



## Pharmacokinetics and metabolism of eprinomectin in cats when administered in a novel topical combination of fipronil, (S)-methoprene, eprinomectin and praziquantel



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### ABSTRACT

Four studies were conducted to determine the pharmacokinetic characteristics and in vitro metabolism of eprinomectin, a semi-synthetic avermectin, in cats. Pharmacokinetic parameters including bioavailability of eprinomectin were determined in a parallel study design comprised of one group of eight cats which were treated once topically at 0.12 mL/kg bodyweight with BROADLINE<sup>®</sup>, a novel combination product (fipronil 8.3% (w/v), (S)-methoprene 10% (w/v), eprinomectin 0.4% (w/v) and praziquantel 8.3% (w/v)), delivering a dose of 0.5 mg eprinomectin per kg body weight, and a group of six cats which received 0.4% (w/v) eprinomectin at 0.4 mg/kg bodyweight once by intravenous injection. For cats treated by topical application, the average eprinomectin (B1<sub>a</sub> component) maximum plasma concentration ( $C_{max}$ ) was 20 ng/mL. The maximum concentrations were reached 24 h after dosing in the majority of the animals (six of eight cats). The average terminal half-life was 114 h due to slow absorption ('flip-flop' kinetics). Following intravenous administration the average  $C_{max}$  was 503 ng/mL at 5 min post-dose, and the mean elimination half-life was 23 h. Eprinomectin was widely distributed with a mean volume of distribution of 2390 mL/kg, and the clearance rate was 81 mL/h/kg. Mean areas under the plasma concentration versus time curves extrapolated to infinity were 2100 ng h/mL and 5160 ng h/mL for the topical and intravenous doses, respectively. Topical eprinomectin was absorbed with an average absolute bioavailability of 31%. In a second parallel design study, the dose proportionality of eprinomectin after single topical administration of BROADLINE<sup>®</sup> was studied. Four groups of eight cats each were treated once topically with 0.5, 1, 2 or 5 times the minimum recommended dose of the combination, 0.12 mL/kg bodyweight. Based on comparison of areas under the plasma concentration versus time curves from the time of dosing to the last time point at which eprinomectin B1<sub>a</sub> was quantified, and  $C_{max}$ , dose proportionality was established. In addition, the metabolic pathway of eprinomectin using cat liver microsomes, and plasma protein binding using cat, rat, and dog plasma were studied in vitro. Results of the analyses of eprinomectin B1<sub>a</sub> described here showed that it is metabolically stable and highly protein bound (>99%), and thus likely to be, as with other species, excreted mainly as unchanged parent drug in the feces of cats.

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## 1. Introduction

Cats are susceptible to a number of internal parasites, such as intestinal worms and heartworms, and external parasites, such as fleas and ticks, which may pose a health risk to the animal and in some cases humans. While clinical signs vary or may be negligible, it is important to treat parasitic infections to reduce any serious and/or long term health effects the cat may experience. There are a number of veterinary products available that target only internal helminth parasites or prevent heartworm infections, and products that target external parasites only. Only a few products concomitantly target both, endo- and ectoparasites, and meet customer expectations with regards to what can be achieved in terms of treatment and prevention of disease (Meinke, 2001; Pipano, 2003). Merial has developed Broadline<sup>®</sup>, a novel topical combination formulation of fipronil 8.3% (w/v), (S)-methoprene 10% (w/v), eprinomectin 0.4% (w/v), praziquantel 8.3% (w/v) for cats suffering from, or at risk of, mixed parasitic infections. In this formulation eprinomectin provides activity for the treatment and control of intestinal and urinary bladder worms, and the prevention of heartworm disease in cats and kittens.

Eprinomectin, developed exclusively for veterinary medicine use, is a potent parasiticide and was developed as the first endectocide for cattle including lactating dairy cows. Eprinomectin was registered as the topical solution EPRINEX<sup>®</sup> Pour-On (Merial) for the treatment and control of certain gastrointestinal roundworms, lungworms, eye-worms, cattle grubs, lice, mange mites and flies in and on cattle (Shoop et al., 1996a; Shoop and Soll, 2002), and more recently as an injectable formulation, LONGRANGE<sup>™</sup> (Merial), providing efficacy against cattle parasites for up to 150 days (Soll et al., 2013). Eprinomectin (eprinomectin B1), 4'-epiacetylamino-4''-deoxyavermectin B1, belongs to the macrolide class of parasiticides and the avermectin family. It is a semi-synthetic derivative of avermectin B1 or abamectin. It consists of two homologs, B1<sub>a</sub> and B1<sub>b</sub>, which differ by a methylene group, and it is defined as no less than 90% B1<sub>a</sub> and no more than 10% B1<sub>b</sub> (EMA, 1996). Eprinomectin B1<sub>a</sub>, the major component was used for quantitative analysis for this study. Although the exact mechanism of action of eprinomectin is not known, this class of macrocyclic lactone drugs mediates its nematocidal effect via interaction with a common receptor molecule; macrocyclic lactones essentially irreversibly open glutamate-gated chloride ion channels resulting in hyperpolarization. Recently, the binding site of a macrocyclic lactone (ivermectin) and the structure of a glutamate gated ion channel were determined (Wolstenholme, 2012). This research contributes to our understanding of these ion channels and their modulation by macrocyclic lactones such as eprinomectin.

While the use of eprinomectin has been characterized in several species other than cattle, it is a new anthelmintic compound for cats. In both laboratory and field studies, it has been shown to be well tolerated in adult cats and kittens. To further characterize the use of eprinomectin in cats, two *in vivo* studies were conducted to evaluate the pharmacokinetic profile, and two *in vitro* studies

were conducted to evaluate biotransformation and protein binding.

## 2. Materials and methods

### 2.1. Evaluation of pharmacokinetics including dose proportionality

#### 2.1.1. Experimental animals

In total, 56 purpose-bred European Short Hair cats were selected for use in the two studies. Animals were randomly allocated to treatment groups following blocking based on pre-treatment body weight within sex category (male and male castrated; female and female spayed). Details of the animals as grouped following allocation in both studies are given in Table 1. None of the animals had been treated with parasiticides in the three months prior to treatment. Cats were acclimated to the study facilities for seven days and were housed individually in cages. Food was provided once daily as per the manufacturer's recommendation; drinking water was available at all times. All cats were observed hourly for 4 h after treatment application and once daily throughout study for health problems or adverse events. All animals were managed similarly and with due regard for their welfare. Animals were handled in compliance with local Ethics Committee approvals and other applicable local regulations and requirements.

#### 2.1.2. Dosing and sample collection

In a parallel design study, one group of eight cats was treated with the combination product, fipronil 8.3% (w/v), (S)-methoprene 10% (w/v), eprinomectin 0.4% (w/v) and praziquantel 8.3% (w/v) (Broadline<sup>®</sup>, Merial), once topically at 0.12 mL/kg bodyweight delivering a dose of 0.5 mg eprinomectin per kg bodyweight, and one group of six cats was treated with 0.4% (w/v) eprinomectin at 0.10 mL/kg bodyweight (equivalent to 0.4 mg eprinomectin per kg bodyweight) once by intravenous (IV) injection. The topical dose corresponded to the intended minimum label dose (0.5 mg/kg) of the novel combination formulation and was applied directly onto the skin in the midline of the neck, between the base of the skull and the shoulder blades in a single spot. The IV injection (0.4 mg/kg eprinomectin) was given in the cephalic vein. Blood samples were collected prior to treatment and at 5 and 30 min (IV-treated cats only), 1, 2, 4, 8 (topical-treated cats only), 10, and 24 h, and 2, 3, 5, 7, 10, 12, 14, 16 and 18 days following treatment administration.

Dose proportionality of eprinomectin after single topical administration of the combination product was studied in a parallel design study. Four groups of eight cats each were treated once topically with the topical combination product at 0.06 mL/kg, 0.12 mL/kg, 0.24 mL/kg or 0.60 mL/kg bodyweight, respectively (equivalent to 0.25, 0.5, 1.0 or 2.5 mg eprinomectin per kg bodyweight), representing 0.5, 1, 2 and 5 times the intended minimum label dose. Treatments were applied as described before. Blood samples were collected prior to treatment and at 1, 2, 4, 8, 10, and 24 h, and 2, 3, 5, 7, 10, 12, 14, 16 and 18 days following treatment application. In both studies, blood samples were drawn from the jugular veins and collected in lithium

**Table 1**

Summary characteristics of experimental cats: treatment and treatment dosage, sex, age and pre-treatment bodyweight.

Study/group/dosage	Sex <sup>c</sup>	~Age (months)	Pre-treatment bodyweight (kg)
Study 1 – Pharmacokinetics and bioavailability			
Topical FMEP <sup>a</sup> at 0.12 mL/kg	4MC, 4FS	7–36	3.18–5.25
Intravenous E <sup>b</sup> at 0.10 mL/kg	3MC, 3FS	18–29	3.44–4.91
Study 2 – Dose proportionality			
Topical FMEP <sup>a</sup> at 0.06 mL/kg	2M, 2MC, 2F, 2FS	9–27	2.74–5.77
Topical FMEP <sup>a</sup> at 0.12 mL/kg	1M, 3MC, 3F, 1FS	12–34	2.94–5.06
Topical FMEP <sup>a</sup> at 0.24 mL/kg	1M, 3MC, 3F, 1FS	8–31	2.62–5.07
Topical FMEP <sup>a</sup> at 0.60 mL/kg	1M, 3MC, 2F, 2FS	11–27	2.79–5.50

<sup>a</sup> Fipronil 8.3% (w/v), (S)-methoprene 10% (w/v), eprinomectin 0.4% (w/v) and praziquantel 8.3% (w/v).

<sup>b</sup> Eprinomectin 0.4% (w/v).

<sup>c</sup> M = male, MC = male castrated, F = female, FS = female spayed.

heparinized tubes. Plasma was separated from each sample and stored frozen in aliquots until analysis.

### 2.1.3. Plasma sample analysis

A validated, solid-phase extraction method was used for the determination of eprinomectin B<sub>1a</sub> (and other actives) from plasma, which is briefly described here. After pre-conditioning/equilibrating a solid-phase extraction plate (BIOTAGE® Evolute ABN, 25 mg, Biotage, LLC, Charlotte, NC, USA) with 1 mL each of acetonitrile and 0.1% formic acid, the acidified plasma sample (250 µL plasma + 750 µL 1% formic acid + 50 µL internal standard + 50 µL acetonitrile) was loaded onto the plate. The plate was subsequently rinsed with 1 mL of 5% methanol in water. Eprinomectin was eluted with 2 × 100 µL of acetonitrile, and 50 µL of water was added to the eluate. An aliquot was injected onto the liquid chromatography–mass spectrometry (LC–MS) system. In addition to incurred samples, control and fortified quality control (QC) plasma samples were also assayed with each set. An eprinomectin stock standard was prepared by dissolving 25 mg (corrected for purity and water content) in 25 mL of acetonitrile. A 10 µg/mL mixed standard solution (fipronil, (S)-methoprene, eprinomectin and praziquantel) was then prepared, from which a series of mixed standards were prepared. Standard curves were prepared with each set by adding 0.050 mL of standard solution (4.0–2500 ng/mL) to 0.250 mL of control plasma to yield a standard curve ranging from 0.8 ng/mL to 500 ng/mL. Quality control (QC) samples were prepared by adding 0.050 mL of 5.0, 10, 125 and 2000 ng/mL standards to 0.250 mL of control plasma to yield 1.0, 2.0, 25, and 400 ng/mL samples. The standards were injected before and after each set of samples. All reagents (Merck KgaA, Darmstadt, Germany) were of the appropriate grade.

The LC–MS system consisted of a Waters Alliance® HT 2795 coupled with a Waters Micromass® Quattro Micro (Micromass UK Limited, Wythenshawe, Manchester, UK). Separation was achieved on a Varian Pursuit Diphenyl column (150 mm × 2 mm, 3 µm particle size, catalog number A3041150x020) coupled with a Sure-Guard disposable In-Line filter (stainless steel frit, 0.5 µm, catalog number 0611-SS05) and Varian Metaguard Pursuit Diphenyl precolumn (2 mm, 3 µm particle size, catalog number A3041MG2) using a mobile phase consisting of 70:30 acetonitrile/2 mM ammonium acetate buffer (pH 4) at a flow rate of 0.2 mL/min. The column was maintained at 55 °C,

and the autosampler chamber was maintained at 10 °C. The retention time of eprinomectin B<sub>1a</sub> was ~4 min, and the transition of 914.5–186.2 *m/z* was monitored in the positive mode. Ethiprole PESTANAL® (Sigma–Aldrich, Batch SZE8168X, purity 98.3%) was used as the internal standard and was monitored in the negative mode. The validated limit of quantitation of eprinomectin B<sub>1a</sub> in cat plasma was 1 ng/mL.

### 2.1.4. Chromatographic data analyses

MassLynx® 4.1 software was used to generate and process chromatograms. QuanLynx® 4.1 was used to perform the linear regression analysis (weighted 1/x, concentration versus peak area ratio), and to calculate the concentration of eprinomectin B<sub>1a</sub> and the accuracy of the standards and QC samples. The slope, y-intercept, and coefficient of determination were determined for each set, and the unknown concentrations were calculated from the linear equation. The curve was considered acceptable if the coefficient of determination was ≥0.99 and contained at least five different standard concentrations. Accuracy and precision were evaluated as percent recovery and reproducibility (coefficient of variation) of fortified sample results. The individual and average percent recoveries in conjunction with the coefficients of variation of the fortified samples were calculated. The overall average recovery was considered acceptable if it was between 85% and 115% with a coefficient of variation of ≤15%.

### 2.1.5. Pharmacokinetic analysis

Eprinomectin B<sub>1a</sub> plasma concentrations were determined for each animal for each sampling time; mean plasma levels were then determined for each time point by treatment. Samples with values below the quantitation limit were not used. The following pharmacokinetic parameters were calculated for each individual animal using the non-compartmental analysis function of the WinNonlin® pharmacokinetic software (version 5.0.1, Pharsight Corp., Mountain View, CA, USA) and then averaged for all animals under the same treatment: the area under the plasma concentration versus time curve (AUC) from the time of dosing to the last time point at which drug concentration was quantified, AUC(0–*t*<sub>last</sub>); the AUC extrapolated to infinity, AUC(0–∞), the terminal half-life, *t*<sub>1/2</sub>; the volume of distribution at steady-state, *V*<sub>ss</sub>; the total body clearance/clearance rate, CL; and the absolute

topical bioavailability,  $F$ . Maximum plasma concentrations ( $C_{\max}$ ) and time to maximum concentration ( $T_{\max}$ ) were the observed values for each animal. The AUC was calculated using the linear/logarithmic trapezoidal method which uses the linear trapezoidal rule up to  $C_{\max}$  and the logarithmic trapezoidal rule for the remainder of the curve. Absolute bioavailability was calculated using the actual topical dose for each animal and the mean IV dose and  $AUC(0-t_{\text{last}})$  (due to the parallel study design) using the formula  $F = (AUC_{\text{topical}}/Dose_{\text{topical}})(Dose_{\text{IV}}/AUC_{\text{IV}})$ .

To assess the relationship between dose and exposure (Study 2, dose proportionality), a comparison of the average dose and exposure ratios ( $AUC(0-t_{\text{last}})$  and dose, and  $C_{\max}$  and dose) was made; in addition, the Pearson product moment correlation coefficient ( $r$ ) for linear regression and the two-sided  $p$ -value were determined (Wessa, 2012. Free Statistics Software, Office for Research Development and Education, version 1.1.23-r7, URL <http://www.wessa.net/>). The regression lines were based on four treatment groups of eight animals each.

## 2.2. Evaluation of in vitro metabolism and plasma protein binding

### 2.2.1. In vitro metabolism

In vitro metabolic pathways were characterized using two lots each of pooled male ( $n=3$ ) and pooled female ( $n=3$ ) cat liver microsomes and S9 fractions (Celsis In Vitro Technologies Inc., Baltimore, MD, USA) in the presence of unlabelled eprinomectin. Two positive control incubations using 7-hydroxycoumarin and midazolam were included to verify oxidation, glucuronidation, and sulfation capabilities of the male and female cat liver microsomes and S9 fractions. The enzyme cofactors tested included the NADPH regeneration system (the cofactor for P450 enzymes), UDPGA (the cofactor for uridine glucuronyl transferases), and PAPS (the cofactor for sulfotransferases). Eprinomectin (125  $\mu\text{L}$ , 0.4  $\mu\text{M}$ ) in 50 mM phosphate buffer was incubated with 250  $\mu\text{L}$  male or female microsomes (1 mg/mL) or S9 fractions (2 mg/mL) and 125  $\mu\text{L}$  of the cofactor at 37 °C in a shaking water bath. Aliquots of 50  $\mu\text{L}$  were taken from the incubation solution at 0, 30, 60, 120, and 240 min. The reaction was stopped by the addition of 150  $\mu\text{L}$  of acetonitrile containing the internal standard. The samples were vortex mixed followed by centrifugation for 30 min at 3310  $\times g$  and then analyzed by LC–MS. The positive controls used 50  $\mu\text{L}$  of 2 mg/mL male or female liver microsomes or S9 fraction in buffer, 25  $\mu\text{L}$  of 4  $\mu\text{M}$  midazolam or 100  $\mu\text{M}$  7-hydroxycoumarin (7-HC) in buffer, and 25  $\mu\text{L}$  of the NADPH regeneration solution, UDPGA, or PAPS. Alamethicin (25  $\mu\text{g}/\text{mL}$ ) was included in the UDPGA incubation cocktail to facilitate transport of UDPGA to the active site. After incubation in a water bath with gentle shaking for 1 h, the reaction was stopped by the addition of 300  $\mu\text{L}$  acetonitrile containing the internal standard. The supernatants were analyzed by LC–MS after centrifugation for 30 min at 3310  $\times g$ .

### 2.2.2. Protein binding

Equilibrium dialysis (Dianorm Multi-Equilibrium DIALYZER™) was used to determine the degree of protein

binding of eprinomectin. The experiments were conducted with three different lots of cat, dog, and rat plasma (EDTA treated; Bioreclamation, West Meadow, NY, USA) fortified at three concentrations, 10, 50, and 500 ng/mL. Aliquots of 1 mL of fortified plasma were placed in one side of the chamber and 1 mL of isotonic phosphate buffer (pH 7.4) was placed in the other side. The chambers were separated by a semi-permeable cellulose membrane with a molecular weight cutoff (MWCO) of 10,000 Da. After incubation for approximately 4 h at 37 °C, aliquots from both sides of the chamber (plasma and buffer) were taken and analyzed by a LC–MS method to determine the concentration of unbound eprinomectin.

## 3. Results

### 3.1. Plasma sample analysis

The bioanalytical method was selective, accurate, and reproducible for the analysis of eprinomectin B1<sub>a</sub> from cat plasma. There were no interferences from endogenous plasma compounds or the other analytes. Fortified and unfortified control plasma samples were analyzed with each set. Samples were fortified at 1, 2, 25, and 400 ng/mL, and the average percent recovery (interday accuracy) at each level was 102, 92, 94, and 97, respectively, with the coefficient of variation (interday precision) less than 13% in all cases. The grand average across all fortified samples ( $n=47$ ) was  $96 \pm 10\%$ . For the dose proportionality study, the average percent recovery at each level was 98, 97, 94, and 98, respectively, with a coefficient of variation of 8% or less for each level. The grand average ( $n=73$ ) was  $96 \pm 7\%$ .

### 3.2. Pharmacokinetic analysis

Topical eprinomectin was absorbed with an absolute bioavailability of 31%. An average  $C_{\max}$  of 20 ng/mL was obtained at an average  $T_{\max}$  of 48 h.  $AUC(0-t_{\text{last}})$  was 1880 ng h/mL on average, and the half-life was 114 h (4.75 days). The clearance rate of intravenously administered eprinomectin was 81 mL/h/kg and the volume of distribution at steady state was 2390 mL/kg. The elimination followed a bi-exponential decay with a half-life of 23 h. Pharmacokinetic parameters from both the topical and IV routes of administration are summarized in Table 2. The plasma concentration time profiles of the individual animals are shown in Figs. 1 and 2, and the average concentration–time profiles are plotted in Fig. 3. Animal 293 in Treatment Group 1 had the highest plasma concentrations of all topically treated animals, perhaps due to ingestion from grooming.

Drug exposure was proportional to dose in the range of 0.25–2.5 mg/kg bodyweight, or 0.5–5 times the recommended minimum dose, as illustrated by the average plasma concentration time curves (Fig. 4), and by comparison of the dose and exposure ( $AUC(0-t_{\text{last}})$  and  $C_{\max}$ ) ratios (Table 3 and Fig. 5), though slightly under proportional at 5 $\times$ . The square of the Pearson product moment correlation coefficient ( $r$ ) for linear regression between  $AUC(0-t_{\text{last}})$

**Table 2**

Summary of the pharmacokinetic parameters of eprinomectin following a single topical dose administration of Broadline® (including eprinomectin at 0.5 mg/kg bodyweight) or an intravenous eprinomectin formulation (0.4 mg/kg eprinomectin) to cats.

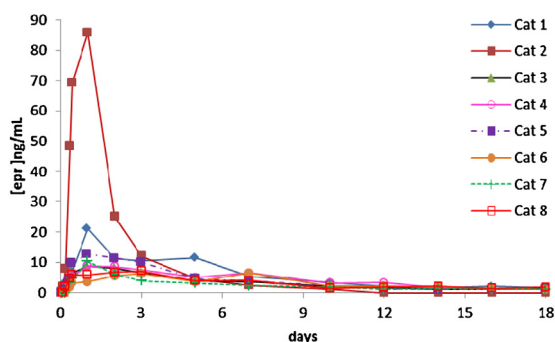
Parameter <sup>a</sup>	Topical eprinomectin, n = 8		Intravenous eprinomectin, n = 6	
	Mean ± SD <sup>b</sup>	CV <sup>c</sup> (%)	Mean ± SD	CV (%)
T <sub>max</sub> (h)	48 ± 51	107	NA <sup>d</sup>	NA
C <sub>max</sub> (ng/mL)	20.1 ± 26.9	133	503 ± 88	17
C <sub>0</sub> (ng/mL)	NA	NA	548 ± 107	19
AUC(0–t <sub>last</sub> ) (ng h/mL)	1880 ± 813	43	5080 ± 987	19
AUC <sub>inf</sub> (ng h/mL)	2100 ± 768	37	5160 ± 983	19
% AUC extrapolated	11.9 ± 5.4	46	1.69 ± 1.17	69
Terminal half-life (h)	114 ± 37	33	23.1 ± 7.0	30
CL (mL/h/kg)	NA	NA	80.8 ± 14.9	18
V <sub>ss</sub> (mL/kg)	NA	NA	2390 ± 595	25

<sup>a</sup> T<sub>max</sub>, time to maximum concentration; C<sub>max</sub>, maximum plasma concentration; C<sub>0</sub>, concentration extrapolated to time 0; AUC(0–t<sub>last</sub>) or (0–∞), area under the plasma concentration versus time curve from the time of dosing to the last time point at which drug concentration was quantified or extrapolated to infinity; %AUC extrapolated, percent of AUC, extrapolated to infinity; CL, total body clearance; V<sub>ss</sub>, volume of distribution at steady-state.

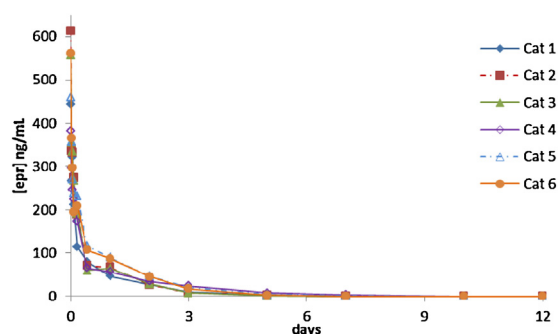
<sup>b</sup> Standard deviation.

<sup>c</sup> Coefficient of variation.

<sup>d</sup> Not applicable.



**Fig. 1.** Plasma concentration over time (days) of eprinomectin B1<sub>a</sub> [epri] in individual cats after topical administration of a single dose of 0.5 mg/kg bodyweight in the combination formulation consisting of fipronil 8.3% (w/v), (S)-methoprene 10% (w/v), eprinomectin 0.4% (w/v) and praziquantel 8.3% (w/v).



**Fig. 2.** Plasma concentration over time (days) of eprinomectin B1<sub>a</sub> [epri] in individual cats after intravenous administration of a single dose of 0.4 mg/kg bodyweight in a 0.4% (w/v) eprinomectin formulation.

and dose and C<sub>max</sub> and dose was 0.9703 and 0.9393, respectively.

### 3.3. In vitro metabolism and protein binding

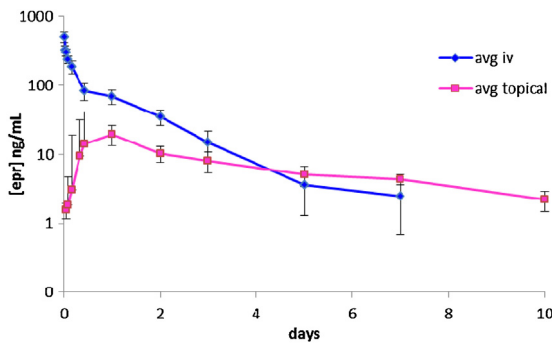
To assess in vitro metabolic pathways, eprinomectin was incubated with one lot of pooled male and one lot of pooled female cat liver microsomes and S9 fractions. The resulting samples were analyzed by LC–MS to quantify eprinomectin B1<sub>a</sub> and determine the disappearance rate. In general, the liver microsomes showed greater metabolic activity than the S9 fractions. It was determined that eprinomectin was very metabolically stable and not subject

to phase II metabolism. Conjugates of eprinomectin were not observed after incubation with the glucuronidation and sulfation cofactors UDPGA and PAPS. In the male and female liver microsomes, the in vitro half-life was 7.8 or 29 h, respectively, and the intrinsic clearance was 3.5 or 1.8 mL/min/kg, respectively. Eprinomectin was determined to be a low hepatic extraction ratio drug, with values of 0.12 or 0.06, respectively. To further evaluate gender related metabolism, an additional lot each of male and female microsomes were obtained and evaluated. It was inconclusive as to whether there was gender dependent eprinomectin metabolism; it appeared to correlate with relative CYP3A4 activity data. One lot of pooled microsomes (male) had CYP3A4 activity almost threefold greater than the other lots (one male and two female).

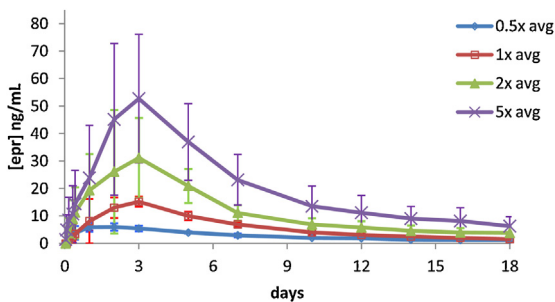
**Table 3**

Nominal dose ratios versus calculated mean exposure ratios (C<sub>max</sub> and AUC) of eprinomectin following single dose administrations of Broadline® to cats.

Eprinomectin dose	Dose ratio	C <sub>max</sub> (ng/mL)		AUC(0–t <sub>last</sub> ) (ng h/mL)	
		Mean	Ratio	Mean	Ratio
0.25 mg/kg topically	0.5	6.90	0.43	1140	0.44
0.5 mg/kg topically	1	16.1	1.00	2570	1.00
1 mg/kg topically	2	33.7	2.09	5040	1.97
2.5 mg/kg topically	5	54.1	3.35	9000	3.51

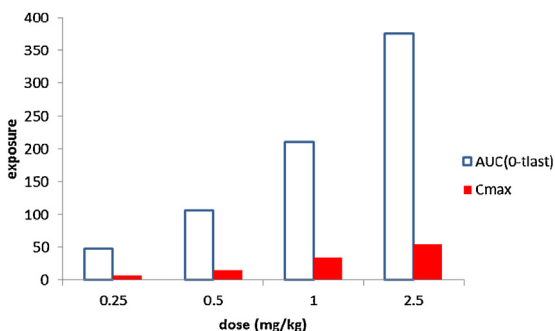


**Fig. 3.** Average ( $\pm$ standard deviation) plasma concentration over time (days) of eprinomectin B<sub>1a</sub> [epr] in cats after topical (0.5 mg/kg bodyweight,  $n = 8$ ) or intravenous (IV, 0.4 mg/kg bodyweight,  $n = 6$ ) administration.



**Fig. 4.** Average ( $\pm$ standard deviation,  $n = 8$ , each) plasma concentration over time (days) of eprinomectin B<sub>1a</sub> [epr] after a single topical administration of eprinomectin at 0.5, 1, 2, or 5 times the recommended dose of 0.5 mg/kg bodyweight in the combination formulation consisting of fipronil 8.3% (w/v), (S)-methoprene 10% (w/v), eprinomectin 0.4% (w/v) and praziquantel 8.3% (w/v).

The amount of drug bound to plasma proteins was determined as the percent of total drug added (%protein binding =  $100 \times (\text{total} - \text{unbound})/\text{total}$ ). The average binding of eprinomectin to cat plasma proteins at concentrations ranging from 10 ng/mL to 500 ng/mL was 99.5%, and the variability in binding between concentrations was negligible. The percent bound was also similar across species, 99.4% in rat and 99.6% in dog.



**Fig. 5.** Proportionality of dose and exposure for both area under the plasma concentration versus time curve from the time of dosing to the last time point at which drug concentration was quantified, AUC(0– $t_{\text{last}}$ ), and maximum plasma concentration ( $C_{\text{max}}$ ).

## 4. Discussion

The pharmacokinetics of eprinomectin have also been studied in rabbits, donkeys, sheep, and goats (Dupuy et al., 2001; Kožuh Eržen et al., 2007; Wen et al., 2010a; Gokbulut et al., 2012). Alongside other macrocyclic lactones such as milbemycin, selamectin, and ivermectin that are used to treat cats (Nolan and Lok, 2012), eprinomectin exhibits slow topical absorption, high protein binding, extensive distribution, minimal biotransformation, and slow biliary excretion (Sarasola et al., 2002; Chittrakarn et al., 2009; González Canga et al., 2009). In contrast, the dermal bioavailability of selamectin in cats is much higher (74%) than eprinomectin (Sarasola et al., 2002). The absolute bioavailability of topically applied eprinomectin in cats is comparable to that in cattle, 31% versus 29%, respectively, (Merial, unpublished data). Licking or self-grooming behaviors may result in an increased variability of exposure (Wen et al., 2010a; Rehbein et al., 2012). Plasma concentration profiles of the individual cats and coefficients of variation of pharmacokinetic parameters indicate some variation in exposure between cats which might be related to a different extent of grooming and thus oral ingestion of topically applied eprinomectin, as grooming was observed for several cats in each treatment group.

In the first study to compare topically and intravenously administered eprinomectin in cats, the concentration time profiles indicate an absorption dependent decay in terminal plasma concentrations of topically administered eprinomectin, what is commonly termed ‘flip-flop’ kinetics. The average half-life is 114 h (4.75 days) for topical dosing and 23 h for intravenous dosing. A prolonged absorption is a common characteristic of topically applied macrocyclic lactones, for example, selamectin applied topically to cats achieves a maximum concentration 15 h after treatment and has a half-life of approximately eight days, which is substantially longer than that following intravenous administration (Sarasola et al., 2002). The terminal half-life of eprinomectin varies among species. The eprinomectin half-life in cats (topical) is approximately 2.7 times longer than the half-life of topically applied eprinomectin in rabbits (Wen et al., 2010a), approximately 1.4 times longer than that in cattle unrestricted from grooming (Merial, unpublished data), variable in cattle restricted from grooming (Wen et al., 2010b; Rehbein et al., 2012) and similar or slightly shorter than that in sheep and donkeys (Hodoscek et al., 2008; Gokbulut et al., 2012). These variations may be due to differences in the pharmacokinetic analysis model, formulations, dermal characteristics, species related differences, and/or additional absorption via grooming for some species. On the other hand, the half-life of eprinomectin is much shorter than that reported for selamectin (Sarasola et al., 2002), and may be due in part to eprinomectin’s molecular structure and resulting effect on plasma and tissue kinetics, for example, eprinomectin has a low milk to plasma ratio (Shoop et al., 1996b).

While one of the least lipophilic ( $\log P = 4$ ) macrocyclic lactones (Kiki-Mvouaka et al., 2010), eprinomectin has a volume of distribution of 2390 mL/kg, which suggests it is well distributed throughout the body. In fact, this value is rather large compared to the blood volume of cats which

is ~75 mL/kg (Chittrakarn et al., 2009). This was substantiated by the results of multiple efficacy studies targeting intestinal, urinary bladder, and cardiopulmonary worms completed during the registration process.

As reported for rats and cattle, eprinomectin is excreted mainly unchanged in the feces, with the major metabolite being deacetylated eprinomectin, a result of phase I metabolism (nonsynthetic metabolism) (EMA, 1996; Zeng et al., 1996). This pattern of minimal biotransformation and formation of deacetylated eprinomectin was also observed in *in vitro* studies using sheep, cattle, and goat liver microsomes (Merial, unpublished data). A similar pattern was demonstrated in our *in vitro* metabolism studies using cat liver microsomes which indicated that eprinomectin was not subject to phase II metabolism, for example, glucuronide conjugates were not synthesized. This is in line with the fact that cats are deficient in the ability to glucuronidate many drugs to facilitate elimination (Court and Greenblatt, 1997a; Tanaka et al., 2006). Glucuronidation reactions are catalyzed by a group of more than 26 isozymes (Court and Greenblatt, 1997b) and it appears that cats have a less diverse pattern of UGT1A isoforms. It is postulated that this could be due to the fact that they are obligatory carnivores, that is, it is related to their diet and other metabolic processes (Court and Greenblatt, 2000).

In a study to compare the *in vitro* metabolic activity of the various P450 enzymes in cats, dogs, horses, and humans, the least activity for cats was observed for the 2C8/9 subfamily (Chauret et al., 1997). This was also confirmed in a study by Shah et al. (2007). Phenacetin O-deethylase activity (CYP1A) was greatest in cats (Chauret et al., 1997). Work by Tanaka et al. (2006) to characterize the P450 cytochromes responsible for phase I metabolism in cats indicated that the CYP1A isoform is active and involved in the oxidation of many drugs. Furthermore, Shah et al. (2007) reported this as a gender independent pathway. In addition, it has been reported that ivermectin, an avermectin closely related to eprinomectin, is primarily metabolized by the CYP3A4 isozyme by human liver microsomes (Merck & Co., 2010) which correlates with the degree of eprinomectin metabolism noted for different lots of the cat liver microsomes with different CYP3A4 activity. While Shah et al. (2007) reported that the *in vitro* CYP3A4 activity was fivefold higher in male cats compared to female cats, in our study *in vitro* metabolic gender differences were inconclusive and were attributed to the activity of CYP3A4. In our studies, one lot of male liver microsomes had CYP3A4 activity (262 pmol/min/mg) approximately 3 times greater than that of the other lot of male liver microsomes and the two lots of female liver microsomes.

## 5. Conclusion

Eprinomectin administered topically to cats is slowly and moderately absorbed. Following topical administration, the half-life is approximately 5 times longer than after intravenous administration and indicates absorption dependent or 'flip-flop' kinetics. Clearance was dose independent, and drug exposure was proportional to dose over a tenfold dose range. In cats, eprinomectin is highly

protein bound, widely distributed, and undergoes minimal biotransformation.

## Conflict of interest

The work reported herein was funded by Merial Limited, GA, USA. All authors are current employees of Merial, designed and assisted with the conduct of the studies, collected the data, and drafted the manuscript.

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