trainers or owners who have lost races and prize money; and, arguably, there would be no advantage to the racing authority.

Consider, also, a third hypothetical laboratory that can detect agents that could pose a serious threat to the integrity of racing, for example α2-agonists, benzodiazepines or amphetamines, only at concentrations that do not act as an effective deterrent against their misuse. By not developing appropriate methods, this laboratory would be governed more by methodological considerations than by the need to avert the threat to racing. Horses, punters and administrators all lose out here.

Analytical chemists require guidance on the potential of chemical agents for abuse and on priorities for method development. Dialogue between racing authorities/veterinarians and laboratories provides guidance on the potential of chemical agents for abuse and can thus establish priorities for method development for drugs.

One solution may be for racing authorities to issue guidelines to their laboratories indicating the reporting limits believed to be appropriate. It would then be the responsibility of the laboratories to decide whether this could best be achieved by methodological manipulation or by semi quantification. The appropriate limits would be determined by a full veterinary and pharmacological review and could most easily be set for agents which are routinely used for the legitimate veterinary treatment of horses and which have a predictable systemic effect. Essentially, the reporting limit would be set to distinguish between exposure to the agent at some time in the past and the possibility that it might be present in the body in a sufficient quantity to produce a pharmacological effect.

Exceptions to this approach would be drugs used in combination, or those with no legitimate veterinary use. Here, detection would be desirable at the lowest possible level and it may be advantageous to detect exposure to some of these agents by methods other than post race sampling, for example by hair analysis. Similarly, the use of medication in training should be controlled by tests carried out in the training environment, rather than by the detection at levels which indicate exposure to the agent, rather than pharmacological effect.

Defined reporting limits should not be confused with thresholds. For threshold substances, sub-threshold levels of a substance are not considered prohibited. All non-threshold substances would remain prohibited once their presence is confirmed in a sample, but it would be the racing authorities, not the analytical method, which would determine the reporting limit for a particular substance. Findings below this level would be of no concern and therefore confirmation would not be necessary.

'NO-EFFECT LIMITS': AN OVERVIEW AND A PHARMACOLOGICAL APPROACH

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ABSTRACT

The sensitivity of current equine drug testing methods enables detection of pharmacologically insignificant residues of therapeutic medications. In 1995, the Association of Racing Commissioners International resolved that members should *"address **trace** level detection so as not to lead to disciplinary action based on **pharmacologically insignificant traces** of these substances"*. This paper describes an approach to implementing this resolution using local anaesthetics as a model system.

First, the target pharmacological effect (local anaesthesia) is identified and then the dose response relationship is quantified. From these data the 'highest no effect dose' (HNED) is calculated and administered. The target analyte is identified, synthesised if required and quantified in blood or urine. The concentrations measured after administration of the HNED are, by definition, pharmacologically insignificant.

Regulatory 'limits' below this concentration are therefore 'no effect limits' or 'no effect thresholds' (NETs), or 'no effect cut-offs'. This approach can also identify highly potent medications for which improved testing may be required.

More recent work (Lehner *et al.* 2001), describes the use of LC/MS/MS technology for serum based detection of clenbuterol. This suggests a rapidly increasing role for serum based 'limits', thereby avoiding many of the technical problems associated with urinary limits.

The data obtained create an analytical/ pharmacological database that should assist industry professionals in interpreting the pharmacological significance of medication residues in official samples. The optimal outcome of this approach is a more appropriate use of therapeutic medication in performance horses, with associated benefits for the health and welfare of these horses.

BACKGROUND

This review will propose the need for 'limits' on the sensitivity of testing for certain medications recognised as therapeutic agents in horses. Such a need is already well established for environmental, dietary and endogenous substances, where the unanimous approach has been the development of specific 'thresholds' (Tobin 1995).

This paper will present the case for similar limits for certain therapeutic medications. For the purposes of this review, a therapeutic medication is an agent identified as such by the American Association of Equine Practitioners (Table 1).

In 1995, the Association of Racing Commissioners International (ARCI) adopted a resolution, the final 2 paragraphs of which are as follows:

"The Association of Racing Commissioners International strongly recommends that its membership adopt a policy that all chemical findings in official test samples undergo a documented review process by the official veterinarian or appropriate veterinary consultant prior to the initiation of any regulatory action.

And, further, the RCI recommends that its members specifically implement procedures to have an official veterinarian or veterinary consultant review findings for RCI class 4 and 5 substances to address 'trace' level detection so as not to lead to disciplinary action based on pharmacologically insignificant traces of these substances."

The final phrase of these paragraphs: *"pharmacologically insignificant traces of these substances"* highlights the scientific challenge, which is to show, with reasonable certainty, that science can distinguish between pharmacologically significant and pharmacologically insignificant concentrations of these analytes. This review outlines a theoretical and practical basis for such distinctions.

TABLE 1: American Association of Equine Practitioners' Therapeutic Medications List

Name	ARCI Class	Name	ARCI Class	Name	ARCI Class
Diazepam	2	Terbutaline	3	Ketoprofen	4
Fluphenazine	2	Xylazine	3	Meclofenamic Acid	4
Hydroxyzine	2			Methocarbamol	4
Ketamine	2	Acetysalicylic Acid	4	Methylergonovine	4
Lidocaine	2	Aminocaproic Acid	4	Methylprednisolone	4
Mepivacaine	2	Betamethasone	4	Nandrolone	4
Reserpine	2	Boldenone	4	Naproxen	4
		Dantrolene	4	Phenytoin	4
Acepromazine	3	Dembroxol	4	Prednisolone	4
Albuterol	3	(Dembrexine)		Prednisone	4
Aminophylline	3	Dexamethasone	4	Stanozolol	4
Atropine	3	Dipyrone	4	Testosterone	4
Butorphanol	3	Flumethasone	4	Thiosalicylate	4
Clenbuterol	3	Flunixin	4	Triamcinolone	4
Detomidine	3	Guaifenesin	4	Trichlormethiazide	4
Glycopyrrolate	3	Hydrocortisone	4		
Pentazocine	3	(Cortisol)		Cimetidine	5
Pentoxifylline	3	Ibuprofen	4	Cromolyn	5
Procaine	3	Isoflupredone	4	Dimethylsulfoxide	5
Promazine	3	(Fluroprednisolone)		Dimethylsulphone	5
Pyrilamine	3	Isoxsuprine	4	Ranitidine	5

This table was generated by circulating a list of several hundred medications to AAEP members and asking them to indicate which agents they used routinely in their practice. The data were collected and reviewed by the AAEP and presented for publication as Appendix G in the Proceedings of the 'Testing of Therapeutic Medications, Environmental and Dietary Substances in Racing Horses', pp191-192, 1995, Lexington, Kentucky.

Definitions

For the purposes of this review, a 'limit', 'threshold', 'cut-off' or 'reporting level' is any defined drug or metabolite concentration in a biological fluid that relates to a regulatory event. The terms 'limit', 'threshold', 'cut-off', and 'limitation on the sensitivity of testing' are equivalent in scientific and regulatory terms. The term 'limit' will be used as the standard descriptor for this concept.

The goal of this research is to identify 'no effect points', which are specific, pharmacologically defined concentrations in biological fluids at or below which the residues of the agents in question are *'pharmacologically insignificant'*, as set forth in the 1995 ARCI resolution. Any defined concentration of the medication or its metabolite found in a biological sample below this 'no effect point' is a 'no effect limit', a 'no effect threshold', a 'no effect trace', a 'no effect cut-off', a 'no effect limitation on the sensitivity of testing', a 'sub-therapeutic residue' or a no-effect 'reporting level' and is a *pharmacologically insignificant trace*, as set forth in the ARCI resolution above.

The use of these broadly equivalent definitions of what one author (TT) initially chose to call a 'no-effect threshold' may seem redundant. This problem, however, has been approached by many groups worldwide (Tobin 1995) and each group has selected different descriptors. To make the close relationship between these descriptors apparent, their scientific equivalence is emphasised in this review. Additionally, for the purposes of the review, the word 'limit' will be used to identify the 'no effect limits', 'no effect cut-offs', 'no-effect thresholds', 'sub-therapeutic residues' or no-effect 'reporting levels', all of which represent *pharmacologically insignificant traces.*

Number of drug molecules

One source of misunderstanding is the lack of understanding that all drugs and medications are present in biological samples collected from horses long after the pharmacological effects are over, and long after analytical methods cease to detect them (Tobin *et al.* 1982).

A useful perspective on this problem is to start with the actual number of drug molecules administered, which can be as high as about 10^{22} (Table 2). This extremely large number explains the principal reason for some medications being retained in horses at low but detectable concentrations for relatively long periods.

An example is a typical dose of phenylbutazone. If a half-life for phenylbutazone of 7.22 h is assumed, 90% of the remaining drug, or one log unit of phenylbutazone, is eliminated each day (Fig 1a). A typical dose of phenylbutazone in a

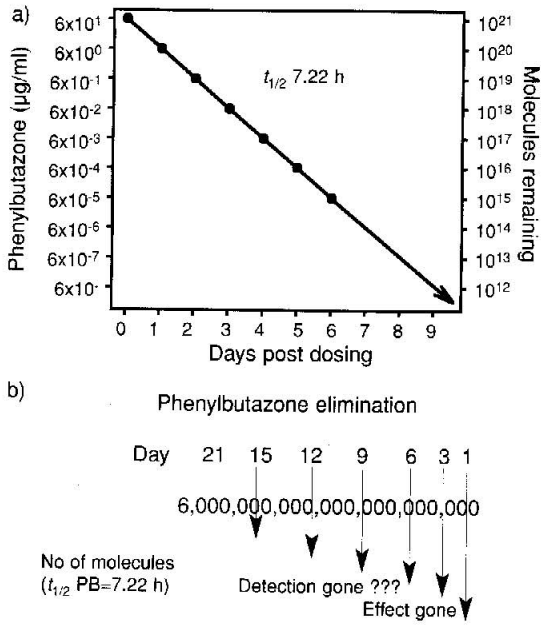

Fig 1: (a) Elimination of phenylbutazone molecules following administration assuming a half-life of 7.22 h; (b) illustration of the loss of pharmacological effects in relationship to the point where the agent is no longer detectable. According to these simple models, the last phenylbutazone molecules are being eliminated at about 21 days post dosing.

horse contains about 10^{21} molecules, and it will take 21 days to eliminate the entire amount of drug. In practice, phenylbutazone or its metabolites can be detected in urine samples for up to 14 days after administration, while its pharmacological effect lasts only 24–36 h (Fig 1b; Tobin 1981).

At this point, therefore, the pharmacologist must establish when the pharmacological effect is over. Before doing this, however, the pharmacological effect itself must be identified and the ability to quantify it must be established.

Identification and quantification of the 'critical pharmacological effect'

Before data on analytical/pharmacological relationships can be developed, the pharmacological effect of concern to racing must be identified. For some agents, this may be relatively straightforward. For example, the local loss of pain sensation is clearly the pharmacological effect of concern for local anaesthetic agents. With other agents, however, it may be less straightforward. For example, after oral administration of isoxsuprine to horses, no pharmacological responses have yet been identified (Harkins *et al.* 1998a). However, identification and quantitation of the 'critical pharmacological effect' of concern to racing is a critical part of this process and one on which there are currently active research programmes.

TABLE 2: Number of therapeutic medication molecules administered/dose

Drug	No. of molecules
Naproxen	10^{22}
Furosemide	10^{20}
Fentanyl	10^{18}
Etorphine	10^{16}
Hyaluronic acid	10^{16}

TABLE 3: Critical therapeutic medication metabolites synthesised

Parent drug	Metabolite/analogue
1) Lidocaine	3-OH-Lidocaine
2) Bupivacaine	3-OH-Bupivacaine
3) Mepivacaine	3-OH-Mepivacaine
4) Pyrilamine	O-Desmethylpyrilamine
5) Acepromazine	2-(1-Hydroxyethyl)promazine sulfoxide
6) Acepromazine	2-(1-Hydroxyethyl)promazine
7) Promazine	3-OH-Promazine
8) Phenylbutazone	Phenylbutazone D9

The 'highest no effect dose' (HNED)

Once the critical pharmacological effect has been identified, the highest no effect dose (HNED) is determined. For example, using a heat lamp/local anaesthesia/abaxial sesamoid block model, dose and time response curves for procaine were developed. The data showed that the HNED of procaine is about 5.0 mg/site and that its duration of action as a local anaesthetic is brief (Fig 2). In this way, a family of dose response curves (Fig 3) and HNEDs have been identified for bupivacaine (Lehner *et al.* 2000), ropivacaine (Harkins *et al.* 2000, 2001), mepivacaine (Woods *et al.* 2000; Harkins *et al.* 1999), lidocaine (Harkins *et al.* 1998b), procaine, cocaine and benzocaine (Harkins *et al.* 1996), Sarapin (Harkins *et al.* 1997a), and fentanyl (Harkins and Tobin 1999). Some of these agents are highly potent, some are of intermediate potency and some are pharmacologically inactive in this model. In other studies, behaviour chambers and alternative laboratory models have been used to determine the HNED of medications (Harkins *et al.* 1997b).

The critical analyte/metabolite

The analyte to be quantified, generally in urine, must be identified. Unfortunately, the residue found in horse urine is generally not the parent drug, but a metabolite for which standards are not readily available. Several of these metabolites,

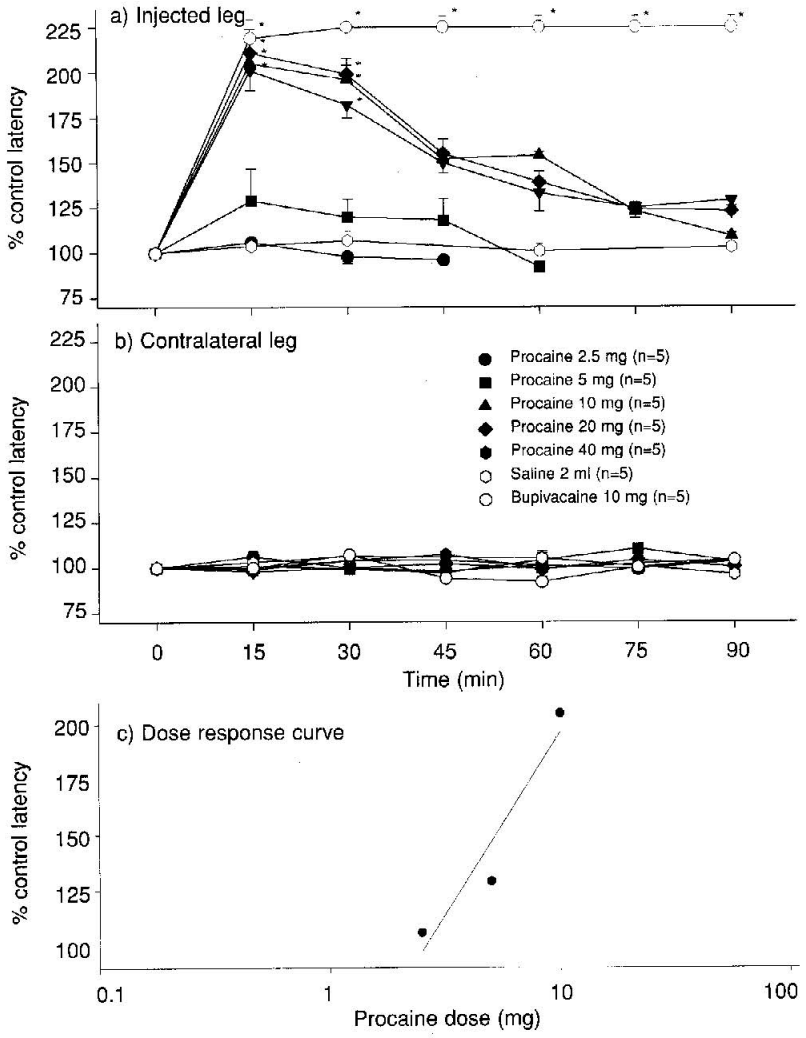

*Fig 2: a) Change in hoof withdrawal reflex latency (HWRL) following injection of procaine; b) change in HWRL in contralateral leg; c) dose response curve. *significantly different from control values (P<0.05). Reproduced with permission from Harkins* et al *(1996).*

therefore, including 3-hydroxymepivacaine, 3-hydroxylidocaine, 3-hydroxybupivacaine, 2-(1-hydroxyethyl)promazine sulfoxide, O-desmethylpyrilamine and 3-hydroxypromazine (Table 3) have been synthesised, purified, characterised and authenticated.

These metabolites are used as standards for identification and quantification, as calibrators in routine screening, as reference standards and for the preparation of specific qualitative and quantitative samples for proficiency or double blind testing and quality assurance work. Also, the specific metabolites synthesised in this research have the ability to form the basis of new and more effective tests for these agents, if such tests were needed (Harkins *et al.* 1998b; Harkins *et al.* 1999).

Assembling the data

Once the HNED and the critical metabolite have been identified, the relevant 'no effect point' can be determined in plasma or urine. For example, the HNED for procaine in the abaxial model was determined to be about 5 mg/site. That dose was administered to horses and the free procaine or free procaine plus its glucuronide metabolite ('total procaine') were quantified in urine. The peak concentration of free procaine recovered from these urine samples was about 28 ng/ml, thereby establishing a basis for a 'no effect point' for free procaine in equine urine. Similarly, the peak concentration recovered after enzyme hydrolysis (conjugated plus non-conjugated) was 45 ng/ml (Fig 4), thereby establishing a basis for a 'no effect point' for total procaine (free plus conjugated) in equine urine.

Because procaine is a basic drug (pK_a=8.98) and these horses were producing alkaline (pH=8.5) urine, a 30 ng/ml cut-off for parent procaine must be regarded as very conservative. Urinary concentrations of free procaine are likely to be substantially increased if the urine pH is more

TABLE 4: Current status of 'limits' project

Agent*	Metabolite**	HNED dose***	Limit****
		No measurable pharmacology	
1) Benzocaine	Not required	No effect	Ineffective (Harkins *et al.* 1996)
2) Sarapin	Not required	No effect	Ineffective (Harkins *et al.* 1997)
3) Isoxsuprine	Not required	No activity orally	(Harkins *et al.* 1998a; Harkins *et al.* 1996)
4) Fentanyl	Not required	No effect	No nerve blocking effect on pain perception (Harkins and Tobin 1999)
		'Limits' on testing sensitivity	
5) Procaine	Not required	5 mg/site	30–50 ng/ml (Harkins *et al.* 1996)
6) Cocaine	Available commercially	>5 mg/site	Various approaches in place (Harkins *et al.* 1996)
7) Lidocaine	Synthesised (3- and 4-OH-lidocaine)	4 mg/site	310 ppb (Harkins *et al.* 1998b; Tobin *et al.* 1998)
8) Mepivacaine	Synthesised (3- and 4-OH-mepivacaine)	2 mg/site	65 ppb (Harkins *et al.* 1999)
9) Bupivacaine	Synthesised (3- and 4-OH-bupivacaine)	0.25 mg/site	~ 28 ppb (Lehner *et al.* 2000; Harkins *et al.* 1996)
10)Clenbuterol	Not required	N/A	30 pg/ml, Serum
		'Limits' work in progress	
12)Acepromazine	Synthesised (HEPS)	1 mg/1000lb	In progress
13) Detomidine	Available	<0.2 mg/kg	Harkins *et al.* (1997b)
14) Pyrilamine	Synthesised (Desmethylpyrilamine)	In progress	In progress
15) Promazine	Synthesised (3-OH-promazine)	In progress	In progress

*Agent for which a 'limit' is being determined; ** Status for metabolite synthesis; *** Status of the Highest No-Effect Dose (HNED) determinations; **** Status of the final phase, 'limit' determination, references

Notes on metabolites:

1) Many drugs are excreted in horse urine linked to glucuronic acid, which makes them highly water-soluble and accelerates their elimination in urine.
2) To recover a linked (conjugated) drug from urine, the chemist first splits (hydrolyses) the glucuronide link by incubating the urine with ß-glucuronidase, an enzyme which breaks the drug-glucuronide bond.
3) If the glucuronide molecule is linked directly to the drug, the chemist recovers unchanged drug, which is the case for procaine and isoxsuprine. Therefore, synthesis of specific metabolites is not required.
4) However, most drugs are metabolised before they are linked to glucuronic acid. Because the metabolites are not available commercially, they were synthesised in our laboratory for the 'limits' programme.
5) The 'limit' range for procaine (30–50 ng/ml) represents both the free drug concentration (30 ng/ml) and the total concentration of free and conjugated drug (50 ng/ml).
6) The presence of conjugated drug in the urine makes it more likely that the drug actually went through a horse, whereas the absence of conjugated drug creates suspicion that the drug did not pass through a horse.

acidic, the usual condition in post race urine samples. Therefore, it is expected that the concentration of free procaine in acidic post race urines will be higher than this concentration, as has been shown for urinary lidocaine (Sams 1996).

Other analytical/pharmacological relationships for the local anaesthetics

Other recently completed work has determined a HNED of lidocaine (5 mg/site) and a relatively high urine concentration of 3-hydroxylidocaine of about 310 ng/ml in horses administered this dose (Harkins *et al.* 1998b). Similarly, the HNED of mepivacaine was determined to be 2 mg/site, and the peak urinary concentration of 3-hydroxymepivacaine was about 65 ng/ml from horses administered that dose (Harkins *et al.* 1999). The HNED of bupivacaine was determined to be 0.25 mg/site and the concentration of the major urinary metabolite of bupivacaine (3-hydroxybupivacaine) was about 28 ng/ml. Preliminary work with ropivacaine suggests that this agent is similar to bupivacaine regarding local anaesthetic potency (Harkins *et al.* 2000, 2001). Table 4 summarises the progress to date for developing 'cut-offs' or 'no effect limitations'.

Plasma or urinary cut-offs?

It is recognised generally that plasma concentrations of medications are more predictable and easier to relate to pharmacological effects than urinary concentrations. This is because urinary concentrations are subject to the influences of urine volume, flow rate, specific gravity and pH. However, there were and can be practical limitations with respect to plasma 'limits' that make their application difficult. The classic example is procaine. Based on research by the authors' group, the estimated plasma 'limit' for procaine would be

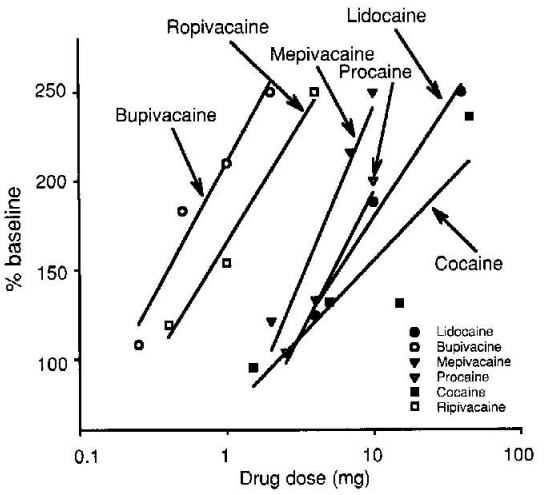

Fig 3: Dose response curves determined by the heat lamp/local anesthesia/abaxial sesamoid block model.

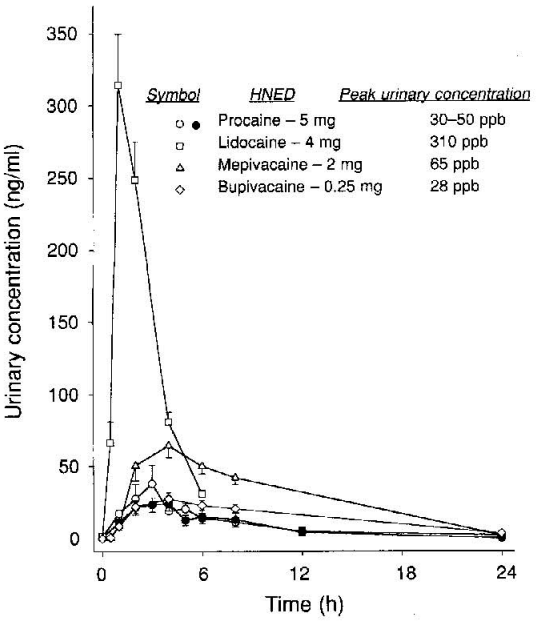

Fig 4: The symbols indicate urine concentrations of recovered 3-hydroxylidocaine (□), recovered 3-hydroxymepivacaine (△), recovered 3-hydroxybupivacaine (◇), recovered free procaine (●), and procaine following enzymatic hydrolysis (○) after subcutaneous injection of the HNEDs of each agent.

about 1 ng/ml (Harkins *et al.* 1996), well below the limit of quantitation of current routine analytical methods when this work was started in 1993.

A second problem with procaine is that it is hydrolysed very rapidly by plasma esterases. Therefore, a plasma 'limit' for procaine requires the use of specially prepared collection tubes containing esterase inhibitors. Finally, and of particular significance in Kentucky, was the fact that until recently the Kentucky post race testing programme did not include blood collections, which made the concept of a plasma 'limit' moot for procaine.

When this work started in 1993, the available analytical techniques were insufficient to quantify serum concentrations of local anaesthetics, and urinary thresholds were developed by default. It was, however, recognised that serum thresholds, when practical, remain scientifically more acceptable than a urinary threshold.

In this regard, the recent application of LC/MS/MS technology to the detection and qualification of very low pg/ml. concentrations of clenbuterol in equine serum, as presented by Lehner *et al.* (2001) clearly opens the door to serum limits for therapeutic medications.

LIMITATIONS OF THESE DATABASES/APPROACHES

The database reported in this communication best applies primarily in the context of these experiments or related circumstances. These data are sensitive to dose and route of administration of the agent in question. If the agent is administered by a different route, as repeated doses, as a different formulation or with another therapeutic rationale, the analytical-pharmacological database reported here may not necessarily apply to the specific regulatory circumstances.

Furthermore, the database is method-dependent. When this work began, no validated quantitative analytical methods were available for any of these therapeutic medications or their urinary metabolites. In fact, few, if any, of these urinary metabolites were available until they were synthesised for this programme. Therefore, the methodologies on which these data were developed are specific to this work and any variation, adaptation or substitution of these methods may affect the interpretation of the database. The analytical methodologies used, however, are based on, or adapted from, those currently used in racing chemistry. The enzymatic hydrolysis method was adapted from the original reports (Combie *et al.* 1982) and is based on that used by Truesdail Laboratories, which performs post race testing for Kentucky, California and other states.

In this regard, the development of standardised validated enzyme hydrolysis methods for glucuronidated agents and their metabolites would significantly improve the reproducibility of quantitative analytical data involving a glucuronide hydrolysis step. Similarly, no validated quantitative

analytical methods were available for any of the hydroxylated metabolites that form the basis of this work. Progress in this field would be facilitated by the development of validated quantitative methods for these drugs and metabolites to increase the level of confidence in quantitative analytical data.

Finally, it must be remembered that these data have been developed in a research setting. Therefore, only experienced regulators, who can determine the utility of a research database, should interpret and apply these data in a regulatory setting.

FUTURE DIRECTIONS

With regard to future directions, virtually all of the limitations pointed out above could be avoided by the use of serum testing. In this regard, our research group in Kentucky is focusing on the development of LC/MS/MS based serum tests as the basis for rapid test development for new drugs, and also for the development of 'thresholds'/ 'limits' for many therapeutic medications in performance horses.

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REFERENCES

Combie, J., Blake, J.W., Nugent, E. and Tobin, T. (1982) Morphine glucuronide hydrolysis: superiority of ß-glucuronidase from *Patella vulgata*. *Clin. Chem.* **28,** 83-86.

Harkins, J.D., Mundy, G.D., Stanley, S., Woods, W.E., Rees, W.A., Thompson, K.N. and Tobin, T. (1996) Determination of highest no-effect dose (HNED) for local anesthetic responses to procaine, cocaine, bupivacaine, and benzocaine. *Equine vet. J.* **28,** 30-37.

Harkins, J.D., Mundy, G.D., Stanley, S., Sams, R.A. and Tobin, T. (1997a) Lack of local anesthetic efficacy of Sarapin in the abaxial sesamoid block model. *J. vet. Pharmacol. Therap.* **20,** 229-232.

Harkins, J.D., Queiroz-Neto, A., Mundy, G.D., West, D. and Tobin, T. (1997b) Development and characterization of an equine behavior chamber and the effects of amitraz and detomidine on spontaneous locomotor activity. *J. vet. Pharmacol. and Therap.* **20,** 396-401.

Harkins, J.D., Mundy, G.D., Stanley, S., Woods, W.E., Sams, R.A., Richardson, D.R., Grambow, S.C. and Tobin, T. (1998a) Absence of detectable pharmacologic effects after oral administration of isoxsuprine. *Equine vet. J.* **30,** 294-299.

Harkins, J.D., Mundy, G.D., Stanley, S., Woods, W.E., Lehner, A.F., Karpiesiuk, W., Rees, W.A., Dirikolu, L., Bass, S., Carter, W., Boyles, J. and Tobin, T. (1998b) Lidocaine: Pharmacological effects and their relationship to analytical findings. *J. vet. Pharmacol. Therap.* **21,** 462-476.

Harkins, J.D. and Tobin, T. (1999) Lack of local anesthetic efficacy of fentanyl in the abaxial sesamoid block model. *J. vet. Pharmacol. and Therap.* **22,** 52-55.

Harkins, J.D., Mundy, G.D., Karpiesiuk, W., Woods, W.E., Lehner, A.F., Rees, W.A., Dirikolu, L., Bass, S., Carter, W., Boyles, J. and Tobin, T. (1999) Mepivacaine: Its pharmacological effects and their relationship to analytical findings in the horse. *J. vet. Pharmacol. and Therap.* **22,** 107-121.

Harkins, J.D., Karpiesiuk, W., Tobin, T. and Lehner, A.F. (2000) Direct identification of hydroxyropivacaine glucuronide by ESI+/MS/MS in post administration equine urine. *Can. J. vet. Res.* **64,** 178-183.

Harkins, J.D., Karpiesiuk, W., Woods, W.E., Lehner, A.F., Dirikolu, L., Carter, W., Boyles, J. and Tobin, T. (2001) Ropivacaine in the horse: its pharmacological responses, urinary detection and mass spectral confirmation. *J. vet. Pharmacol. Therap.* In Press.

Lehner, A.F., Woods, W.E., Karpiesiuk, W., Harkins, J.D. and Tobin, T. (2000) Detection and quantitation of 3-hydroxybupivacaine in horse urine: A preliminary report. *Proc. 12th int. Conf. racing Anal. Vet.* Eds: B. Laviolette and M.R. Koupai-Abyazani, R&W Publications, Newmarket, p 13.

Lehner, A.F., Harkins, J.D., Karpiesiuk, W., Woods, W.E., Robinson, N.E., Dirikolu, L., Fisher, M. and Tobin, T. (2001) Clenbuterol in the horse: Confirmation and quantification of serum clenbuterol by LC/MS/MS after oral and intra-tracheal administration. *J. anat. Toxic.* In press.

Sams, R.A. (1996) Pharmacokinetic studies of drugs in racehorses. *Proc. 11th int. Conf. racing Anal. Vet.* Eds: D.E. Auer and E. Houghton, R&W Publications, Newmarket, 345-354.

Tobin, T. (1981) In: *Drugs and the Performance Horse,* Charles C. Thomas, Springfield, IL. pp 95-96.

Tobin, T. (1995) Thresholds/limits on testing for therapeutic medications: viewpoints of the regulated. In: *Testing for Therapeutic Medications, Environmental and Dietary Substances in Racing Horses.* Eds: T. Tobin, G.D. Mundy, S.D. Stanley, R. A. Sams and D. L. Crone. Published by The Maxwell H. Gluck Equine Research Center, Lexington, Kentucky, pp 23-32.

Tobin, T., Combie, J. and Nugent, T.E. (1982) 'Detection times' and 'clearance times' for drugs in horses and other animals: a reappraisal. *J. vet. Pharmacol. Therap.* **5,** 195-197.

Woods, W.E., Lehner, A.F., Karpiesiuk, W., Boyles, J., Carter, W., Harkins, J.D. and Tobin, T. (2000) Detection and quantitation of 3-hydroxymepivacaine in horse urine: a preliminary report. *Proc. 12th int. Conf. racing Anal. Vet.* Eds: B. Laviolette and M.R. Koupai-Abyazani, R&W Publications, Newmarket, p 324.