PENTOBARBITAL: HORSE RACING'S SOLUTION, GREYHOUND RACING'S PROBLEM

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ABSTRACT

There has been an apparent increase in adverse analytical findings of pentobarbital in the urine of racing greyhounds in Europe. Many greyhound trainers advocate the feeding of raw or cooked meat, usually obtained as by-products from the meat industry. Recent events have affected this supply, including undeclared entry of horse meat to the food chain in Europe requiring more stringent control; and less use of firearms increasing the use of pentobarbital for euthanasia, for example in horseracing. The existing literature on pentobarbital in dogs was reviewed, together with findings from recent British cases where the amount of pentobarbital present was estimated from historical studies and screening analytical data. Whilst it has not been possible to control the use of pentobarbital in British racing greyhound solely by use of Official Controls, it has been possible to control using a residue limit that assures racing integrity and animal welfare. The historical scientific literature proved useful in setting this limit, and the persistence of some barbiturates in greyhound urine after exposure is noteworthy.

KEYWORDS

Greyhound, pentobarbital, contamination, meat, anti-doping

INTRODUCTION

Greyhound trainers commonly feed raw or cooked meat. This may be for a variety of reasons, including tradition, perception it is needed for racing performance, or cost. If meat is fed, it is common for the meat to be sourced from animals slaughtered but not fit for human consumption, as the cost is considerably reduced. Such animals may well have been treated with medicines, which give rise to residues, and/or been killed by a lethal injection of the veterinary euthanasia drug pentobarbital.

Such exposure to pentobarbital is undesirable for a number of animal welfare reasons. It may result in death of the greyhounds (Reid, 1978), loss of consciousness (Sams, Muir, Detra & Robinson, 1985), or subclinical sedation (O'Connor, Stowe & Robinson, 1985). If subclinical sedation results from such exposure this may negatively affect racing performance, and so disrupt integrity.

Clearly pentobarbital should be regarded as a prohibited substance that should not be found in racing greyhounds, and can be controlled by surveillance using analytical testing as well as other controls.

The Greyhound Board of Great Britain (GBGB) operates an anti-doping and medication control program, with pre and post-race urine samples being collected and analysed. There has been a sharp rise in pentobarbital findings by the GBGB over recent years, with 3 findings in 2013, 7 in 2014, 12 in 2015 and around 25 in 2016. A similar, but less marked rise has been seen in Ireland, but not in Australia (AORC, 2016).

AIM

To review the anti-doping control of pentobarbital in greyhound racing and propose effective methods of control that assure integrity and animal welfare.

MATERIALS AND METHOD

Analytical findings in urine from the GBGB anti-doping and medication control program were reviewed. Analysis was by LC-MS with high-resolution accurate mass spectrometry after solid phase extraction. Levels of pentobarbital and pentobarbital metabolites, principally two diastereoisomers of 5-ethyl-5-(3-hydroxy-1-methylbutyl) barbituric acid (Titus & Weiss, 1955) were estimated by comparison to internal standards.

Findings of pentobarbital in a range of specific situations were collated and reviewed:

Post-meal: Samples taken around 8 hours after the feeding of meat containing residues, to a group of 8 dogs.

Contaminated meat: Samples representative of situations where there were admissions of feeding meat containing residues.

Pre-Sales: Samples taken prior to the sales of greyhounds that had just arrived from Ireland (where, at that time, the feeding meat containing residues was allowed).

Derby; early heat: Samples taken from participating greyhounds around 1-2 weeks after the commencement of heats for the 2016 Greyhound Derby.

Derby; later heat: Samples taken from participating greyhounds around 2-4 weeks after the commencement of heats for the 2016 Greyhound Derby.

The findings of an internal report from the UK racing laboratory on a pentobarbital greyhound administration study were reviewed and extrapolated (Dumasia, 2002). Here pentobarbital was administered intravenously to two greyhounds at two low doses of approximately 0.25 mg and 0.5 mg total dose to determine times of detection in plasma and urine. The primary data were then re-examined and levels of pentobarbital and pentobarbital metabolites (Titus & Weiss, 1955) were estimated by comparison to internal standards (Figure 1).

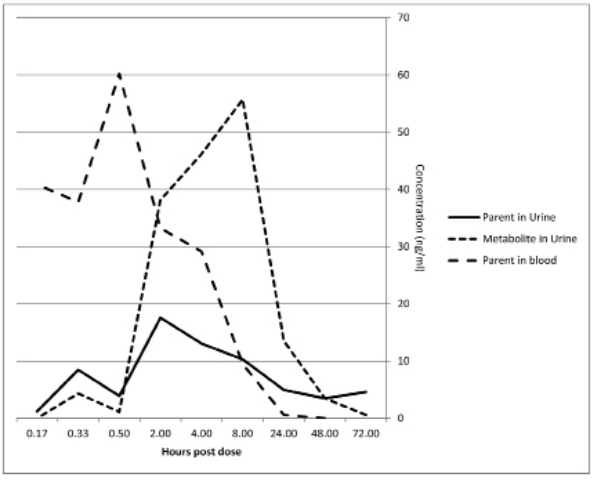

Figure 1: Estimated pentobarbital and pentobarbital metabolite concentrations in plasma and urine of greyhounds after pentobarbital was administered intravenously to two greyhounds at two low doses of approximately 0.25mg and 0.5mg total dose.

The existing published literature and 'grey' materials produced by organisations outside of traditional commercial or academic publishing (Schöpfel & Farace, 2010) on pentobarbital in dogs were reviewed.

RESULTS

Analytical findings

Table 1 summarises the findings of pentobarbital in a range of specific situations. In the context that the levels of pentobarbital and pentobarbital metabolite are estimated from screening data they provided useful information. When meat containing residues is routinely fed, i.e. 'post-meal', 'contaminated meat', 'Pre-Sales', then urine levels of pentobarbital in the order of 10's ng/ml, and urine levels of pentobarbital metabolites in the order of 100's ng/ml are routinely found. When samples were taken from participating greyhounds around 1-2 weeks after the commencement of heats for the 2016 Greyhound Derby no parent pentobarbital was found but pentobarbital metabolites in the range 10-100 ng/ml were found. When samples were taken from participating greyhounds around 2-4 weeks after the commencement of heats for the 2016 Greyhound Derby no parent pentobarbital was found but pentobarbital metabolites in the range 1-10 ng/ml were found.

Pentobarbital greyhound administration study

Figure 1 summarises the levels of pentobarbital after pentobarbital was administered intravenously to two greyhounds at two low doses of approximately 0.25 mg and 0.5 mg total dose (Dumasia, 2002). In the context that the levels of pentobarbital and pentobarbital metabolite are estimated from detection time data, this provided useful information. Whilst levels of parent pentobarbital in blood fell to 1 ng/ml or less by 24 hrs, both pentobarbital and pentobarbital metabolites were present in urine for at least 72 hours, terminally in the 1-10 ng/ml range.

	Post-meal n=8		Contaminated meat n=10		Pre-sales n=7		Derby early heat n=8		Derby later heat n=5	
	parent ng/ml	metabo-lite ng/ml	parent ng/ml	metabo-lite ng/ml	parent ng/ml	metabo-lite ng/ml	parent ng/ml	metabo-lite ng/ml	parent ng/ml	metabolite ng/ml
Mean	18.2	434.6	9.0	703.9	3.4	565.7	0.0	13.5	0.0	2.4
Median	14.0	398.9	7.3	473.4	0.0	347.7	0.0	12.0	0.0	2.7
Min	7.0	199.7	0.0	175.9	0.0	50.5	0.0	107.7	0.0	1.4
Max	43.0	878.4	27.7	1763.8	15.0	1581.8	0.0	22.9	0.0	3.7
SD	11.2	211.9	9.1	559.6	5.8	502.4	0.0	5.8	0.0	1.0

Table 1: Summary of findings of estimated pentobarbital and pentobarbital metabolite concentrations in urine of greyhounds in a range of situations.

Literature review

A range of useful publications were found including these summarised below.

Reid (1978) recorded death and sedation following ingestion of meat contaminated with barbiturates. O'Connor, Stowe & Robinson (1985) showed that pentobarbital in meat rendered at high temperatures remains active and can be present at sedative doses. The Food and Drug Administration in 2002 surveyed dry dog food samples to measure how much pentobarbital might be present; and to estimate no-effect levels.

Sams, Muir, Detra & Robinson (1985) conducted pharmacokinetic and anaesthetic effect studies of pentobarbital on greyhounds. Dumasia's 2002 study could be utilised to extrapolate terminal levels of pentobarbital in urine. Ehrnebo (1974) studied pharmacokinetics of intravenously and orally administered pentobarbital in humans and provided clues to the reasons for the protracted finding of pentobarbital metabolites in greyhounds.

Titus & Weiss (1955) provided information on the metabolites of pentobarbital in the dog, and Dickert, Shea & McCarty (1966) showed these diastereoisomers of 5-ethyl-5-(3-hydroxy-1-methylbutyl) barbituric acid were inactive in the dog.

DISCUSSION

The control of pentobarbital in racing greyhounds should be straightforward, especially in Europe where there are Official Controls (EU 2004). Pentobarbital is now only used in Europe as a veterinary drug for euthanasia. As well as being Prescription Only, it is also a Controlled Drug, both of which greatly restrict access to the drug and make direct administration of pentobarbital a very unlikely source of findings of pentobarbital in greyhounds.

When pentobarbital is used for euthanasia of farm animals and horses in Europe the carcass cannot be used for processed pet food and the meat must be marked with a stain and designated a Category 2 Animal By Product. Such Category 2 meat can be fed to dogs, subject to registration and licensing requirements (Department for Environment, Food & Rural Affairs, 2014).

The GBGB, and the Irish Greyhound Board, have both advised trainers against feeding Category 2 Animal By-Products(Greyhound Board of Great Britain 2012, Irish Greyhound Board, 2015).

However it would appear from information obtained in Disciplinary hearings, (Greyhound Board of Great Britain, 2016), that there is both poor compliance with this advice, but also findings of pentobarbital when Category 3 Animal By-Products (which should not contain drug residue) or meat for human consumption are fed.

It therefore would appear that there are two challenges for straightforward control of pentobarbital by zero tolerance to any finding of this substance.

The first is that there appears to be some diversion of Category 2 Animal By-Products to Category 3 Animal By Products. This may be the result of more stringent controls on horse meat introduced by governments to address public health concerns following the diversion of horse meat into the human food chain across Europe in 2013 (Food Standards Agency, 2015). This issue is beyond the control of greyhound racing regulators and these concerns have been reported to the UK authorities by the GBGB.

The second is that there may also be some persistence of pentobarbital metabolites for some time after Category 2 Animal By-Products have been withdrawn. The intelligence received after the Derby heats samples (Figure 1) indicated that meat for human consumption had been fed prior to these heats. Ehrnebo (1974) showed marked tissue binding of pentobarbital in studies in humans. Interestingly, Jarrett (2015) in his report at this meeting also showed '*remarkable longevity*' of phenobarbital findings post-administration in greyhounds.

One approach to controlling substances that result from feed contamination is to set residue limits, such as recently publicised for horseracing (International Federation of Horseracing Authorities, 2015).

Using the available information some assumptions can be drawn from the available historical data. A 500 microgram pentobarbital dose leads to a urine hydroxy pentobarbital concentration of around 50ng/ml (Dumasia, 2002), which is 1/300 of a sedative dose, (O'Connor, Stowe & Robinson, 1985), and when this amount was given once daily for eight weeks it led to statistically higher liver weights (Food and Drug Administration, 2002). Increased liver weights are associated with the increased production by the liver of cytochrome P450 enzymes. Induction of cytochrome P450 enzymes is a normal response to many substances that are naturally found in foods. It is not an indication of harm, but was selected as the most sensitive indicator to detect any biological effect due to pentobarbital. A 50 microgram pentobarbital dose should then lead to a urine hydroxy pentobarbital concentration of around 5ng/ml, which is 1/3000 of a sedative dose and when given once daily for eight weeks: this was a no-observable-effect level (Food and Drug Administration, 2002). The hydroxy metabolite is not pharmacologically active (Dickert, Shea & McCarty, 1966).

This information has been used by the GBGB to establish control of pentobarbital, with a confidential residue limit for urinary hydroxy pentobarbital, utilised only in the absence of urinary parent pentobarbital.

There are two important considerations for this study. Whilst the historical literature from a diverse range of sources is useful, it must be remembered that the levels

so utilised are semi-quantitative at best. In addition, at present, with controls using residue limits appearing to be effective, it is difficult ethically to justify a quantitative pentobarbital administration study.

CONCLUSION

Whilst it has not been possible to control the use of pentobarbital in racing greyhounds solely by use of Official Controls, it has been possible to control using a residue limit that assures racing integrity and animal welfare. The historical scientific literature proved useful in setting this limit, and the persistence of some barbiturates in greyhound urine after exposure was noteworthy.

AUTHOR'S DECLARATION OF INTERESTS

The author is the Independent Scientific Advisor to the Greyhound Board of Great Britain and has provided consultancy advice to LGC Ltd, receiving fees for both activities.

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