting local practises and constraints. The logic used in establishing the theobromine threshold was different; it consisted of feeding horses with feed contaminated with different theobromine concentrations knowing that the maximal expected food contamination cannot be higher than 1.2 mg/kg. When horses were fed with this diet, the maximal urine concentrations were less than 0.60 μg/mL and the threshold was fixed to 2 μg/mL (Houghton and Crone 2000).

7 Testing Exposure and the End of a Zero Tolerance Approach for Medication Control

For doping drugs, i.e. illicit substances, with no accepted medical use in horses, the goal is to control any drug exposure (parent drug or metabolites) using the most powerful analytical methods. Although ideal for doping control, the "zero tolerance rule" is not suitable for medication control (Smith 2000; Spencer et al. 2008).


a
Types of prohibited substances (Hazard)
Medications
Doping agents
Endogenous (hormones)
Dietary & environmental contaminants
Control of drug exposure
Exposure assessment (population survey)
Zero tolerance approach for exposure
Selection of statistical risk to establish a cutoff value to predict exposure
Permanent refinement of analytical methods (parent compounds, metabolites...)
Published international threshold (hormones)
Published international or regional.... (theobromine)
In house analytical cutoff threshold (cocaine, morphine)
Recommendation to food manufacturers to improve quality control
Arguments supporting the step
1-Societal values (fairness, level playing field ethics, animal welfare...)
2-Policy - Regulation
3- Risk analysis (EHSLC approach)
3.1-Risk assessment (science)
3.2-Risk management (scientifically sound)
3.3-Risk communication
b
Types of prohibited substances (Hazard)
Doping agents
Endogenous (hormones)
Dietary & environmental contaminants
Medications
Control of drug effect
Systemic (e.g. NSAID)
Systemic & Non Systemic (e.g. corticosteroids)
Non Systemic (e.g. local anesthetics)
• A single plasma drug concentration can be an univocal biomarker of drug effect
• Urine is a surrogate of plasma
Determination of irrelevant plasma and urine concentration (IPC & IUC) using a PK/PD approach
A single plasma or urine concentration cannot be an univocal biomarker of drug effect
An IPC/IUC cannot be ascertained from PK/PD
Decision of a HSL i.e. of a cutoff concentration that guarantees no effect
In house LOD
Control of some exposure
Agreed operational HSL for the control of good veterinary practices
Establishment of a detection time by racing organization using HSL
Establishment of a DT corresponding to selected good veterinary practices
Recommendation of a withholding time by the treating veterinarian (detection time + safety span)
Arguments supporting the step
1-Societal values (fairness, level playing field ethics, animal welfare...
2-Policy - Regulation
3-Risk analysis (EHSLC approach)
3.1-Risk assessment (science)
3.2-Risk management (scientifically sound)
3.3-Risk communication

Currently, the same powerful analytical processes are used to screen for all substances, regardless of their potencies or their regulatory status. The consequence is that trace concentrations of therapeutic substances, totally irrelevant in terms of clinical or physiological effects, may now be detected for a long time (days or weeks) after their therapeutic administration. As such the zero tolerance policy is inappropriate for medication control and this opens the way to a new approach for legitimate medication based upon PK/PD principles to estimate the order of magnitude of the so-called irrelevant drug concentrations in plasma and urine (Toutain and Lassourd 2002a) and to limit the sensitivity of analytical techniques used for medication control (*vide infra*). Smith (2000) addressed the background for a

Fig. 1 Risk analysis applied to doping and medical control. Depending on the values claimed by the various organisations, prohibited substances are classified under 4 categories: doping (illicit), endogenous (hormones, CO_2, etc), dietary or environmental contaminants and medications (legitimate drugs). The final policy applied to each substance results from a risk analysis consisting of three different steps: RA, RM and RC. The RA is a wholly scientific exercise performed by risk assessors (scientists) and aimed at providing the risk manager with initial scientific data to perform the RM exercise. For doping substances, there is no scientific assessment as these drugs are a priori considered as illicit. For endogenous or dietary contaminants, scientists have to qualify the exposure in the target population (population distribution) and for medication control scientists have to provide the order of magnitude for which an exposure has an effect or not. This is done by computing irrelevant plasma (IPC) or IUC. The next step is the RM. This step is performed by risk managers (typically racing authorities) who are not scientists but nevertheless the RM should be a scientifically sound exercise. The risk manager has to decide the statistical level of risk for an endogenous product, or to decide an analytical cut-off value for screening of medications. For this, the risk manager may mitigate the IPC and IUC as determined by risk assessors considering the possibility or not of harmonising, the cost of analytical techniques, and the feasibility etc. The final decision will be a threshold value (endogenous substance or contaminant) or a harmonised screening level (HSL). For some drugs acting both locally and systemically, there is no single concentration value which covers every situation and the risk manager has to select some options (liberal or conservative). For example, to control intra-articular corticoid administration, it would be necessary to fix the HSL at a very low level (few pg/mL in urine) but in doing so, it would be impossible to use the same corticoid for a systemic administration because a urine concentration of a few pg per mL for a systemic treatment has no meaning. Conversely, determining the IPC and IUC corresponding to a systemic treatment using the PK/PD approach would not be conservative enough for an intra-articular administration. The strategy adopted by the manager may be to determine an HSL in order to support good veterinary practise and not to guarantee a lack of any effect for any route of administration for that drug (local or systemic). For example, for a local anaesthetic, good veterinary practise would consist in not using it within the 2 days preceding a race. To be consistent with this rule, the risk manager can adopt an HSL high enough to have no positive control for those observing this delay. Actually, to control good veterinary practise consists firstly, to select a withholding time (regulatory or professional considerations) and then, to establish the corresponding plasma/urine concentration. The last step is a communication step. For doping control it is explained to all stakeholders that the institution controls any exposure and that there will be a continuing improvement of the analytical techniques. For medication control, the pivotal item of communication is the detection time. Detection time is established by racing authorities (or FEI) to give to the practitioner an order of magnitude of the future withholding time. This withholding time is a veterinary decision and the practitioner should perform his own risk analysis to fix the withholding time by adding a safety span to the detection time established by racing organisations

consideration of this approach (see also Houghton (1994); Tobin et al. 1998, 1999); (Spencer et al. (2008) for reviews).

8 The Decision Making Process on No Significant Effect Levels: A Risk Analysis Integrated Approach

To solve the dilemma of whether or not to report trace levels of drugs used legitimately for therapeutic medication, the EHLSC developed a general approach following the principle of risk analysis. Figure 1 gives an overview of risk analysis for doping/medication control.

A risk analysis is a structured (formalised) approach using risk concepts. I It includes three steps: risk assessment (RA), risk management (RM) and risk communication (RC). The reasons for adopting a risk analysis are when harmonisation is a requirement, regulatory decisions need to take into account competing interests using an unbiased and transparent approach.

Before developing any risk analysis, an institution (FEI, EHLSC, IFHA) should express formally what its values and standards are, i.e. its ideal rules of conduct. For the EHSLC, this includes giving priority to protect the welfare of the horse, to defend the integrity of the sport, to protect the breed and to reassure the public.

8.1 Risk Assessment

The RA is a scientifically based process comprising the following steps: (1) hazard identification, (2) hazard characterisation, (3) exposure assessment and (4) risk characterisation.

The hazard identification consists of identifying the hazardous agent that may result in a negative (harmful) impact and the "receptors", that is the specific things or entities affected by the hazard. For medication and doping control, hazardous agents are legitimate and illegitimate drugs, contaminants and endogenous products. Receptors are horses (animal welfare), punters and public (betting, public concern), Institutions (trust and confidence in regulation) and owners and trainers (business).

Hazard characterisation is the qualitative and/or quantitative evaluation of the nature of the adverse effects associated with the hazard. For medication control, a non-experimental PK/PD approach to determine irrelevant drug plasma concentrations (IPC) and IUC has been proposed (Toutain and Lassourd 2002a, b).This non-experimental method consists of retrieving published PK parameters and variables to calculate IPC and IUC as follows: consider that an effective dose is a PK/PD hybrid variable determined by two PK parameters (plasma clearance and

bioavailability) and one PD parameter i.e. the effective plasma concentration (EPC) as given by (1)

$$\text{Effective dose rate} = \frac{\text{plasma clearance} \times \text{effective plasma concentration}}{\text{bioavailability}}. \quad (1)$$

It was suggested that (1) can be rearranged to estimate the EPC for a standard approved dosage regimen as (2):

$$\text{EPC} = \frac{\text{standard dose (per dosing interval)}}{\text{plasma clearance (per dosing interval)}}, \quad (2)$$

where plasma clearance is the genuine PK parameter that expresses the ability to eliminate a drug.

As only the intravenous route of administration was considered for this RA, bioavailability in (1) was fixed at 1. EPC may be taken as the average plasma concentrations over the dosing interval following the administration of an effective dose and, as such, EPC is a relevant surrogate of PD or clinical endpoints.

The next step involves transforming an EPC into an IPC and then into an IUC.

The IPC and IUC are defined as drug plasma (serum) or urine concentrations that guarantee the absence of any relevant drug effect.

The IPC can be deduced from the EPC by applying a safety (uncertainty) factor (SF) to EPC (3):

$$\text{IPC} = \text{EPC}/\text{SF}. \quad (3)$$

The selection of a SF is both a scientific and a regulatory decision. A default value of 500 (i.e. 50 $\times$ 10) has been suggested for the following reasons: firstly a factor of 50 was selected to transform a mean EPC into a mean IPC. This figure assumes that horse medications are marketed at a dose corresponding to (or at least similar to) their ED_{50} i.e. to a dose level able to achieve half the maximal possible effect of that drug; and that the dose effect relationship is described by a classical E_{max} (hyperbolic) model. If these two hypotheses hold, then dividing the ED_{50} by 50 leads to the estimation of an ED_2 i.e. the dose corresponding to only 2% of the maximal possible effect of that drug. Secondly, in order to take into account the inter-individual variability of PK and PD parameters in the horse population, a factor of 10 was selected (i.e. 3.3 for PK variability and 3.3 for PD variability). This factor of 3 is the common standard in toxicology and is known as the rule of 3s (CV$\approx$ 25%) regardless of the source of uncertainty. Thus, the IPC may be viewed as a lower boundary of a population plasma concentration corresponding to a residual drug effect of 2%. It may be noted that fixing the SF to infinity would be equivalent to following the zero tolerance rule i.e. from an operational point of view, to control drug exposure with the highest performance analytical techniques, as for illicit substances.

Next, the IUC is derived from the IPC using (4):

$$\text{IUC} = \text{IPC} \times \text{Rss}. \tag{4}$$

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

The main difficulty with IUC is the uncertainty of the retrieved Rss. Rss is seldom reported and difficult to evaluate. It is influenced by several biological factors (see Sect. 6) and a given snapshot urine concentration may correspond to very different plasma concentrations (and effects), because there is no guarantee that the horse is in a pseudo-equilibrium condition (single dose) or under steady-state conditions (multiple doses) at the time of sampling.

This inexpensive and straightforward approach requires that the marketed effective dose rate is actually appropriate. Drugs eligible for the PK/PD model must act systemically, i.e. the pharmacological effect should be directly governed by the plasma concentration. Thus, local anaesthetics, substances administered intra-articularly or by inhalation are not considered suitable candidates for analysis using this PK/PD model.

Finally, the proposed hazard characterisation method aims at determining an order of magnitude of the required sensitivity of the analytical technique, and IPC and IUC are starting values that will be used during the RM process to decide a screening limit.

8.1.1 Exposure Assessment

There is no risk without exposure, and for medication or doping control, the question of the origin of the exposure, i.e. how will exposure occur (sources assessment), is generally simple to identify. Inquiries following positive cases show that most often it is some kind of error (e.g. inappropriate large dose for intra-articular corticosteroid administration), bad veterinary practise (use of a drug without marketing authorisation and for which no information exists for rational use), lack of observance of a minimal withholding time by the trainer... and also cheating. Sometimes the source of exposure is more difficult to identify. It was observed that drugs can be detected in horse urine for a longer time than is expected from their intrinsic PK properties (Lees et al. 1986). Norgren et al. (2000) and Wennerlund et al. (2000) reported that flunixin or naproxen were detected in the urine of untreated horses that resided for several days in boxes previously allocated to horses treated with flunixin or naproxen. This suggests some cross contamination via the bedding. Possible contamination by ingesting straw contaminated by urine was also observed for dipyrone, chlorpheniramine and procaine and well documented for meclofenamic acid (Popot et al. 2007). Hence, it was concluded that spurious urinary drug rebound may lead to some positive controls after the recommended withholding time.

The question of exposure assessment is more demanding for compounds requiring the establishment of a regulatory threshold because the statistical distribution of the analyte of interest should be qualified.

8.1.2 Risk Characterisation

Risk characterisation is the last step of RA. It is an estimation of the severity of the "adverse effect" and it involves integration of the hazard, hazard characterisation, and exposure and should be expressed numerically to the risk manager. For illicit substances, it will be the "minimal" limit of detection (LOD) required for an analytical technique or the minimal performance to achieve for any other marker of exposure such as an antibody for growth hormone or EPO. For medication control, the IPC and IUC as calculated during the RA step will be provided to allow risk managers to propose a screening LOD. For endogenous analytes, a statistical distribution of the concentration of interest will be given, allowing risk managers to fix a local, regional or international threshold. For feed contaminants, it should be proposed to risk managers that they either fix a threshold (using the same approach as for endogenous product) or, alternatively, that some measure of correction be suggested to the manufacturer.

8.2 Risk Management

RM is the process of weighing policy alternatives in the light of the RA in order to minimise or reduce the assessed risk. It consists of selecting and implementing appropriate options such as prevention, control and regulatory measures. RM is not a scientific exercise but it should be scientifically sound. Sound science does not exist as a "ready for use" entity in the policy development process, so that scientific data should be subjected to a reasoned interpretation for regulatory purposes. RM is carried out by risk managers i.e. the racing authorities. They have to make decisions based not only scientific facts, but also on all relevant information from other sources including the specific values and criteria of their own organisation. For example, the FEI considers that omeprazole, a proton pump inhibitor extensively used for ulcer control, is not a prohibited substance for international horse sporting competitions and, as such, does not have to be included in a screening programme for medication control. On the other hand, it is a prohibited substance for European racing organisations. Similarly, furosemide is accepted by many US jurisdictions for the prevention of EIPH but is prohibited in Europe for reasons explained in Sect. 4. At first glance, this seems illogical and inappropriate but it should be acknowledged that science is not always able to resolve societal choices concerning what decisions to take in the case of competing interests. Science can describe the world, but science cannot determine what the world should be. Therefore, different regulatory jurisdictions may reach markedly different regulatory conclusions, based upon the same set of scientific data. For this reason, international standardisation should focus on the process of RA, which is primarily a scientific task, rather than on the harmonisation of risk criteria and RM.

For medication control, the main task at the RM step is the establishment of agreed HSL for all laboratories engaged in the EHLSC programme. The HSL is a

confidential instruction to laboratories from Racing Authorities to screen at a plasma or urine level for the presence of drugs commonly used in equine medication. The HSL is deduced from the IPC (for plasma) or from the IUC (for urine), established during the RA exercise but the HSL may be (slightly) higher or lower than the IPC/IUC to take into account other relevant factors than residual drug efficacy as the common goal to achieve harmonisation. Thus, it is verified that all countries are in a position to enforce the selected HSL. It should be stressed that HSLs are not equivalent to specific quantitative thresholds; they are decisions resulting from a RM exercise. Monitoring the HSLs through screening procedures will greatly simplify the analytical process compared to the use of absolute quantification. HSLs are not considered as threshold values.

For food contaminants, the most efficient means of avoiding inadvertent positive cases is to test the feeds. When this is impossible in practise, an analytical threshold is selected and the value selected by the risk manager is the statistical risk (usually approximately 1 in 10,000).

8.3 Risk Communication: Detection Times Versus Withdrawal Times

RC is an integral part of the risk analysis process: it is the interactive exchange of information and opinions between risk assessors, risk managers and stakeholders. Efficient RC requires the provision of meaningful, relevant and accurate information in clear and understandable terms. It is targeted at specific audiences (trainers, veterinarians, punters, etc) in order to improve the overall effectiveness of the control process. For medication control, it is evident that a screening limit does not fulfill these requirements, because a cut-off plasma/urine concentration does not comprise “ready for use” information for veterinarians who must advise owners or trainers on appropriate withholding times. Therefore, the EHLSC decided to keep this information confidential and rather to communicate the duration of the DT of the main medications when screening is performed, with the harmonised but unavailable screening limit. This led the EHLSC to embark on a programme to define DTs for the major veterinary medicinal products by conducting a series of standardised excretion studies.

A DT, according to the EHSLC definition, is the time at which the urinary (or plasma) concentrations of a drug, in all horses involved in a particular trial conducted according to the EHSLC guidance rules, are shown to be lower than the HSL when controls are performed using routine screening methods. It should be stressed that the DTs, as issued by the EHSLC (and followed by the FEI, see the FEI web site), are not synonymous with withdrawal times (WT). A DT is a raw experimental observation, whereas a WT is a recommendation and, as such, requires the professional judgement of the treating veterinarian. A WT should be longer than a DT because the WT should take into account the impact of all sources of animal variability (age, sex, breed, training, racing) as well as the variabilities associated with the medicinal product

actually administered (formulation, route of administration, dosage regimen, duration of treatment) in order to avoid a positive test.

One of the possible limitations of the information provided by published DTs in horses is the fact that they are determined from classic PK studies conducted in animals at rest and performed under laboratory conditions on a limited number of horses (generally 6 or 8). Under field conditions, training and exercise programmes may influence the rate of drug elimination. In horses, there is virtually no experimental data on the direct effect of exercise on drug disposition and hence on DT. To explore the possible influence of exercise on the DT as obtained under the EHSLC conditions, a trial was conducted to compare the PK disposition of two tests drugs [(phenylbutazone (PBZ) and dexamethasone (DXM)] under resting conditions and in conditions involving a 3-h endurance-type exercise. It was shown that a sustained mild test exercise moderately decreased the plasma clearance of both drugs (approximately 25% for DXM and 37% for PBZ). However, as the volume of distribution was correlatively decreased, the plasma terminal half-life, which is a hybrid parameter of plasma clearance and of volume distribution, remained unchanged overall (Authié et al. 2009). This is of relevance for establishing DTs and WTs, as plasma and urine half-lives, not clearance, are the main determinants of the length of the DT. More generally, it can be hypothesised that a race lasting a few minutes only will not markedly alter residual drug concentrations in plasma or urine at the control sampling times i.e. at a time when most of the drug has already been eliminated. Indeed, under European racing rules, the shortest WT is 48 h because a race horse cannot be treated with any drugs within the 2 days preceding a race.

9 From a Detection Time to a Withdrawal Time

It should be re-emphasised that a DT, as issued by the EHLSC, is not equivalent to a WT. An appropriate safety span must be considered when extrapolating a WT from a DT published by the EHLSC. The length of the safety margin required to transform a DT to a WT remains unclear. To help the veterinarian select a WT from a published DT the question of a safety span was explored using Monte Carlo Simulations (MCSs) (Toutain 2009). A Monte Carlo simulation is a numerical method with a built-in random process that involves assessing the impact of variability due to different sources. In this instance there are two main sources of variability. Firstly, there is intrinsic biological variability between horses for PK parameters controlling plasma and urine drug disposition (i.e. plasma clearance, volume of distribution, urine-to-plasma ratio). These sources of variability are explained by factors such as breed, age, sex, weight. Secondly, there are the various sources of uncertainty associated with the veterinary decision and/or trainer practise concerning the actual administered dose, uncertainty due to approximate estimation of the actual body weight, the administration of a dosage form different from that tested by the EHLSC, modalities of administration, trained/untrained conditions

etc. Using MCSs, 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. In other words, MCSs may replace a large population survey aimed at establishing a WT experimentally.

Using MCSs, it was shown that for a low variability of PK parameters (CV = 20%), an uncertainty span of about 40% may be selected to transform a mean EHLSC DT to a WT (i.e. WT = 1.4DT), which encompasses 90% of the horse population. In contrast, for a highly variable drug (CV = 40%), the uncertainty factor is of the order of 2.1–2.2 (i.e. the WT should be approximately twice the DT). In addition, MCSs suggested that the variability in DTs will be influenced mainly by inter-animal variability and that either more or less reliable veterinary practises will have only a minimal impact on DT, because the main sources of variability for a DT are of a biological nature. A consequence of this is that DTs, as released by the EHLSC, are likely to be of generic value for other countries having different veterinary practises (slightly different dosage regimens, different formulations or routes of administration) but having similar horses to those used in the EHLSC trials. This could be a relevant argument to promote and support harmonisation between countries.

It should be stressed that the ultimate goal of the ESHLC is to propose a DT, for which a lack of drug effect can be assumed at the time of racing. For the EHLSC, the regulated parameter must be a screening LOD that guarantees a lack of drug effect at the time of racing. An HSL is a property of the drug (*substance*) that may easily be reported by a single universal (international) value while DTs are a *formulation* property (except for administration by the IV route). Consequently, there can be no universal DT value for a given drug but rather as many DTs as there are commercial formulations and indeed for a given formulation as many DTs as possible routes of administration, dosage regimens etc. This renders an international harmonisation of DT with a necessary statistical protection an unachievable illusion.

10 Conclusion

Athletes decide for themselves if they wish to take drugs, horses do not (Higgins 2006) and practically all equine organisations and jurisdictions (racing, sport) claim that horse welfare is the priority. Despite this goal substantial differences in approach still exist between America and most other countries in the world.

The pivotal aspect of these shared values is a clear distinction between doping control and medication control, with the requirement to limit the sensitivity of the analytical techniques to prevent positive cases that could be due to residual presence of legitimate drugs at concentrations without any biological relevance.

As this goal has now almost been achieved, new horizons are opening through new doping practises (including gene doping) and by the use of substances difficult to screen and/or to detect by traditional approaches.

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