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SMALL ANIMALS

ORIGINAL ARTICLE

Pharmacokinetics of carprofen and firocoxib for medication control in racing greyhounds

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Animals used in sport should be treated as required to ensure animal welfare but any such use of medication should also be controlled to ensure integrity. Pharmacokinetic studies on groups of six greyhounds were performed to measure plasma and urine levels of carprofen and firocoxib to inform medication control advice. Using the standard methodology for medication control the Irrelevant Plasma Concentration was determined as 20 and 2 ng/mL for carprofen and firocoxib, respectively. The Irrelevant Urine Concentration was also determined as 0.3 and 2 ng/mL for carprofen and firocoxib, respectively. These Irrelevant Plasma and Urine Concentrations will allow laboratory Screening Limits, Detection Times and Withdrawal Time advice to be determined and publicised by regulators of greyhound racing. The Screening Limits will also inform Recommended Limits of Detection if meat-containing residues of these medications are fed to greyhounds.

Keywords carprofen; firocoxib; greyhound; pharmacokinetics; racing

Abbreviations COX, cyclooxygenase; DT, detection time; IPC, irrelevant plasma concentration; IUC, irrelevant urine concentration; NSAIDs, non-steroidal anti-inflammatory drugs; PG, prostaglandins; SL, screening limit; WT, withdrawal time

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Non-steroidal anti-inflammatory drugs (NSAIDs) are important therapeutic agents for animals used in sports. They act to reduce the biosynthesis of prostaglandins (PG) by inhibiting cyclooxygenase (COX) which exists in two isoforms, COX-1 and COX-2. Preferential activity against COX-2 may have fewer adverse effects due to mitigation of concurrent COX-1 inhibition in dogs, with favourable COX1:2 ratios having been reported for carprofen and firocoxib.[1]

Animals used in sport should be treated as required to ensure their welfare, for example, with NSAIDs, but any such medication use should also be controlled to ensure integrity. The accepted approach for medication control of drugs used in animal sports, is the control of analytical sensitivity based on administration studies, with urine being the sample matrix of choice for medication control.[2] For a particular drug, it is especially important to understand the need for an extended administration study that includes urine as well as plasma sampling to allow contemporaneous plasma and urine levels that fully define the terminal phases of drug excretion, with a lower limit of drug detection in the part per billion range (ng/mL) that is appropriate for the different perspectives between therapy and medication control.[3] In addition to the therapeutic use of NSAIDs in dogs, the role of their exposure via feeding meat from animals that contain residues of NSAIDs to greyhounds is an issue that dictates the need for a limit of detection that is also appropriate for control of these residues.

In Australia, carprofen is licensed for use in dogs, cats and horses, in Europe it is also licensed for use in cattle. There have been a number of studies that examined the levels of carprofen in the plasma of dogs.[4-9] None of these studies appeared to fully extend into the terminal phase of excretion, they did not have limits of quantification down to an appropriate range of 1 ng/mL, nor did they measure urine levels. One study measured urinary levels of carprofen in two greyhound dogs, finding it for up to 72 h with a limit of quantification of approximately 0.1 ng/mL, but it did not measure plasma levels.[10]

In Australia, firocoxib is licensed for use in dogs, in Europe it is licensed for use in dogs and horses. There are limited published studies of the pharmacokinetics of firocoxib in dogs. Plasma pharmacokinetics have been reported for 32 h after administration, with plasma levels at around 100 ng/mL at that time.[11] A method for quantifying firocoxib in canine urine with a limit of detection of 3.0 ng/mL did not report any pharmacokinetic data.[12] No published studies have been found that measured urine levels in dogs.

The aims of this study were: to characterise the plasma and the urinary pharmacokinetics of two commonly used NSAIDs, carprofen and firocoxib, for the purpose of medication control in racing greyhounds at doses relevant to their prescription for use by trainers of racing greyhounds; ensure analytical detection extended into the terminal phase and at a higher sensitivity than previous studies; derive Irrelevant Plasma and Urine Concentrations; produce regulatory advice for greyhound racing nationally and internationally in respect of both medication and meat residue control limits; and so enable science-based advice to veterinarians prescribing for racing greyhounds.

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Materials and methods

Animal care and use

Six greyhounds,[13] three females, three males, in good health were selected from the SCEC research colony for inclusion in each study. They were fed a commercial dry dog food (Dogpro PLUS Working Dog, Hypro Petcare Pty. Ltd., Wilberforce, NSW, Australia) twice daily with an additional portion of fresh knackery meat (Luddenham Pet Meat, Luddenham, NSW, Aust), and had access to water at all times. Drug administration preceded the morning feed, excluding the final oral dose, which was given with a small amount of food. The studies were conducted in accordance to the principles of the VICH GCP guidelines.[14] Ethics approval was obtained from the Secretary's Animal Care and Ethics Committee of the NSW Department of Primary Industries (Approval reference TRIM #15/1406(158)). The dogs used in the carprofen study had received Flunixin meglumine (Ilium Flunixil® Injection 50 mg/mL; Troy Laboratories Australia Pty. Ltd, Glendenning NSW, Australia) intravenously on one occasion at the label dose of 1 mg/kg (0.6 mg/kg flunixin free acid) 30 days previously.[15] The dogs used in the firocoxib study had not been previously used in other studies involving NSAIDs or any study within the previous 7 days.

Drug administrations and sample collections

Carprofen (Rimadyl® 50 mg/mL and Rimadyl® Tablets for Dogs 50 and 20 mg, Zoetis Australia Pty. Ltd., Rhodes, NSW, Australia) was administered as 4 mg/kg single intravenous injection followed 1 day later by 4 mg/kg once daily oral administrations, utilising the two tablet presentations that continued for 5 days. Three female and three male animals were studied, with a mean bodyweight of 32.4 kg and mean age of 3.8 years. Blood and urine were collected before each daily drug administration over the first 6 days. Blood samples were collected at 0, 0.25, 0.5, 1, 2, 4, 6, 8, 12, 24, 48, 72, 96 and 120 h, and then continued after the last administration at 120.25, 120.5, 121, 122, 124, 126, 128, 130, 132, 144, 168 and 192 h. Urine samples were collected at 0, 2, 4, 6, 8, 10, 12, 24, 48, 72, 96 and 120 h, and then continued after the last administration at 122, 124, 126, 128, 130, 132, 144, 168 and 192 h.

Firocoxib (Previcox® 57 mg and 227 mg Hi-Select COX-2 Flavoured Tablets, Merial Australia Pty. Ltd., Macquarie Park NSW, Australia) was administered at a dose rate of 5 mg/kg orally once daily for 5 days, utilising the two tablet presentations. Three female and three male animals were studied, with a mean bodyweight of 31.6 kg and mean age of 3.8 years. Blood and urine were collected at times as for the carprofen administration study.

The blood samples were heparinised and plasma obtained by centrifugation. All samples were then stored and transported at −20°C until analysis.

Sample preparation, chemical analysis, quantification, calibration and quality control

Concentrations of carprofen and firocoxib were measured in the pre- and post-administration plasma and urine samples by Racing Analytical Services Limited, Victoria, Australia.

Urine and plasma sample extraction. For carprofen in urine: samples (3 mL) were hydrolysed by adding sodium hydroxide (2 M, 500 uL) and allowed to stand at room temperature for 30 min. Sodium acetate buffer (1 mL) was then added and the pH adjusted to 3. Samples were liquid/liquid extracted with hexane/dichloromethane/ethyl acetate (40:30:30) for 15 min. After centrifugation, the organic layer was removed and washed with saturated sodium bicarbonate (3 mL). Samples were centrifuged and the organic layer removed and passed through anhydrous sodium sulphate. Extracts were evaporated to dryness under nitrogen at 60°C. Dried residues were methylated with diazomethane, followed by reconstitution in ethyl acetate (100 µL).

For carprofen in plasma: samples (300 µL) were diluted with deionised water (700 µL), followed by the addition of hydrochloric acid (6.4%, 50 µL). Samples were then vortexed, followed by liquid/liquid extraction with diethyl ether (4 mL) for 15 min. After centrifugation, the organic layer was removed, passed through anhydrous sodium sulphate and evaporated to dryness under nitrogen at 60°C. Dried residues were methylated with diazomethane, followed by reconstitution in ethyl acetate (100 µL).

For firocoxib in urine: samples (1 mL) were diluted with ammonium acetate buffer (0.5 M, 3 mL) followed by enzyme hydrolysis. Samples were adjusted to pH 6 and liquid/liquid extracted with hexane/dichloromethane/ethyl acetate (40:30:30) for 15 min. After centrifugation, the organic layer was removed passed through anhydrous sodium sulphate. Extracts were evaporated to dryness under nitrogen at 60°C, followed by reconstitution in formic acid (0.1%) and methanol (95:5, 100 µL).

For firocoxib in plasma: samples (300 µL) were diluted with deionised water (700 µL), followed by the addition of hydrochloric acid (6.4%, 50 µL). Samples were then vortexed, followed by liquid/liquid extraction with diethyl ether (4 mL) for 15 min. After centrifugation, the organic layer was removed, passed through anhydrous sodium sulphate and evaporated to dryness under nitrogen at 60°C. Dried residues were reconstituted in formic acid (0.1%) and methanol (95:5, 100 µL).

Sample analysis. Samples for carprofen analysis were processed by gas chromatography mass spectrometry using an Agilent 7000B triple quadrupole mass spectrometer (Agilent, Santa Clara, CA, USA) coupled to an Agilent 7890 gas chromatograph (Agilent). Chromatographic separation was achieved using a SGE BPX5 (25 m × 0.2 mm I.D. × 0.25 um film thickness) capillary column (SGE Analytical Science, Ringwood, VIC, Aust) with helium as the carrier gas. Sample injections (2 µL) were carried out in splitless mode with an inlet temperature of 220°C. The column oven was held at 75°C for 1 min and then ramped at a rate of 30°C/min until a final temperature of 320°C was reached. This temperature was held for 5 min. The carrier gas was maintained at a constant flow rate of 1.2 mL/min. The mass spectrometry (MS) was operated in electron ionisation mode with a transfer line temperature of 300°C and source temperature of 280°C. Carprofen was monitored using the transitions m/z 228 > m/z 193 (for quantitation), m/z 287 > m/z 228 and m/z 287 > m/z 193 (for identification). The internal standard, carprofen-d3, was monitored using the transition m/z 231 > m/z 196.

Samples for firocoxib analysis were processed by liquid chromatography mass spectrometry using a Shimadzu 8050 triple quadruple mass spectrometer (Shimadzu Corp., Kyoto, Japan) coupled to a Nexera LC-30AD (Shimadzu Corp.) liquid chromatograph. Chromatographic separation was achieved using a Poroshell 120 EC-C18 column (3 mm × 50 mm, 2.7 µm particle size) (Agilent Technologies). The mobile phase consisted of 0.1% formic acid (A) and methanol (B). The initial composition was 5% B, which was held for 0.3 min and then ramped to reach 98% B at 3.5 min. This was held for 0.5 min before being returned to 50% B and equilibrated for 1.5 min. The mass spectrometer was operated in multiple reaction monitoring mode with positive polarity. Electrospray ionisation was carried out with heater block, interface and dimorphite-DL (DL) temperatures of 300, 300 and 300°C, respectively. The nebulizer, heating and drying gas flow rates were 3, 5 and 15 L/min, respectively. Firocoxib was monitored using the precursor ion *m/z* 337.1 and daughter ions *m/z* 265.1 (for quantitation), *m/z* 237.1 and *m/z* 130.1 (for identification). The internal standard, Firocoxib-d_4, was monitored using the transition *m/z* 341.1 > *m/z* 265.1.

Calibration and quality control. Calibration and quality control samples for carprofen were used in the range of 2.5–1000 ng/mL for urine and 1–1000 ng/mL plasma, and the methods were shown to be linear with correlation coefficients greater than 0.99. The urine and plasma methods produced a lower limmit of quantification (LLOQ) of 2.5 and 1 ng/mL respectively, and were shown to be accurate and reproducible with acceptable inter-batch variability of precision and accuracy. The accuracy of the urine method was demonstrated to be 7.6% at LLOQ, and the precision 6.7% at LLOQ. The accuracy of the plasma method was demonstrated to be 19.2% at LLOQ, and the precision 4.3% at LLOQ.

Calibration and quality control samples for firocoxib were used in the range of 2.5–1000 ng/mL for urine and 1–1000 ng/mL plasma, and the methods were shown to be linear with correlation coefficients greater than 0.99. The urine and plasma methods produced a LLOQ of 2.5 and 1 ng/mL respectively, and were shown to be accurate and reproducible with acceptable inter-batch variability of precision and accuracy. The accuracy of the urine method was demonstrated to be 2.7% at LLOQ, and the precision 11.6% at LLOQ. The accuracy of the plasma method was demonstrated to be 2.7% at LLOQ, and the precision 15.1% at LLOQ.

Urine pH was measured for carprofen and firocoxib, with a pH meter (SevenEasy, Mettler Toledo, Schwerzenbach, Switzerland).

Pharmacokinetic analysis

Phoenix WinNonlin 7.0 (Pharsight Corporation, Cary, NC, USA) was used for; a non-compartmental analysis of the plasma data for firocoxib; a 2-compartmental pharmacokinetic analysis for the 0–24 h plasma data for carprofen; a non-compartmental analysis for the last oral dose of carprofen from 120–192 h – in order to determine average steady-state plasma concentrations and terminal half-life. Phoenix WinNonlin 7.0 (Pharsight Corporation) was used to compute the area under the urine carprofen concentration-time curve, a measure of carprofen exposure in the urine, which was compared to the time weighted average urine pH for each dog using a non-compartmental approach. Both parametric (student *t*-test) and non-parametric (Mann-Whitney) tests were used to determine any statistical differences in pharmacokinetic parameters between male and female dogs.

Effective plasma concentrations were calculated by dividing the standard dose (per dosing interval) by the plasma clearance (per dosing interval), a safety factor was then applied to derive an irrelevant plasma concentration, and finally an irrelevant urine concentration derived from applying the steady-state urine to plasma concentration ratio.[3]

Results

Carprofen

Following an intravenous administration, continued 1 day later by five once daily oral administrations, plasma carprofen concentrations rapidly peaked and then declined rapidly over 4 h, and after the last administration then progressed into a longer terminal phase. Each pre-dose plasma concentration between 24 and 120 h showed no significant accumulation (Figure 1A). Plasma pharmacokinetic parameters for carprofen are summarised in Tables 1 and 2 with further information in corresponding tables in the supplementary material. The average bioavailability for the last oral dose relative to the IV dose is 102%, that is, complete absorption of carprofen via the oral route. Urine carprofen concentrations after IV administration reach a peak at 4 h before progressing into a decay phase (Figure 1B. Each pre-dose urine concentrations between 24 and 120 h showed no significant accumulation. After the last oral administration levels peak and decline in a similar manner to the intravenous terminal phase. In plasma, all samples were above the LLOQ, and a single sample from one of the six dogs was not obtained at 120 h after the final carprofen administration. In urine, all samples were above the LLOQ, and two samples from the six dogs could not be obtained at 2 h after the final drug administration and at 10 h after the final drug administration in another dog. This latter dog was withdrawn late in the study due to pyrexia requiring treatment, so the last sample at 72 h after the final drug administration was not obtained. There appears to be no relationship between the carprofen exposure in urine and the average urine pH (Figure S1). There were no statistical differences between male and female dogs for the pharmacokinetic (PK) parameters determined for carprofen. The computed effective plasma concentration (EPC), Irrelevant Plasma Concentration (IPC), Irrelevant Urine Concentration (IUC) and corresponding Detection Times for carprofen are given in Table 3.

Firocoxib

Following oral administration once daily for 6 days to five greyhounds, plasma firocoxib concentrations increase rapidly over 4–8 h before declining. There appears to be no accumulation of firocoxib upon once daily dosing as shown by the immediate pre-dose concentrations between 24 and 120 h (Figure 2A). Plasma pharmacokinetic parameters for firocoxib are summarised in Table 4 with further information in the corresponding table in the supplementary material. Urine firocoxib concentrations also peak within 4–8 h before declining in a similar manner to the plasma pharmacokinetics with urine concentrations generally mirroring those of plasma (Figure 2B). In plasma, firocoxib was below the LLOQ in two of the

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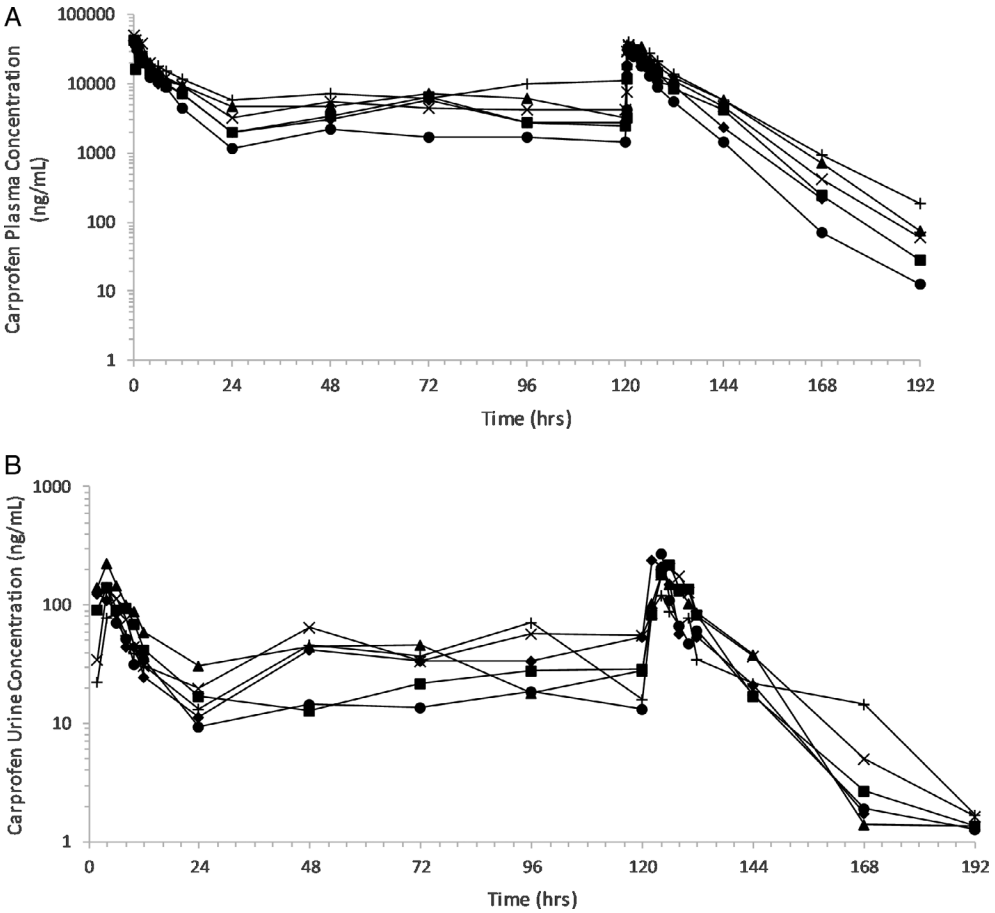

Figure 1. (A) Plasma concentrations in ng/mL of carprofen following a 4 mg/kg single intravenous injection followed 1 day later by 4 mg/kg five once daily oral administrations to 6 greyhounds, (B) urine concentrations in ng/mL of carprofen following a 4 mg/kg single intravenous injection followed 1 day later by 4 mg/kg five once daily oral administrations to 6 greyhounds,

Table 1. Plasma pharmacokinetic parameters for carprofen following a 4 mg/kg single intravenous injection fitted to a 2 compartmental pharmacokinetic model

Data from 6 greyhounds	V1 (L/kg)	V2 (L/kg)	First phase HL (h)	Terminal HL (h)	MRT (h)	Vss (L/kg)	CL (mL/min/kg)
Mean	0.098	0.066	1.11[a]	8.49[a]	11.17	0.16	0.26
Median	0.100	0.064	0.99	7.94	9.92	0.17	0.26

[a] Geometric mean.
CL is the plasma clearance for carprofen; HL are the respective half-lives of the first and second, terminal, decay phases; MRT is the mean residence time (average time spent in animal) of carprofen; V1 and V2 are the physiological volumes of each respective compartment; Vss is the steady-state volume of distribution for carprofen.

Table 2. Plasma pharmacokinetic oral parameters of carprofen following the last oral dose (120–192 h) using a non-compartmental PK model. $AUC_{120\text{-}144}$ and $AUC_{120\text{-}\infty}$ are the area under the plasma curve for 120–144 h (24 h post last dose) and 120-infinity hours, respectively

Data from 6 greyhounds	$AUC_{120\text{-}144}$ (ng h/mL)	$AUC_{120\text{-}\infty}$ (ng h/mL)	HL (h)
Mean	317,893	378,650	7.63[a]
Median	307,855	369,493	7.50

[a] Geometric mean.
HL, half-life of the terminal phase.

five dogs 168 h after the last drug administration. In urine, firocoxib was below the LLOQ in one dog 168 h after the last drug administration, in three dogs 192 h after the last drug administration, and in the remaining dog 216 h after the last drug administration. The data from one dog were removed from the plasma PK analysis as no samples were taken after the first 24 h due to it being excluded from the study due to

an injury unrelated to the study (a fight wound from another dog). The AUC for the first 24 h in the case of this dog was approximately 2 times higher than the $AUC_{120-144}$ for the other dogs. There were no statistical differences between male and female dogs for the PK parameters determined for firocoxib. The computed EPC, IPC, IUC and corresponding Detection Times for firocoxib are given in Table 5.

Table 3. Computed EPC, IPC, IUC and corresponding detection times for carprofen

Mean plasma clearance	0.26 mL/min/kg
Dosing interval	24 h
EPC	10,684 ng/mL
IPC	21 ng/mL
IPC on ordinal scale	20 ng/mL
Plasma detection time	103 h
Rss	0.015
IUC	0.32 ng/mL
IUC on ordinal scale	0.3 ng/mL (300 pg/mL)
Urine detection time	105 h

Discussion

Carprofen

Pharmacokinetic parameters and excretion profiles in this study were broadly similar to previous studies for plasma.[4-9] and also to the limited urine data.[10] The lack of a relationship between the carprofen exposure in urine and the average urine pH is not surprising as the pH range is narrow (5.4–6.6) and higher than the pKa. The pH range is lower than the pH of blood suggesting that the neutral form of carprofen dominates in urine leading to greater reabsorption of carprofen back into blood. This will lead to lower urine levels of carprofen relative to the situation where urine pH is neutral or alkaline. Conversely, a greyhound with sustained neutral to alkaline urine may have a higher concentration of carprofen in their urine compared to the levels observed in this study.

The pre-dose plasma samples had no detectable levels of carprofen, as expected as in Australia carprofen is licensed for use in dogs, cats and horses, but not in other farm livestock. In Europe, it is also licensed for use in cattle with the EU MRL for carprofen in bovine being 500 µg/kg for muscle and 1000 µg/kg for liver/kidney.[16] Assuming that a greyhound fed meat eats up to 600 g of meat per day at a level of 500 µg/kg then that would be equivalent of

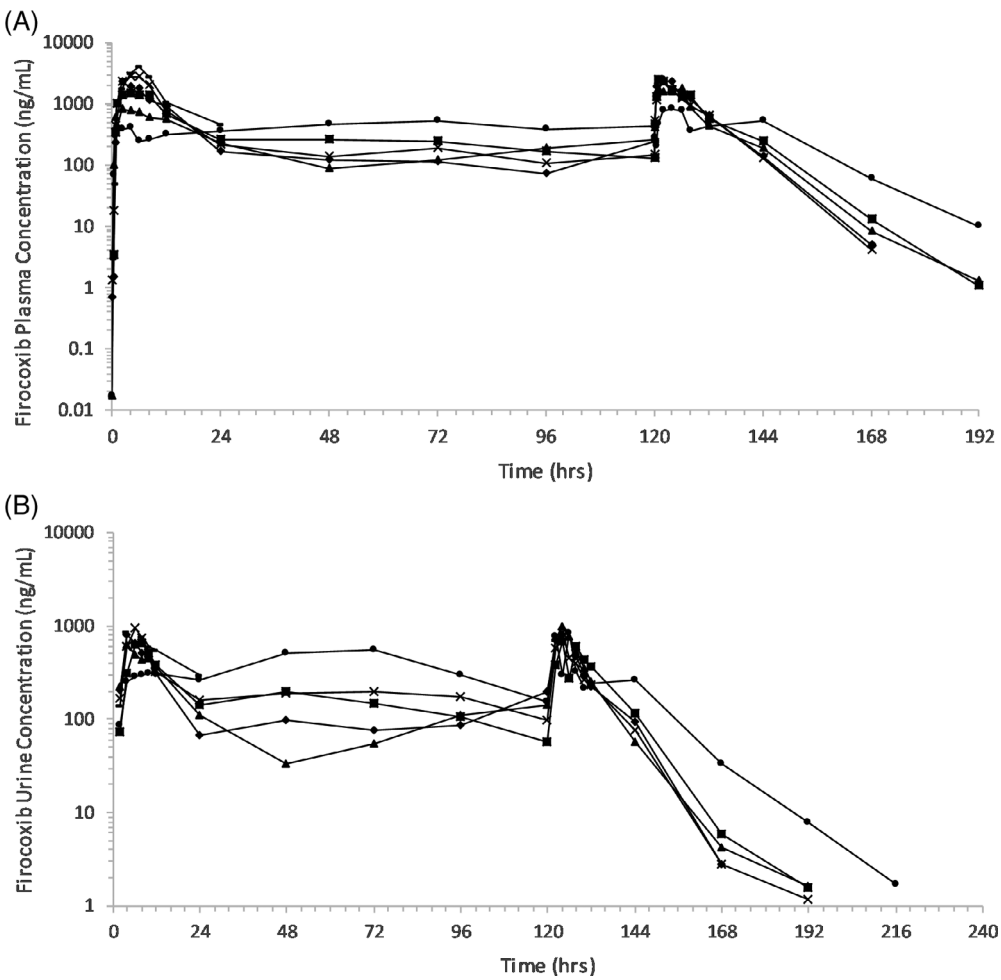

Figure 2. (A) Plasma concentrations in ng/mL of firocoxib following six 5 mg/kg once daily oral administrations to 6 greyhounds. Symbols on each line, which are different between lines, indicate the data from individual animals. (B) Urine concentrations in ng/mL of firocoxib following six 5 mg/kg once daily oral administrations to 6 greyhounds. Symbols on each line, which are different between lines, indicate the data from individual animals.

Table 4. Pharmacokinetic parameters of firocoxib following oral administration of 5 mg/kg once per day for 6 days to 6 greyhounds using a non-compartmental PK model

Data from 6 greyhounds	$AUC_{120\text{-}144}$ (ng h/mL)	$AUC_{120\text{-}\infty}$ (ng h/mL)	Terminal HL (h)
Mean	18,923	22,313	6.5[a]
Median	19,392	21,018	6.5

[a] Geometric mean.
$AUC_{120\text{-}144}$ and $AUC_{120\text{-}\infty}$, the area under the plasma curve for 120–144 h (24 h post last dose) and 120-infinity hours, respectively; HL, half-life of the terminal phase.

Table 5. Computed EPC, IPC, IUC and corresponding detection times for firocoxib

Mean $AUC_{120\text{-}144}$	18,923 ng h/mL
Dosing interval	24 h
EPC	788 ng/mL
IPC	1.6 ng/mL
IPC on ordinal scale	2 ng/mL
Plasma detection time	96 h
Rss	1
IUC	1.6 ng/mL
IUC on ordinal scale	2 ng/mL
Urine detection time	96 h

delivering 300 µg of carprofen. If the meat was eaten once per day for a few weeks then accumulation of approximately 20% for carprofen would occur. Therefore, the effective dose of carprofen would be 360 µg or approximately 12 µg/kg Greyhound. This administration study delivered 4 mg/kg (= 4000 µg/kg) of carprofen, that is, 333 times higher than the 12 µg/kg from ingestion of meat. Therefore, the expected plasma levels due to ingestion of cattle muscle at 500 µg/kg carprofen, 24 h after the last feed, would be approximately 18 ng/mL and the expected urine levels would be 0.12 ng/mL. The expected urine levels would be below the LLOQ for urine and therefore not be detected, whereas the expected plasma levels of 18 ng/mL due to feed would be detected. However, 18 ng/mL is comparable to the Irrelevant Plasma Concentration (see below) of 20 ng/mL and theoretically there is the possibility of the limit being exceeded. However, this would only occur if the meat contained the highest level of the MRL when animal byproducts from treated animals are fed.

Firocoxib

The plasma concentrations of firocoxib are moderately higher as compared to the information previously available where plasma concentrations were measured for up to 32 h.[11] In the present study, the $AUC_{120\text{-}144}$ was 18,923 ng h/mL, whereas AUC $_{0\text{–}24}$ is reported as 4630 ng h/m,[17] although for this latter AUC it is not stated whether this is for a single administration value or the AUC of the dosing interval for multiple administrations. This Summary of Product Characteristics[17] states the tablets can be administered with or without food, the last dose was given with a small amount of food in the present study, but the product assessment,[18] states feeding is unlikely to impact on the bioavailability of firocoxib. In the present study, the half-life of the terminal phase was 6.5 h, as compared to the elimination half-life of 7.59 h reported in the Summary of Product Characteristics.[17] As also found by Biddle (personal communication) hydroxyfirocoxib and desalkyl firocoxib are reported here as urinary metabolites and this information on these metabolites in the dog, which is not readily obtainable elsewhere, can be useful as evidence of direct exposure, metabolism, and excretion if there is challenge on this aspect in sporting regulatory investigations. As firocoxib is a neutral molecule and therefore does not ionise then any changes in urine pH will have no effect on the urine pharmacokinetics thus reducing variability.

The half-life of firocoxib in horses is 64.5 h with an EU MRL of 10 µg/kg for muscle and 60 µg/kg for liver.[18] Assuming that a Greyhound fed meat eats up 600 g of meat per day at a level of 10 µg/kg then that would be equivalent of delivering 6 µg of firocoxib. If the meat was eaten once per day for a few weeks then accumulation of approximately 8% for firocoxib would occur. Therefore, the effective dose of firocoxib would be 6.5 µg or approximately 0.22 µg/kg. This administration study delivered 5 mg/kg (= 5000 µg/kg) of firocoxib, that is, 22,727 times higher than 0.22 µg/kg from ingestion of meat. Therefore, the expected plasma levels due to ingestion of horse muscle at 10 µg/kg firocoxib, 24 h after the last feed, would be approximately 23 pg/mL and the expected urine levels would be 12 pg/mL. The expected urine levels would be below the LLOQ for urine and therefore not be detected, whereas the expected plasma levels of 23 pg/mL due to feed may be detected. However, 23 pg/mL is much lower than the Irrelevant Plasma Concentration (see below) of 2 ng/mL and therefore will not lead to a positive finding.

Both these studies yielded adequate data, especially combining plasma and urine levels for the first time, to inform medication control in racing greyhounds.

Medication control calculations

Using the standard methodology for medication control,[3] the IPC was determined as 20 and 2 ng/mL for carprofen and firocoxib, respectively. The IUC was also determined as 0.3 and 2 ng/mL for carprofen and firocoxib, respectively. Extrapolation for the terminal phase was used for carprofen computations. The calculations for

these determinations are provided in the on-line supplementary material.

The actual screening limit (SL), being the reporting levels used by the analytic laboratories, and detection time (DT), being the time after drug administration when the SL is not exceeded, as determined and publicised by regulators will not necessarily be absolutely based on these Irrelevant Concentrations.[19] Regulatory Risk Management is not a scientific exercise but it should be scientifically sound, and the SL may be (slightly) higher or lower than the IPC/IUC to take into account other relevant factors other than residual drug efficacy as the common goal to achieve harmonisation.[20] For example, it is reported from the efficacy studies completed during the registration process of firocoxib that the threshold for effect was approximately 100 ng/mL for the dog,[11] and the contrast with the calculated IPC of 2.0 ng/mL shows the different perspectives between therapy and medication control. The carprofen IC_{50} in the plasma of dogs is 2.7 μg/mL,[21] again in contrasting perspective with the calculated IPC of 20 ng/mL.

The DT, as issued by the regulator is a regulatory decision based on experimental data, whereas a withdrawal time (WT) is a recommendation and, as such, is a matter for professional judgement of the treating veterinarian. The WT should be longer than the DT because the WT should take into account the impact of all sources of animal variability (age, sex, breed, training, racing, etc.) and those of the medicinal product actually administered.[13]

Given that dogs are fed meat, and if this contains drug residues, a regulatory SL for medication control of carprofen and firocoxib, derived from the IPC and IUC, can also serve as a Recommended Limit of Detection for drug residues when feeding animal byproducts.[19]

Conclusion

These administration studies characterised the plasma and the urinary pharmacokinetics of carprofen and firocoxib, with sufficient duration and sensitivity to enable the IPCs and IUCs to be determined after the exposure of greyhounds to clinical doses of carprofen and firocoxib. This information will allow greyhound regulators to provide regulatory advice for greyhound racing in respect of both a SL for medication control and a Recommended Limit of Detection for drug residues if meat containing such residues is fed, and for veterinarians to advise a WT.

Conflicts of interest and sources of funding

Greyhound Racing Victoria funded this study, the administration study was performed at Eurofins SCEC, the chemical analysis was performed at Racing Analytical Services Limited, and the pharmacokinetic analysis was performed at the University of Nottingham. Tim Morris is Independent Scientific Adviser to the Greyhound Board of Great Britain and receives fees for this activity, holds an unpaid appointment the University of Nottingham and has received fees from Greyhound Racing Victoria. Steven Karamatic is Chief Veterinarian at Greyhound Racing Victoria and receives payment for this activity. Paul Zahra is an employee of Racing Analytical Services Limited and Eric Li was an employee at the time the work was performed. Stuart Paine is an employee of the University of Nottingham and has received fees for advice from Greyhound Racing Victoria. Sally Colgan was an employee of Eurofins SCEC.

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Supporting information

Additional Supporting Information may be found in the online version of this article at the publisher's web-site: http://onlinelibrary.wiley.com/doi/10.1111/avj.13014/suppinfo.

Data S1. Supplementary Information.

Figure S1. Area under the urine carprofen concentration-time curve versus time weighted average urine pH for each dog. Dotted line represents pKa of carprofen (4.3).

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