**PHARMACOKINETIC REPORT**

**Single and multiple dose pharmacokinetics of a novel mirtazapine transdermal ointment in cats**

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**Abstract**
Single and multiple dose pharmacokinetics (PK) of mirtazapine transdermal ointment applied to the inner ear pinna of cats were assessed. Study 1 was a randomized, cross-over single dose study (n = 8). Cats were treated once with 0.5 mg/kg of mirtazapine transdermal ointment applied topically to the inner ear pinna (treatment) or administered orally (control) and then crossed over after washout. Plasma was collected predose and at specified intervals over 96 hr following dosing. Study 2 was a multiple dose study (n = 8). Cats were treated daily for 14 days with 0.5 mg/kg of mirtazapine transdermal ointment applied topically to the inner pinna. Plasma was collected on Day 13 predose and at specified intervals over 96 hr following the final dose. In Study 1, single transdermal administration of mirtazapine resulted in mean $T_{max} = 15.9$ hr, $C_{max} = 21.5$ ng/mL, $AUC_{0-24} = 100$ ng*hr/mL, $AUC_{0-\infty} = 260$ ng*hr/mL and calculated half-life = 26.8 hr. Single oral administration of mirtazapine resulted in mean $T_{max} = 1.1$ hr, $C_{max} = 83.1$ ng/mL, $AUC_{0-24} = 377$ ng*hr/mL, $AUC_{0-\infty} = 434$ ng*hr/mL and calculated half-life = 10.1 hr. Mean relative bioavailability (F) of transdermal to oral dosing was 64.9%. In Study 2, daily application of mirtazapine for 14 days resulted in mean $T_{max} = 2.1$ hr, $C_{max} = 39.6$ ng/mL, $AUC_{0-24} = 400$ ng*hr/mL, $AUC_{0-\infty} = 647$ ng*hr/mL and calculated half-life = 20.7 hr. Single and repeat topical doses of a novel mirtazapine transdermal ointment achieve measurable plasma concentrations in cats.

1 | INTRODUCTION

Weight loss and anorexia can occur in cats due to various causes and are a relatively common occurrence in feline medicine (Agnew & Korman, 2014). While obtaining a diagnosis and treating the underlying condition causing weight loss and anorexia, it may be important to consider symptomatic therapy. In these circumstances, appetite stimulation via pharmacotherapy can play a valuable role to bolster the patient’s nutritional status and improve the ability for them to recover from the underlying illness or injury while maintaining body weight and condition (Agnew & Korman, 2014). Mirtazapine transdermal ointment (Kindred Biosciences, Inc.) is approved by the Food and Drug Administration Center for Veterinary Medicine to manage weight loss in cats and is classified as a weight gain drug. The pharmacokinetics of oral mirtazapine have previously been described in cats, dogs, and horses and are quite variable among veterinary species (Quimby, Gustafson, & Lunn, 2011; Quimby, Gustafson, Samber, & Lunn, 2011; Giorgi & Yun, 2012; Rouini et al., 2013). Oral mirtazapine has been demonstrated to increase appetite in normal cats as well as increase appetite and weight and decrease vomiting in chronic kidney disease cats (Quimby, Gustafson, Samber et al., 2011; Quimby & Lunn, 2013).

Feline patients can be challenging to orally medicate. Transdermal medications are an attractive option in feline patients because they...
can improve compliance (Hill et al., 2011). In one study, administration compliance was only 65% with oral carbimazole versus 100% for transdermal methimazole (Hill et al., 2011). However, not all medications are suitable to transdermal application. The pharmacokinetics of transdermal amlodipine, amitriptyline, buspirone, cyclosporine, dexamethasone, fluoxetine, methimazole, mirtazapine, ondansetron, and phenobarbital have been studied (Hoffmann, Marks, Taboada, Hosgood, & Wolfsheimer, 2003; Mealey et al., 2004; Helms, 2007; Hill et al., 2011; Delamaide Gasper, Barnes Heller, Robertson, & Trepanier, 2015; Benson, Zajic et al., 2017; Eichstadt et al., 2017; Zajic et al., 2017). Of these, transdermal methimazole and mirtazapine have demonstrated the most promise to achieve therapeutic blood concentrations and result in a pharmacodynamic effect (Hoffman, Yoder, & Trepanier, 2002; Hoffmann et al., 2003; Hill et al., 2011; Boretti et al., 2014; Benson, Zajic et al., 2017; Quimby, Summers, Benson, Herndon, & Gustafson, 2017). The objective of these studies was to evaluate the pharmacokinetics (PK) of a novel, proprietary mirtazapine transdermal ointment formulation (Kindred Biosciences, Inc., Burlingame, CA) after single and multiple doses in cats. The mirtazapine transdermal ointment (Kindred Biosciences, Inc.) used in this study is a new animal drug (NADA 141-481) approved by the Food and Drug Administration Center for Veterinary Medicine (CVM). The formulation used in these studies has 20 mg of mirtazapine to 1 g of ointment (i.e., 20 mg/g or approximately 20 mg/ml) and was the final commercial formulation.

2 | MATERIALS AND METHODS

2.1 | Cats

Cats were selected from the Vivocore Inc. (Fergus, Ontario, Canada) large cat colony where cats were housed multiple cats per room. Vivocore Inc. obtained purpose-bred, out-bred, domestic short hair or long hair cats from Liberty Research, Inc. (Waverly, NY) for these studies. Study 1 and Study 2 used different cats from the same purpose-bred facility. Eligible laboratory cats had to be apparently healthy, as determined by physical examination and unremarkable CBC and clinical chemistry performed on Day-7, be manageable and cooperative with study procedures, have two accessible jugular veins, and have not participated in another drug trial for at least 2 weeks. Cats were excluded if they were less than 2 kg in weight, were pregnant or lactating, had fractious behavior, or had any medical or surgical condition requiring treatment or intervention.

During study activities, cats were housed in individual cages in compliance with current recommendations of the Guide for the Care and Use of Laboratory Animals, under the standard operating procedures (SOPs) of the test facility, and according to the approved test facility Institutional Animal Care and Use Committee (IACUC) for protocol identifiers VRI92-15061-FP (KB103PK) and VRI92-15083-FP (KB111PK). Cats were acclimated from Day -7 to Day -1 to cages and feed and management practices that were maintained throughout the study. Cats were fed commercial dry cat chow ad libitum and were dosed with study drug without regard to fed state. Water was provided ad libitum with bowls filled at least twice daily. Elizabethan collars were worn throughout both studies in all cats to minimize ear grooming. Cats were acclimatized to the Elizabethan collar for 7 days prior to the first dosing event.

2.2 | Drug product and administration

The drug product consisted of a novel mirtazapine transdermal ointment formulation intended for transdermal (topical) use only. Each 1 g of ointment contains 20 mg mirtazapine (2% mirtazapine, active; i.e., approximately 2 mg/0.1 ml). The formulation contains the following inactive ingredients: polyethylene glycol (PEG) 400, PEG 3350, diethylene glycol monoethyl ether, PEG-8 caprylic/capric glycerides, oleyl alcohol, butylated hydroxytoluene, dimethicone, and Dry Flo TS. A single dose of ointment was administered orally in the single dose study solely for the purpose of a control dose to assess the relative bioavailability of the transdermal compared to the oral as there is no appropriate IV mirtazapine formulation. Calculated dose volumes were determined by weight and drawn up in a separate 1.0 ml tuberculin syringe for each cat. Cats were dosed at 0.5 mg/kg which was selected based on the dose of oral mirtazapine (0.27-0.64 mg/kg) that was previously reported to achieve a pharmacodynamic effect (Quimby, Gustafson, Samber et al., 2011). Once-daily administration was selected based on a previous study in which daily administration of 1.88 mg mirtazapine to young normal cats resulted in negligible drug accumulation, and thus daily would be the shortest clinically applicable dosing interval (Quimby, Gustafson, Samber et al., 2011). Cats were dosed by application of the calculated volume of ointment onto the inner (anterior) surface of the right pinna followed by rubbing and spreading of the ointment to cover the surface area of the pinna. Ear cleaning between doses was not performed in the multiple dose study. The oral administration consisted of opening the cats’ mouth and inserting the syringe to the back of the pharynx.

2.3 | Study 1

This was a randomized, cross-over single dose pilot PK study. Eight apparently healthy purpose-bred adult cats with unremarkable physical examination, CBC and serum biochemistry were used and were acclimated to study conditions for seven days prior to dosing. All eight cats were domestic shorthairs; two neutered males and six spayed females. Ages ranged from 6.0 to 12.3 years (median 10.2 years) and body weight on Day 0 ranged from 2.5 to 8.7 kg (median 5.1 kg). Cats were randomized to treatment group per a sequence provided by the sponsor and stratified based on sex. On Day 0, four cats were randomly assigned to Group A and treated with 0.5 mg/kg of mirtazapine transdermal ointment administered orally, and four cats were randomly assigned to Group B were treated with 0.5 mg/kg of mirtazapine transdermal ointment applied onto the right ear pinna. Blood samples were collected predose and at 1, 2, 4, 6, 8, 12, 24, 48, 72, and 96 hr postdose. On Day 5, ten half-lives from the initial Day 0 dose, the two groups crossed over to the opposite treatment and the same blood sampling regimen was performed. On Day 5 in Study 1,
prior to the second dose, all cats had mirtazapine levels below quantifiable limits.

2.4 | Study 2

This was a multiple dose PK study. Eight apparently healthy purpose-bred adult cats with unremarkable physical examination, CBC and serum biochemistry were used. All eight cats were domestic short hairs; four neutered males and four spayed females. Ages ranged from 6.3 to 13.0 years (median 7.3 years) and body weight on Day -1 ranged from 3.7-7.3 kg (median 5.3 kg). Cats received once-daily transdermal application of 0.5 mg/kg mirtazapine transdermal ointment on Day 0 through Day 13. Blood samples were collected pre-dose and at 1, 2, 4, 6, 8, 12, 24, 48, 72, and 96 h after the final Day 13 administration of mirtazapine.

2.5 | Blood collection

Colony cats were trained to be accustomed to handling and venipuncture and blood was collected with positive reinforcement throughout the study. Blood sampling via venipuncture was performed in compliance with current recommendations of the Guide for the Care and Use of Laboratory Animals, under the standard operating procedures (SOPs) of the test facility, and according to the approved test facility IACUC for protocol identifiers VR92-15061-FP (KB103PK) and VR92-15083-FP (KB111PK). Venipuncture in these colony cats was considered less stressful than catheter placement due to the training methodologies as well as the potential complications with indwelling intravenous catheters in a laboratory setting which may require additional catheterization or additive venipuncture. For each collection, approximately 1 ml of blood was collected and transferred into a 3 ml evacuated tube containing K(2)EDTA as an anticoagulant. Blood tubes were held on ice and within 2 hr of collection, each blood tube was processed by refrigerated centrifugation (~720 g; 4°C; 10 min). After centrifugation, plasma was aspirated with a disposable pipet, and ~0.25 ml volume was transferred to cryovials. Plasma samples were held in frozen storage at ≤−20°C until shipped (on dry ice to maintain frozen state) to the analytical laboratory.

2.6 | Mirtazapine analysis

An LC-MS/MS method for the determination of mirtazapine concentrations in cat plasma was qualified according to prespecified acceptance criteria and was conducted with guidance based on the United States Food and Drug Administration (FDA) Good Laboratory Practice (GLP) Regulations, 21 Code of Federal Regulations (CFR) Part 58 at an independent laboratory (MPI Research, 54943 North Main Street, Mattawan, Michigan 49071). An injection volume of 5 μl was utilized. For calibration curve standard samples, quality control (QC) samples, and incurred study samples, a 25 μl aliquot of each was added to a v-bottom 96-well extraction plate and mixed with 200 μl of working internal standard ([5, 2H4]-Mirtazapine) solution (50 ng/ml) by vortexing. The sample was then centrifuged for 10 min at ~1000 – 2000 g. A 25 μl aliquot of the final supernatant was combined with 225 μl of acetonitrile/water/formic acid (50/50/0.1, v/v/v), mixed by Tomtec, and an aliquot was injected onto an LC-MS/MS system for analysis. The liquid chromatography system used an ACE 5 C18 column, 2.1 × 50 mm (5 μm particle size) with a gradient flow consisting of water/formic acid (100/0.1, v/v) and methanol/formic acid (100/0.1, v/v) at a flow rate of 0.750 ml/min.

The analyte and internal standard were detected using an AB Sciex API 5000 triple quadrupole LC-MS/MS system equipped with an ESI (TurbolonSpray®) ionization source operated in the positive ion mode. The following MRM transitions of the respective [M+H]+ ions were used to monitor mirtazapine and [2H4]-mirtazapine [transition monitored: m/z 266 → 195 and retention time: 1.1-1.3 min]. The calibration range of the method was 0.500–500 ng/ml for mirtazapine using a 25 μl sample aliquot. Accuracy of calibration curve standards within ±15.0% (±20.0% at LLOQ) of the nominal concentration was achieved. Mean intra- and inter-run accuracy of quality control samples were within ±15.0% (±20.0% at the LLOQ) of the nominal concentration and precision was ±15.0% (±20.0% at the LLOQ). Mirtazapine was also demonstrated to be stable during storage, processing, and analysis.

2.7 | Plasma pharmacokinetic analysis and statistical methods

Pharmacokinetic parameters were calculated using standard noncompartmental methods. The following plasma PK parameters for mirtazapine were computed for each animal when possible: T(max) (time to maximum observed plasma concentration), C(max) (maximum peak observed plasma concentration), half-life (t1/2; calculated half-life), AUC(0–24) (area under the concentration–time curve from time 0–24 hr), AUC(0→t) (area under the concentration–time curve from time 0 to the last quantifiable concentration), AUC(0–∞) (area under the concentration–time curve from time 0 to infinity), and F (relative bioavailability of transdermal compared to oral dosing). AUC parameters were calculated using the linear trapezoidal rule. At least three points were used to calculate lambda z, and as a result, AUC(0–∞) could not be calculated for two cats because there were not a minimum of three points. The formula to calculate F (relative bioavailability; comparing transdermal to oral) is:

\[ F = 100\times \left( \frac{AUC_{0–\infty,\text{transdermal}}}{AUC_{0–\infty,\text{oral}}} \right) \]

WinNonlin Professional 5.3 (Pharsight, Inc, Mountain View, CA) was used to calculate the PK parameters and generate typical descriptive statistics. As the data were not normally distributed, PK parameters were statistically compared between single dose treatments using Wilcoxon Signed Rank test with significance set at p < 0.05. Wilcoxon–Mann–Whitney test (at p = 0.05) was used to test for carry-over, period and sequence effects in Study 1.
### RESULTS

#### 3.1 Study 1

The mean plasma concentration–time profiles of mirtazapine following transdermal and oral single dose administration of mirtazapine transdermal ointment are graphically displayed in Figure 1. The PK parameters are presented in Table 1. All cats had measurable levels of mirtazapine following transdermal dosing, with the range of AUC$_{0-\infty}$ from 204 to 397 ng·h/ml ($n = 6$ cats). PK parameters were significantly different between transdermally applied and orally administered mirtazapine transdermal ointment ($T_{\text{max}}$, $p = 0.02$; $C_{\text{max}}$, $p = 0.04$; calculated half-life, $p = 0.03$) with the exception of AUC$_{0-\infty}$ ($p = 0.09$). Significant carry-over, sequence or period effects were not observed for any of the PK parameters in Study 1. The mean relative bioavailability compared to oral dosing was ~65% following a single dose.

#### 3.2 Study 2

The mean plasma concentration–time profile of mirtazapine following multiple dose transdermal application is graphically displayed in Figure 2. The pharmacokinetic parameters are presented in Table 2. Average concentration over the dosing interval was 16.4 ng/ml and the mean fluctuation in plasma concentrations over the dosing interval was 210%. The mean (±standard deviation (SD)) calculated half-life of transdermal mirtazapine after 14 days of daily administration was 20.7 (4.0) h. Some cats experienced mild redness of the skin of the pinna after multiple day dosing.

### DISCUSSION

Based upon the results of these two PK studies, the transdermal application of mirtazapine transdermal ointment to the inner surface of the ear pinna achieves systemic absorption in cats following single or multiple doses, with mean relative bioavailability compared to oral dosing of ~65% following a single dose. Following transdermal application of single doses, mirtazapine absorption was slower than oral administration and exhibited large interindividual variation. The plasma concentration–time curve for transdermal application demonstrates a flatter and more prolonged drug exposure (i.e., lower $C_{\text{max}}$ and longer calculated half-life), particularly after single dosing, compared to oral dosing (Figure 1). For single transdermal dosing, more variable peak ($C_{\text{max}}$) exposure was observed compared to single oral dosing (based on %CV values), but the variability in total (AUC) plasma exposure was more modest and generally comparable to that seen with oral administration. Upon repeat daily transdermal application, variability in peak and total plasma exposure measures was moderate. From the mean calculated half-lives seen in the two studies with transdermal application, steady-state plasma exposure levels would be expected within 5–6 half-lives, which is approximately 4–7 days after initiation of once-daily transdermal dosing. The longer calculated half-lives seen with transdermal application versus oral administration appear consistent with ‘flip-flop kinetics’ (Yanez, Remsberg, Sayre, Forrest, & Davies, 2011), which occurs when the rate of systemic absorption from the transdermal application site is slower than the rate of elimination from the systemic circulation. The pinna of the cats’ ear has been chosen in many studies as the preferred transdermal application site as it has been shown that drugs are well absorbed through the skin for systemic action (Ciribassi et al., 2003; Hoffmann et al., 2003; Mealey et al., 2004; Sartor, Trepanier, Kroll, Rodan, & Challoner, 2004; Bennett, Papich, Hoenig, Fettman, & Lappin, 2005; Lecuyer, Prini, Dunn, & Doucet, 2006; Helms, 2007; Hill et al., 2015). The inner pinna of the cats’ ear is ideally suited to transdermal medications as it is hairless and highly vascular. In addition, the inner surface of the pinna cannot be directly licked during grooming making oral ingestion of the full

### Table 1

PK parameters of mirtazapine following a single dose of mirtazapine transdermal ointment applied transdermally and administered orally (Study 1)

| Parameter | 0.5 mg/kg Transdermal ($n = 8$) | 0.5 mg/kg Oral ($n = 8$) |
|-----------|-------------------------------|--------------------------|
| $T_{\text{max}}$ (hr) | Mean ± SD [%CV] | Median (range) | Mean ± SD [%CV] | Median (range) |
| $C_{\text{max}}$ (ng/ml) | 21.5 ± 43.5 [202.4] | 5.6 (2.1-129) | 83.1 ± 31.2 [37.5] | 83.2 (43.4-128) |
| AUC$_{0-\text{max}}$ (ng·h/ml) | 100 ± 51.7 [51.5] | 84 (29-180)$^1$ | 377 ± 130 [34.5] | 450 (191-510) |
| AUC$_{0-\infty}$ (ng·h/ml) | 225 ± 69.3 [30.8] | 210 (147-378)$^1$ | 418 ± 150 [35.8] | 478 (191-578) |
| AUC$_{0-n}$ (ng·h/ml) | 260 ± 69.8 [26.8] | 247 (204-397)$^1$ | 434 ± 149 [34.4] | 494 (208-590) |
| % AUC Extrapolated (%) | 11 ± 4.2 [38.3] | 12.0 (4.7 - 12.0)$^1$ | 4.3 ± 2.3 [54.4] | 4.8 (1.5 - 4.8) |
| Half-life (hr) | 26.8 ± 6.0 [22.5] | 27.4 (19.0-34.5)$^1$ | 10.1 ± 4.2 [41.4] | 9.1 (4.7-17.0) |

Notes: AUC$_{0-24}$: area under the concentration–time curve from time 0 to 24 hr; AUC$_{0-\infty}$: area under the concentration–time curve from time 0 to the last quantifiable concentration; AUC$_{0-n}$: area under the concentration–time curve from time 0 to infinity; $C_{\text{max}}$: maximum peak observed plasma concentration; %CV: percent coefficient of variation; half-life $t_{\frac{1}{2}}$: terminal elimination half-life; SD: standard deviation; $T_{\text{max}}$: time to maximum observed plasma concentration.

$^1$ $n = 6$; AUC$_{0-\infty}$ was not calculated for 2 cats because the terminal elimination half-life could not be determined.
dose unlikely. However, for the purposes of the study to ensure no grooming occurred, cats wore Elizabethan collars throughout the study. There were few instances (one instance in Study 1 and seven instances in Study 2) where the Elizabethan collar was removed for a period of time and the possibility for oral consumption occurred. We do not believe that significant oral ingestion of ointment occurred in any cat dosed topically. The oral administration of the ointment in Study 1 was solely for the purpose of comparing transdermal to oral bioavailability. The entire transdermal dose was well tolerated when administered orally. Additionally, in a clinical field study in 230 cats using this formulation applied transdermally to the inner pinna at 2 mg per cat without use of an Elizabethan collar, there were no signs of toxicity noted (Longpre et al., 2017).

The PK of mirtazapine in cats has been previously described for an oral capsule formulation in healthy young cats, healthy geriatric cats, and cats with chronic kidney disease, as well as for transdermal lipoderm gel in healthy young cats (Tables 3 and 4) (Quimby, Gustafson, & Lunn, 2011; Quimby, Gustafson, Samber et al., 2011; Benson et al., 2017). Meaningful comparisons between studies with different formulations and populations of cats from various sites are limited, however, the following discussion relates to general concepts. In Study 1, a single oral dose of mirtazapine transdermal ointment was administered at a mg/kg dose similar to that administered in previous PK studies of the oral 1.88 mg capsule formulation of mirtazapine (Table 3) and plasma AUC$_{0-\infty}$ in Study 1 (434 ± 149 ng*h/ml) was generally comparable with serum AUC$_{0-\infty}$ (407.4 ± 102.1) from the previously published PK study (Quimby, Gustafson, Samber et al., 2011). The mean AUC$_{0-24}$ of cats administered mirtazapine transdermal ointment daily in Study 2 (400 ± 100 ng*h/ml) was generally comparable to mean AUC$_{0-24}$ of cats who had a demonstrable increase in appetite after single dose administration of 1.88 mg.

**FIGURE 1**  a & b Drug concentration curves (±SD) for cats after single dose oral administration (Figure 1a) and the transdermal application of mirtazapine ointment (0.5 mg/kg) (Figure 1b). Note that following transdermal application, mirtazapine absorption was relatively slow (compared to oral administration).

**TABLE 2** PK parameters of mirtazapine following transdermal application of mirtazapine transdermal ointment once daily for 14 days (Study 2)

| Parameter | 0.5 mg/kg Transdermal (n = 8) |
|-----------|-------------------------------|
| $T_{\text{max}}$ (hr) | 2.1 ± 1.3 [61.9] 2.0 (1.0-4.0) |
| $C_{\text{max}}$ (ng/ml) | 39.6 ± 9.7 [24.5] 40.9 (27.2-56.5) |
| $C_{\text{avg}}$ (ng/ml) | 16.4 ± 4.01 [24.4] 15.2 (13.0-25.2) |
| AUC$_{0-24}$ (ng*h/ml) | 400 ± 100 [24.4] 364 (312-605) |
| AUC$_{0-24}$ (ng*h/ml) | 614 ± 213 [34.7] 566 (432-1108) |
| AUC$_{0-\infty}$ (ng*h/ml) | 647 ± 225 [34.8] 590 (453-1167) |
| % AUC Extrapolated (%) | 5.1 ± 1.7 [34.2] 4.9 (2.4 – 8.5) |
| Half-life (hr) | 20.7 ± 4.0 [19.3] 21.0 (15.8-25.5) |

Note. AUC$_{0-24}$: area under the concentration–time curve from time 0 to 24 hr; AUC$_{0-\infty}$: area under the concentration–time curve from time 0 to the last quantifiable concentration; AUC$_{0-\infty}$: area under the concentration–time curve from time 0 to infinity; $C_{\text{max}}$: maximum peak observed plasma concentration; %CV: percent coefficient of variation; half-life ($t_{1/2}$): terminal elimination half-life; SD: standard deviation; $T_{\text{max}}$: time to maximum observed plasma concentration.
mirtazapine (440.2 ± 137.5 ng*h/ml) (Quimby, Gustafson, Samber et al., 2011). For single dose transdermal application of mirtazapine ointment in healthy young cats, serum PK parameters seen in a previously published study were again generally comparable with that reported in Study 1 (Table 4) (Benson et al., 2017). However, the previously reported lipoderm gel formulation appeared to produce a somewhat flatter drug concentration curve and thus a lower mean \( C_{\text{max}} \) compared to the novel ointment formulation, even at a higher mg/kg dose. Additionally, a half-life could not be calculated for the lipoderm product due to the variable absorption and resulting atypical drug concentration curve.

Limitations of the PK data presented here are that they were obtained from two separate groups of apparently healthy cats of varying sex and age, and as urinalyses were not performed, the presence of early renal insufficiency could not be ruled out. Although this limitation exists, the population of cats used is representative of the target population of client-owned cats in veterinary practice likely to be treated with mirtazapine transdermal ointment. In previous studies, completed by Quimby and colleagues, they found that cats with chronic kidney disease, and to a lesser extent geriatric healthy cats, had a delayed clearance of oral mirtazapine (Table 3) (Quimby, Gustafson, & Lunn, 2011). In human studies, liver and moderate renal impairment causes a 30% decrease in clearance and severe renal impairment causes a 50% decrease in clearance (Timmer, Sitsen, & Delbressine, 2000). Further PK studies are needed to confirm whether these observations are still valid with transdermal dosing of mirtazapine in cats with chronic kidney disease or geriatric healthy cats.

Ideally, in this study, there would have been an intravenous arm to calculate absolute bioavailability. However, as the use of intravenous mirtazapine is not clinically relevant and the safety is unknown, relative bioavailability comparing oral and transdermal routes of administration was performed instead. A possible

### TABLE 3
Mirtazapine plasma or serum PK parameters from published studies and Study 1 after single dose oral administration

| PK Parameter | Quimby et al. 2011 (Quimby, Gustafson, & Lunn, 2011) Healthy Young Cats Mirtazapine PO Administration (1.88 mg capsule formulation) [0.43 mg/kg] N = 5 | Quimby et al. 2011 (Quimby, Gustafson, & Lunn, 2011) Healthy Geriatric Cats Mirtazapine PO Administration (1.88 mg capsule formulation) [0.44 mg/kg] N = 6 | Quimby et al. 2011 (Quimby, Gustafson, & Lunn, 2011) Cats with CKD Mirtazapine PO Administration (1.88 mg capsule formulation) [0.51 mg/kg] N = 6 | Study 1 Healthy Cats Mirtazapine PO Administration (2.0% novel ointment formulation) [0.5 mg/kg] N = 8 |
|--------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| \( T_{\text{max}} \) (h) | 1.0 (1.0-4.0) | 1.0 (1.0-4.0) | 1.0 (0.5-1.5) | 1.0 (1.0-2.0) |
| \( C_{\text{max}} \) (ng/ml) | 73.1 (45.5) | 79.6 (21.7) | 110.6 (30.8) | 83.1 (31.2) |
| AUC\(_{0-\infty}\) (ng*hr/ml) | 407.4 (102.1) | 589.8 (185.3) | 866.5 (257.9) | 434 (149) |
| Half-life (hr) | 10.3 (2.3) | 12.1 (1.1) | 15.2 (4.2) | 10.1 (4.2) |

Note: When possible, plasma PK parameter values are reported as mean (SD), except for \( T_{\text{max}} \) which is reported as median (range). CKD: chronic kidney disease; NR: not reported.

### TABLE 4
Mirtazapine plasma or serum PK parameters from a published study and the Study 1 study after single dose transdermal application

| PK Parameter | Benson et al. 2016 (Benson et al., 2017) Healthy Young Cats Mirtazapine Aural Transdermal Application (3.75 mg lipoderm gel formulation) [0.90 mg/kg] N = 4 | Study 1 Study Healthy Cats Mirtazapine Aural Transdermal Application (2.0% novel ointment formulation) [0.5 mg/kg] N = 8 |
|--------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| \( T_{\text{max}} \) (hr) | 11.6 (1.0-24.0) | 9.0 (1-48) |
| \( C_{\text{max}} \) (ng/ml) | 7.9 (3.9) | 21.5 (43.5) |
| AUC\(_{0-\infty}\) (ng*hr/ml) | 166.4 (41.7)* | 260 ± 69.8 |
| \( t_{1/2} \) (hr) | NR | 26.8 (6.0) |

Note: When possible, plasma PK parameter values are reported as mean (SD), except for \( T_{\text{max}} \) which is reported as median (range). NR: not reported. *AUC\(_{0-48\,h}\) was reported in this study.
limitation of the study was that doses of ointment were measured and dispensed using a tuberculin syringe and variability in dose could have occurred; however, the dosing methodology was chosen because of its clinical relevance. Ear cleaning between doses was not performed during the multiple dose study. This may have had an effect on potential drug accumulation as well as resulted in residual drug on the ear. Pharmacodynamic (PD) effects were not reported in this study. As the primary endpoint of the studies was PK, the study was not designed to be powered for PD effects and thus data were not included as meaningful conclusions could not be drawn. However, as previously discussed, mean AUC0–24 was PK, the study was not designed to be powered for PD effects and thus data were not included as meaningful conclusions could not be drawn. However, as previously discussed, mean AUC0–24 was generally comparable to the mean AUC0–24 of oral mirtazapine studies in which PD was performed (Quimby, Gustafson, Samber et al., 2011).

Single and repeat doses of a novel mirtazapine transdermal ointment applied to the inner pinna of the ear achieve lower mean peak (Cmax) and total (AUC) plasma exposure measures than oral dosing, whereas mean total exposure was somewhat more comparable between the two routes of administration. Repeat daily topical dosing provided higher mean peak and total plasma exposure measures, when compared to single topical dosing.

ACKNOWLEDGMENTS

The authors gratefully acknowledge Jennifer Davis, DVM, PhD, DACVIM, DACVCP for pharmacokinetic consultation and Steven Radecki for statistical consultation. The study was supported financially by Kindred Biosciences Inc.

CONFLICTS OF INTEREST

Jessica M. Quimby consulted on the data and manuscript preparation for Kindred Biosciences Inc. Valentine S. Williams is an employee of Kindred Biosciences Inc. William Buhles and Daizie Labelle are former employees of Kindred Biosciences Inc.

AUTHOR CONTRIBUTIONS

William Buhles and Daizie Labelle involved in hypothesis generation and experimental design.

Daizie Labelle organized and conducted the experiments. William Buhles, Jessica Quimby, Daizie Labelle, and Valentine Williams interpreted and analyzed the results and wrote the manuscript.

All named authors meet the International Committee of Medical Journal Editors (ICMJE) criteria for authorship for this manuscript, take responsibility for the integrity of the work as a whole, and have given final approval to the version to be published.

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How to cite this article: Buhles W, Quimby JM, Labelle D, Williams VS. Single and multiple dose pharmacokinetics of a novel mirtazapine transdermal ointment in cats. J Vet Pharmacol Therap. 2018;41:644–651. https://doi.org/10.1111/jvp.12691