JOURNAL OF
Veterinary Pharmacology and Therapeutics

J. vet. Pharmacol. Therap. **35** (Suppl. 2), 65–72. doi: 10.1111/j.1365-2885.2012.01407.x

Population pharmacokinetics of transdermal fentanyl solution following a single dose administered prior to soft tissue and orthopedic surgery in dogs

K. J. FREISE*
D. D. LINTON*
G. C. NEWBOUND*
C. TUDAN† &
T. P. CLARK*

**Nexcyon Pharmaceuticals, Inc., Madison, WI, USA; †BioAccurate Enterprises, Inc. Vancouver, BC, Canada*

Freise, K. J., Linton, D. D., Newbound, G. C., Tudan, C., Clark, T. P. Population pharmacokinetics of transdermal fentanyl solution following a single dose administered prior to soft tissue and orthopedic surgery in dogs. *J. vet. Pharmacol. Therap.* **35** (Suppl. 2), 65–72.

A novel, long-acting transdermal fentanyl solution (TFS) that delivers sustained plasma fentanyl concentrations following a single application for the control of postoperative pain has recently been approved for use in dogs. The pharmacokinetics (PKs) of this formulation have been evaluated in healthy laboratory dogs, but they have not been reported in a clinical population of dogs for which it is indicated. Plasma fentanyl concentrations were determined from 215 dogs following a single, small-volume (~50 μL/kg) dose of TFS administered 2–4 h prior to orthopedic or soft tissue surgery. A population PK model was fit, and a 1-compartment open PK model with first-order absorption and an absorption lag-time best described the data. No tested clinical covariates had a significant effect on the PKs. The final model adequately described the population PKs and gave results consistent with laboratory PK studies in healthy dogs. The PKs were primarily characterized by a rapid initial increase in plasma fentanyl concentrations and a long terminal half-life of 74.0 (95% C.I. [54.7–113]) h governed by flip-flop kinetics for the typical subject. The plasma fentanyl concentrations were sustained over days in the range considered to be analgesic for postoperative pain in dogs.

(Paper received 30 January 2012; accepted for publication 16 April 2012)

Dr Terrence Clark, Nexcyon Pharmaceuticals, Inc., 644 W Washington Ave., Madison, WI, 53703, USA. E-mail: clarktp@nexcyon.com

INTRODUCTION

Most analgesics used in veterinary medicine are administered by oral or parenteral routes resulting in fluctuating peak and trough drug concentrations over each dosing interval. Depending on the therapeutic index for a drug, safety concerns may arise near peak concentrations and end-of-dosing-interval lack of effectiveness may emerge at trough concentrations. The recent approval of a novel, transdermal fentanyl solution, however, makes available a convenient, long-acting opioid for the control of postoperative pain in dogs that minimizes fluctuations in drug concentrations over the course of therapy. The efficacy and safety of transdermal fentanyl solution (TFS) has been previously demonstrated in a blinded, multicenter clinical study conducted with client-owned dogs that presented for various types of orthopedic or soft tissue surgery (Linton *et al.*, 2012). The study demonstrated that a single, small-volume (~50 μL/kg) dose of TFS administered prior to surgery was noninferior to buprenorphine intramuscular injections over 4 days.

The minimal fluctuations in the pharmacokinetic profile exhibited by TFS have been demonstrated in several laboratory dog studies (Freise *et al.*, 2012b,c). Following dosing, the systemic concentrations of drug remain relatively constant because of a rapid fentanyl deposition into the stratum corneum, from where fentanyl is then steadily delivered into plasma absent of peaks and troughs. These laboratory studies demonstrated that when applied to the dorsal scapular area, mean plasma fentanyl concentrations remained above 0.5 ng/mL through 96 h postdose administration (Freise *et al.*, 2012b), a concentration that resides in the analgesic range (Kyles *et al.*, 1998; Robinson *et al.*, 1999; Gilbert *et al.*, 2003; Hofmeister & Egger, 2004; Egger *et al.*, 2007). However, the pharmacokinetics (PKs) of TFS in a clinical population of dogs for which it is indicated that includes concomitant medications and other possible comorbidities have not been previously evaluated. Moreover, the pharmacokinetics of the drug has only been previously studied in laboratory Beagles and mixed-breed hounds, which does not represent the variety of breeds that will be treated in the field. Thus, the objective of the current study was to determine the population PKs of a single presurgical application of TFS in a clinical population of dogs undergoing a variety or orthopedic and soft tissue surgeries.

MATERIALS AND METHODS

Clinical study design

The pharmacokinetic data were from a double-blinded, multicentered, positive controlled, field study conducted to good clinical practices (GCPs) according to veterinary international committee on harmonization guideline 9 (VICH GL9; http://www.fda.gov/RegulatoryInformation/Guidances/ucm122050.htm). The efficacy and safety results from this field study will be published at a future date. The investigational drug was TFS (Recuvyra™ transdermal solution (fentanyl); Nexcyon Pharmaceuticals Ltd, London UK). It is a clear, colorless to light yellow solution that contains 5% w/v (50 mg/mL) fentanyl base, 5% w/v (50 mg/mL) octyl salicylate (octisalate [2-ethylhexyl salicylate]), and isopropanol *qs* (*quantum satis*). The positive control drug was oxymorphone hydrochloride (Opana® (oxymorphone hydrochloride) injection; Endo Pharmaceuticals, Chadds Ford, PA, USA).

Dogs that qualified for inclusion into the study were client-owned animals from 24 clinics across the United States. Dogs were required to be normal and healthy with the exception of the condition requiring surgery, 6 months or older, and weigh ≥2.7 kg at the time of treatment. There was no restriction on breed or sex; both intact and castrated males and intact and ovariohysterectomized females were enrolled. Dogs were eligible to enroll if they presented for cranial or caudal cruciate ligament repair to be surgically stabilized (orthopedic) or undergo one of the following soft tissue surgeries: ovariohysterectomy, lateral ear resection, ear crop, or laporotomy (cystotomy, enterotomy, splenectomy [partial or full], liver lobectomy or biopsy, kidney removal or biopsy, or tumor removal [including retained testes]). Other inclusion criteria included no clinically relevant medical abnormalities detected on hematology, serum chemistry, or physical examination, no history of seizures, and a presurgical score of physical status (PS) 1 or PS 2 according to the American Society of Anesthesiologists system (Muir *et al.*, 1995). Dogs were excluded if they failed to meet the inclusion criteria listed above, exhibited an extremely fractious nature, were pregnant or suspected to be pregnant, were lactating, were males intended for breeding, had received short-acting systemic corticosteroids within 14 days of enrollment or a long-acting corticosteroids within 30 days of enrollment, had received nonsteroidal anti-inflammatory drugs (NSAIDs) within 24 h prior to surgery, had another orthopedic, neurological, or uncontrolled systemic disorder that required medical attention, had orthopedic surgery within the last 6 months, and/or had a known sensitivity to opioids, NSAIDs, or to any of the anesthetic articles to be used in the study.

Dogs randomized to TFS were administered a single dose of 2.6 mg/kg (~50 μL/kg) according to a dosing table (Appendix 1) to the skin of the dorsal scapular area 2–4 h prior to surgery using the syringe and applicator tip provided by the manufacturer. Dogs randomized to oxymorphone were administered a dose of 0.1–0.2 mg/kg SC, 2–4 h prior to intubation with additional doses at extubation and then every 6 h through 90 h postextubation. Day 0 was defined as the day in which investigational or positive control drug was first administered. Anesthetic protocols were similar across all clinics in that dogs were anesthetized using a combination of agents according to the veterinarian's preference. Sedation and pain scoring were conducted prior to treatment, on day 0 (1, 2, 4, 6, 8, 12 h postextubation), twice daily on days 1, 2, and 3, and once on day 4. Prior to each pain assessment, sedation was assessed as none (0), slight (1), moderate (2), profound (3), or unresponsive (4). If dogs had a sedation score of 0 or 1, then a composite pain score was computed for each time, based on a modification (deletion of section B) of the Glasgow Composite Pain Scale (GCPS) (Holton *et al.*, 2001). Dogs with a pain score of ≥8 on the GCPS were considered treatment failures and were administered additional analgesia at the investigator's discretion. In dogs randomized to TFS only, a single venous blood sample for plasma fentanyl analysis was collected according to a randomization schedule from day 0 through day 4. Additional (unscheduled) plasma samples were collected at the discretion of the investigator owing to adverse events or other concerns. Plasma was harvested from the blood samples by centrifugation and stored at ≤ −20 °C until analysis.

Sample analysis

Plasma samples were analyzed for fentanyl concentration using liquid chromatography and tandem mass spectrometry (LC-MS/MS) as previously described (Freise *et al.*, 2012b). In brief, control dog plasma (Bioreclamation Inc., Hicksville, NY, USA) was serially diluted with the fentanyl (Cerilliant®, Round Rock, TX, USA) working solution (25 μg/mL in 50:50 water [Milli-Q; Millipore Corp., Billerica, MA, USA]/methanol [Honeywell Burdick & Jackson®, Morristown, NJ, USA]) to create standard curve samples ranging from 0.1 to 10 ng/mL and quality control (QC) samples at concentrations of 0.1, 0.3, 3.5, 40, and 85 ng/mL. A 100 μL each of sample, standard, QC, or control blank was aliquoted directly into a 96-well block, and 20 μL of the internal standard (IS) working solution (200 ng/mL of fentanyl-d_5 (Cerilliant) in 50:50 water/methanol) was added to all wells except for the control blanks. Samples were then vortexed and 400 μL of 5% acetic acid (Mallinckrodt Baker, Phillipsburg, NJ, USA) in water was added to each well. Samples were vortexed again followed by centrifugation at 4 °C. Solid-phase extraction (SPE) then proceeded using Bond Elut® 96 Certify, 50 mg sample extraction blocks (Varian Corp., Palo Alto, CA, USA). Following SPE, the evaporated samples were reconstituted with 200 μL of 1% formic acid (EMD Biosciences, Darmstadt, Germany) in acetonitrile (Honeywell Burdick & Jackson®).

Reconstituted samples were quantified using an API 3000 triple quadrupole mass spectrometer (Applied BioSystems/MDS SCIEX, Foster City, CA, USA) with peak area integration conducted using Analyst Software v 1.4 (Applied BioSystems/MDS SCIEX) data acquisition system. High-performance liquid chromatography (HPLC) separation was achieved using LC-10AD HPLC pumps (Shimadzu Co., Kyoto, Japan) and a Thermo Betasil Silica-100 column (50 × 3 mm, 5 μm) (Thermo

Fisher Scientific, Waltham, MA, USA) with gradient method separation and flow rate of 0.5 mL/min. Mobile phase A consisted of 1% formic acid in water, and mobile phase B consisted of 1% formic acid in acetonitrile. The injection volume was 1 μL, and mass spectrometer detection was conducted using positive ionization mode and monitoring of the transitions 337.2 m/z → 188.3 m/z for fentanyl and 342.2 m/z → 188.3 m/z for the IS fentanyl-d_5. Standard curves were determined using linear regression with $1/x^2$ weighting using Watson v7.0.0.01 (Thermo Fisher Scientific) and had typical squared correlation coefficient (R^2) values of 0.9987. The intra- and interassay precision (i.e., coefficient of variation) was ≤ 7.3%, and the accuracy (i.e., relative error) ranged from −13.5% to 5.9%. The lower limit of quantification (LLOQ) was 0.100 ng/mL.

Dataset preparation and patient demographics

Two hundred and twenty of 249 dogs randomized to TFS treatment had one or more plasma samples collected postadministration for fentanyl analysis. Three of these dogs each had one plasma samples collected post-TFS dosing that were below the LLOQ and were all excluded from the analysis. These samples <LLOQ were collected at 8.5, 99, and 98 h postdosing. Two additional samples from two different dogs were excluded from the analysis that were >15 ng/mL, but were not associated with profound or greater sedation. These samples were judged to be spurious based on previous studies where 100% dogs exhibited profound or greater sedation above this plasma concentration (Freise *et al.*, 2012; Savides *et al.*, 2012). In total, 224 plasma fentanyl samples from 215 dogs (1–2 samples/dog) were included in the analyzed dataset.

Of the 215 dogs in the dataset, 108 underwent orthopedic surgery and 107 had a soft tissue surgery procedure. A total of 86 males and 129 female dogs were included in the dataset. Eighty-eight dogs were sexually intact presurgery and 127 were neutered. A wide range of body weights and ages were represented from 2.72 to 56.2 kg and 0.5 to 13 years of age (Table 1). The mean day 0 alkaline phosphatase (ALP), alanine aminotransferase (ALT), aspartate aminotransferase (AST), blood urea nitrogen (BUN), and serum creatinine (CREAT) resided within the normal range (Table 1). A wide variety of breeds were also represented (Table 2), with crossbreeds (30.2%) and Labrador Retrievers (14.0%) the most highly represented. No covariates were missing from any of the subjects.

Data analysis

The plasma fentanyl concentration data were analyzed using a nonlinear mixed-effects population PK model. Tested PK structural models were parameterized in terms of rate constants (as opposed to parameterization in terms of clearances). The individual subject PK parameters were assumed to follow a multivariate log-normal distribution in order to constrain these parameters to be positive. Thus, on a log scale, the individual subject parameters were distributed by a multivariate normal distribution with a mean vector log (θ), where θ is the vector of population median PK parameters, and a variance–covariance matrix Ω.

In the first step of model building, four structural PK models were compared each with first-order absorption: a 1-compartment open model without an absorption lag-time, a 1-compartment open model with an absorption lag-time, a 2-compartment open model without an absorption lag-time, and a 2-compartment open model with an absorption lag-time. In all cases, the apparent first-order absorption rate constant (k_a) was constrained to be greater than the apparent first-order elimination rate constant (k_e). A diagonal variance–covariance matrix and a proportional residual error model were initially assumed in this first step. Subsequent to the structural PK model selection, four residual error models were compared: additive, proportional, general (additive and proportional), and exponential. The structural PK and residual error models chosen were those that gave the lowest Bayesian Information Criterion (BIC) value.

Next, the covariate model was constructed by stepwise forward inclusion followed by backward elimination of the selected covariates according to the procedure outline by Karlsson and colleagues (Wahlby *et al.*, 2002). Covariates tested included the breed, sex, body weight, age and sexually intact status as well as CREAT, BUN, ALP, ALT, and AST values collected at the time of inclusion. For a breed to be evaluated in the covariate model building, at least 20 dogs of that breed had to be enrolled, which was considered the minimum number sufficient to accurately determine the subpopulation PK characteristics. Thus, the only breed

Table 1. Summary statistics of the continuous covariates at the time of inclusion into the study (n = 215 dogs)

	Mean	Standard deviation	Minimum	Median	Maximum	Normal low	Normal high
Body weight (kg)	24.3	13.9	2.72	24.0	56.2	NA	NA
Age (years)	3.95	2.95	0.5	3	13	NA	NA
ALP (U/L)	82.9	87.4	0.5	59	747	22	256
ALT (U/L)	59.7	56.1	17	45	474	15	81
AST (U/L)	37.2	11.8	17	35	100	20	68
CREAT (mg/dL)	0.790	0.253	0.1	0.8	2.2	0.1	2.3
BUN (mg/dL)	16.9	5.95	5	17	54	4	31

ALP = alkaline phosphatase; ALT = alanine transaminase; AST = aspartate aminotransferase; CREAT = creatinine; BUN = blood urea nitrogen; NA = not applicable.

Table 2. Breeds of dogs included in the study analysis (n = 215 dogs)

Breed	n	%
Crossbreed	65	30.2
Labrador Retriever	30	14.0
Dachshund	9	4.2
American Pit Bull Terrier	8	3.7
Golden Retriever	8	3.7
Chihuahua	7	3.3
Australian shepherd	6	2.8
Cocker Spaniel	6	2.8
Australian Cattle Dog	5	2.3
Boxer	5	2.3
English pointer	5	2.3
Rottweiler	5	2.3
Bassett hound	4	1.9
German Shepherd Dog	4	1.9
Pomeranian	4	1.9
American bulldog	3	1.4
Doberman Pinscher	3	1.4
Poodle	3	1.4
Treeing Walker Coonhound	3	1.4
Airedale Terrier	2	0.9
Bichon Frise	2	0.9
Shih Tzu	2	0.9
Anatolian shepherd	1	0.5
Beagle	1	0.5
Border Collie	1	0.5
Bouvier des Flandres	1	0.5
Brittany spaniel	1	0.5
Cairn Terrier	1	0.5
Cavalier King Charles Spaniel	1	0.5
Dogue de Bordeaux	1	0.5
English Setter	1	0.5
English Springer Spaniel	1	0.5
German shorthaired pointer	1	0.5
Great Pyrenees	1	0.5
Japanese Chin	1	0.5
Lhasa Apso	1	0.5
Maltese	1	0.5
Mastiff	1	0.5
Newfoundland	1	0.5
Pug	1	0.5
Schipperke	1	0.5
Scottish Terrier	1	0.5
Shetland Sheepdog	1	0.5
Silky Terrier	1	0.5
Standard Poodle	1	0.5
Weimaraner	1	0.5
Welsh Corgi, Pembroke	1	0.5
West Highland White Terrier	1	0.5

evaluated for covariate model building was purebred Labrador Retrievers (n = 30), as no other (pure) breeds had at least 20 or more dogs represented (Table 2). Covariates limited to those with a plausible mechanism of action to PK parameters were tested to control the experimentwise type-I error rate. Fentanyl is eliminated by hepatic biotransformation followed by renal excretion (Ohtsuka *et al.*, 2001). Owing to the 'flip-flop' kinetics of TFS (Freise *et al.*, 2012b,c), the only parameter related to the elimination of fentanyl in the tested models is k_a (i.e., when $k_a > k_e$). Thus, CREAT, BUN, ALP, ALT, and AST covariates were only tested on k_a. All other covariates were tested on all parameters of the selected model because they may affect absorption, distribution, or elimination.

For the stepwise forward inclusion of covariates, plots of the empirical Bayes estimates of the random effects on the log scale (η_i) vs. each covariate were created and a correlation coefficient was calculated. At each step in the covariate model forward inclusion building process, the covariates with the highest absolute value of the correlation coefficient for each parameter were first selected for model inclusion, followed by the next highest and so on. Because covariate models are nested, the likelihood ratio (LR) test was used for covariate model selection. An $\alpha = 0.05$ and $\alpha = 0.01$ significance level of a type-I error rate were used during stepwise forward inclusion and backward elimination procedures, respectively (Wahlby *et al.*, 2002). Following forward inclusion of a covariate, the parameter estimation was conducted again and the plots of the conditional expectation of the random effects vs. each covariate were recreated and new correlation coefficient was calculated before continuing with the stepwise forward inclusion covariate model building. All continuous covariates were centered at the covariate mean value. Finally, following completion of the residual error model building, a full variance–covariance matrix was compared to the initially assumed diagonal variance–covariance matrix. Again, the variance–covariance structure was chosen using the BIC.

Model convergence was assessed graphically based on the plots of the parameter iteration history. The model fit to the data was visually evaluated from the following goodness-of-fit (GOF) plots: visual predictive check (90% prediction intervals), observed vs. predicted concentrations, individual fit, and normalized prediction distribution errors (NPDE) plots. The NPDE were used instead of weighted residuals because the NPDE follow a standard normal distribution without any approximations (Brendel *et al.*, 2006). The shrinkage of the individual subject parameter estimates used in the GOF plots for individual subject predictions was assessed by

$$\text{Shrinkage} = 1 - \frac{\text{Var}(\hat{\eta})}{\hat{\omega}^2}$$

where Var ($\hat{\eta}$) is the variance of the empirical Bayes estimates of the random effects (η_i) and $\hat{\omega}^2$ is the estimated population variance parameter.

All data analysis was conducted using Monolix Version 2.3C (http://software.monolix.org). Monolix estimates the parameters using a stochastic approximation-expectation maximization (SAEM) algorithm. The SAEM method was chosen over other frequentist estimation procedures (i.e., procedures that linearize the PK models such as NONMEM® [ICON Development Solutions, Ellicott City, MD, USA] or the *nlme* routine of S-PLUS® [TIBCO Software Inc., Palo Alto, CA, USA]) because of its properties such as maximizing the exact likelihood (Girard & Mentre, 2005), model flexibility, and guaranteed convergence to at least a local minimum (Deylon *et al.*, 1999). Importance

sampling was used for BIC and log-likelihood estimations. The default algorithm settings were used for all parameter and likelihood estimations.

RESULTS

The model building indicated that a 1-compartment open PK model with first-order absorption and an absorption lag-time (t_{lag}) was preferred over the other tested PK models based on BIC. The PK model was parameterized in terms of apparent first-order absorption rate constant (k_a), bioavailability normalized volume of distribution (V/F), and apparent first-order elimination rate constant (k_e). No tested clinical covariates had a significant effect on the PKs of TFS and were not included in the final model using the described stepwise forward inclusion and backward elimination covariate model building strategy (Wahlby *et al.*, 2002). Additionally, based on BIC, the proportional residual error model was preferred over the other tested residual error models and the diagonal variance–covariance matrix was preferred to a full variance–covariance matrix. Figure 1 displays the visual predictive check (VPC) of the model to the fitted data, with the 90% prediction interval shaded and the predicted population median estimates at a dashed line. As can be observed from Fig. 1, the fitted model describes the population data well. Within hours of dose administration, plasma fentanyl concentrations rapidly increased, followed by a prolonged decline over days.

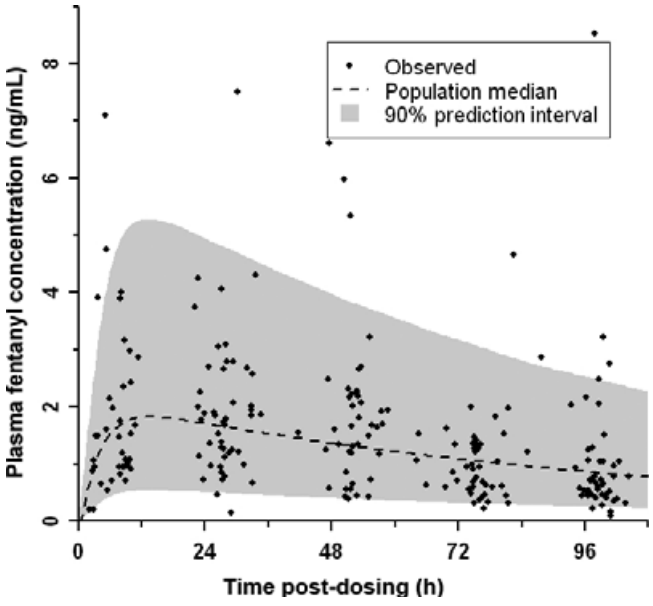

Fig. 1. Visual predictive check of the model fit to the observed data.

The model parameter estimates are summarized in Table 3. The population median V/F and k_e parameters were well estimated with relative standard errors of less than 20%, while the t_{lag} and k_a parameters were not as well estimated with relative standard errors >50%. Again, based on a low relative standard error, the population parameter variance (ω^2) for V/F was well estimated. The other population parameter variance estimates had large (>1000%) relative standard errors. The majority of the subject-to-subject variability occurred in the V/F parameter, with a coefficient of variation (CV) in the population of 64.3%. The subject-to-subject variability for t_{lag}, k_a, and k_e was smaller with CVs of <25%. The residual error scalar estimate of 0.301 corresponds to a residual error CV of 30.1% in plasma fentanyl concentrations.

The spread of the observed vs. predicted concentrations decreases with the individual predicted concentrations compared to the population predictions (Fig. 2); however, bias appears evident as the individual subject predicted concentrations are consistently lower than the observed concentrations in the higher concentration range (right panel). The observed bias is likely an artifact because of the high shrinkage values of the individual subject parameter estimates (Table 4) with three of the four parameters having shrinkage values near 1.0. To confirm that the apparent bias was an artifact of plot creation, data with a study design identical to the observed dataset (i.e., number of subjects, samples, and sampling times) were simulated from the parameter estimates in Table 3. The final model was then fitted back to the simulated dataset and the plot created again. A similar apparent bias existed in the observed vs. predicted plot from the fitted simulated data, indicating that the bias in Fig. 2 is an artifact of plotting because of shrinkage affecting the empirical Bayes estimates used for the model diagnostics and not because of model misspecification (Jonsson *et al.*, 2007; Savic & Karlsson, 2009). No bias of the predicted concentrations vs. time is evident based on the NPDE and nearly all NPDEs fall within ±2 standard deviations, as expected (Fig. 3, left panel). Additionally, the overall distribution of the NPDE

Table 3. Population PK model parameter estimates of median and variance (n = 215 dogs)

	Median		Variance (ω^2)		
Parameter	Estimate	Standard error	Estimate	Standard error	CV
t_{lag} (h)	0.552	1.63	0.0517	12.3	23.0%
k_a (1/h)	0.267	0.167	0.0581	0.745	24.5%
V/F (10^3 L/kg)	1.26	0.130	0.346	0.0694	64.3%
k_{el} (1/h)	0.00937	0.00165	0.00204	0.140	4.52%
b*	0.301	0.0765	NA	NA	NA

CV = coefficient of variation in the population; NA = not applicable.
*Scalar of the proportional residual error model.

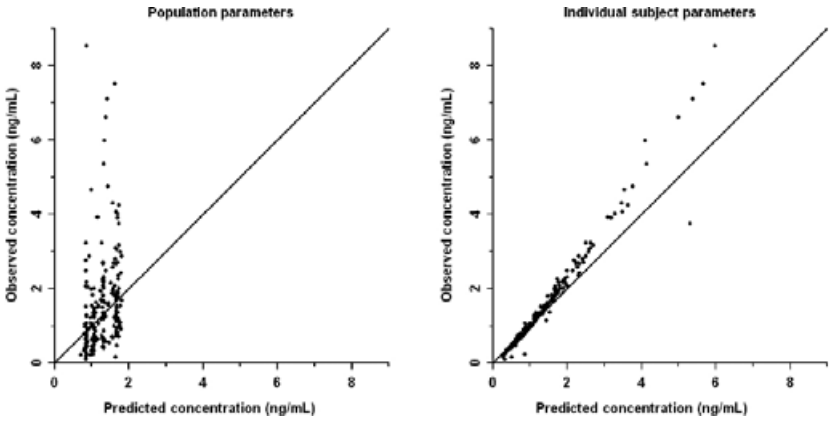

Fig. 2. Observed vs. predicted plasma fentanyl concentrations using the estimated population (left panel) and the individual subject (right panel) parameters. The solid line is the line of identity.

Table 4. Shrinkage of the individual subject PK parameter estimates

Parameter	Shrinkage
t_{lag}	0.987
k_a	0.983
V/F	0.253
k_e	0.983

agrees well with the modeled normal residual distribution, as can be observed from the NPDE histogram plot with the overlaid standard normal distribution (Fig. 3, right panel).

Using the final model population median parameter estimates (Table 3), pharmacokinetic parameters were estimated in a 'typical subject' and are summarized in Table 5. The estimated average concentration from days 0 to 4 days (96 h) for a typical clinical patient was 1.32 ng/mL. The maximum concentration (C_{max}) and time of C_{max} occurrence (t_{max}) were 1.83 ng/mL and 13.6 h, respectively, and the terminal half-life ($t_{½}$) was 74.0 (95% C.I. [54.7–113]) h.

DISCUSSION

The population pharmacokinetics of a single, pre-emptive dose of TFS was determined in the intended clinical population of dogs. A 1-compartment open PK model with first-order absorption and an absorption lag-time best fits the data. The selected structural PK model in this sparsely sampled clinical population was consistent with the monophasic decline observed in the plasma fentanyl concentrations of laboratory dogs that were frequently sampled following TFS administration (Freise *et al.*, 2012b). This PK model described the observed data well based on the VPC, observed vs. predicted concentrations, and NPDE plots, except at the highest fentanyl concentrations. The apparent under prediction at higher fentanyl concentrations appeared to be primarily an artifact of the plot creation, likely due to high shrinkage values of the computed individual subject parameter empirical Bayes estimates affecting the model diagnostics (Savic & Karlsson, 2009). No tested clinical covariates had a significant effect on the PKs.

The PK model population median parameters were well estimated for V/F and k_e but poorly estimated for the k_a and t_{lag} parameters. This is likely due to the limited number of samples collected within the first few hours of fentanyl administration. Except for the V/F population variance parameter that had a low relative standard error, the population variance parameters were poorly estimated and the individual subject parameter estimates suffered from a large amount of shrinkage. Given the reasonable model fit to the data as judged by the VPC plot, the poor variance parameter estimates and high shrinkage were likely due to the limited number of samples per subject in this study.

Using the final model population median parameter estimates, a $t_{½}$ of 74.0 h was calculated for a typical subject (Table 5). This long $t_{½}$ is consistent with the previously recognized 'flip-flop' pharmacokinetics of the TFS established in laboratory dogs (Freise *et al.*, 2012b,c). Intravenously administered fentanyl citrate has an elimination half-life of approximately 0.76–6.0 h (Murphy *et al.*, 1983; Kyles *et al.*, 1996; Sano *et al.*, 2006; Freise *et al.*, 2012b). With 'flip-flop' kinetics and k_a constrained to be k_e, k_a is related to the elimination of fentanyl and k_e is related to the absorption of fentanyl in the 1-compartment open model with first-order absorption. In the present study, the population median estimate of the elimination rate (i.e., k_a) half-life was 2.60 h, which is within the range of estimates of elimination half-life of systemically administered fentanyl.

Except for t_{max}, the PK parameter estimates in a typical subject (Table 5) were similar to that obtained from a laboratory study in beagles using noncompartmental PK analysis methods (Freise *et al.*, 2012b). In that study, 10 males and 10 females were administered a single dose of TFS and each dog was sampled predosing through 504 h postdosing. The mean estimates in that study following application to the dorsal, interscapular region of area under the curve from time 0 to infinity ($AUC_{0-\infty}$), C_{max}, t_{max}, and $t_{½}$ were 198 ng·h/mL, 2.02 ng/mL, 24.8 h, and 117 h, respectively. The different t_{max} values may be due to the

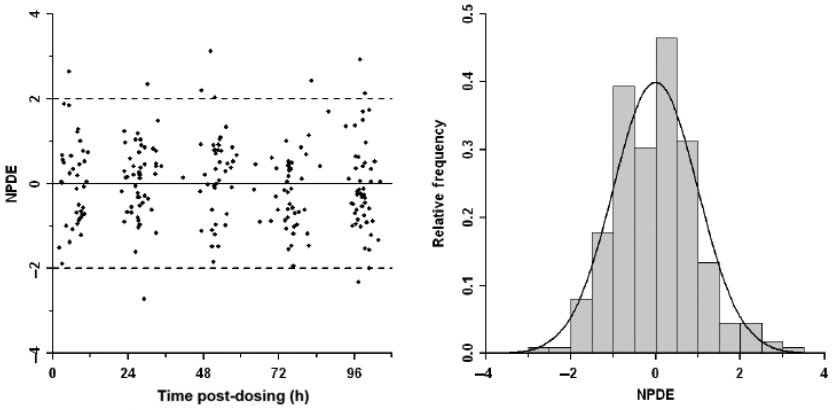

Fig. 3. Normalized prediction distribution errors vs. time (left panel) and histogram (right panel). The dashed lines in the left panel represent ±2 standard deviations. The solid curved line in right panel represents the standard normal distribution.

Table 5. Typical pharmacokinetic parameters in dogs

$AUC_{0-\infty}$	Mean Concentration from 0 to 4 days	Time to reach 0.6 ng/mL*	C_{max}	t_{max}	Terminal half-life
220 ng·h/mL	1.32 ng/mL	1.85 h	1.83 ng/mL	13.6 h	74.0 h

*Minimum analgesic concentration based on Hofmeister & Egger (2004).

sampling schedule used in the laboratory study, as t_{max} estimated by noncompartmental analysis methods is often dependent on the sampling schedule. The $t_{½}$ estimate may be moderately shorter in the clinical studies compared to the laboratory study because of a less extensive and contracted sampling schedule (96 vs. 504 h postdosing, respectively). However, the 95% confidence interval of 54.7–113 h of the population median $t_{½}$ in the clinical studies nearly contains the estimate from the laboratory study. A review and analysis of fentanyl in dogs suggests that a mean plasma fentanyl concentration of 0.6 ng/mL is likely effective at providing analgesia (Hofmeister & Egger, 2004). In the present study, the time to reach 0.6 ng/mL in a typical dog was 1.85 h, and the mean concentration over 4 days was 1.32 ng/mL (Table 5). Thus, the population PK results in this study and the demonstrated efficacy of TFS in a clinical study of postoperative analgesia (Linton *et al.*, 2012) are consistent with the review indicating that plasma fentanyl concentration ≥0.6 ng/mL is analgesic.

CONCLUSION

In summary, a 1-compartment open PK model with first-order absorption and an absorption lag-time best described the PKs of a single application of TFS at a dose of 2.6 mg/kg of fentanyl in the target clinical population. No tested clinical covariates had a significant effect on the PKs of transdermal fentanyl solution. Although several of the population PK parameters were poorly estimated, the population median PK parameters were relatively well estimated and the final model results described the data well. Furthermore, the population PK median parameter estimates gave results consistent with laboratory PK studies in dogs where more frequent and extensive sampling was conducted. Both the observed and predicted plasma fentanyl concentrations were sustained over days in the range considered to be analgesic for postoperative pain in dogs.

ACKNOWLEDGEMENTS

The study was funded by Nexcyon Pharmaceuticals, Inc.

CONFLICTS OF INTEREST

The authors were employees or paid contributors to Nexcyon Pharmaceuticals, Inc.

REFERENCES

Brendel, K., Comets, E., Laffont, C., Laveille, C. & Mentre, F. (2006) Metrics for external model evaluation with an application to the population pharmacokinetics of gliclazide. *Pharmaceutical Research*, **23,** 2036–2049.

Deylon, B., Lavielle, M. & Moulines, E. (1999) Convergence of a stochastic approximation version of the EM algorithm. *The Annals of Statistics*, **27,** 94–128.

Egger, C.M., Glerum, L., Michelle Haag, K. & Rohrbach, B.W. (2007) Efficacy and cost-effectiveness of transdermal fentanyl patches for the relief of post-operative pain in dogs after anterior cruciate ligament and pelvic limb repair. *Veterinary Anaesthesia and Analgesia*, **34,** 200–208.

Freise, K.J., Newbound, G.C., Linton, D.D., Tudan, C. & Clark, T.P. (2012a) Naloxone reversal of an overdose of a novel, long-acting

transdermal fentanyl solution in laboratory Beagles. *Journal of Veterinary Pharmacology and Therapeutics*, **35**(Suppl. 2), 45–51.

Freise, K.J., Newbound, G.C., Tudan, C. & T.P., C. (2012b) Pharmacokinetics and the effect of application site on a novel, long-acting transdermal fentanyl solution in healthy laboratory Beagles. *Journal of Veterinary Pharmacology and Therapeutics*, **35**(Suppl. 2), 27–33.

Freise, K.J., Savides, M.C., Riggs, K.L., Owens, J.G., Newbound, G.C. & Clark, T.P. (2012c) Pharmacokinetics and dose selection of a novel, long-acting transdermal fentanyl solution in healthy laboratory Beagles. *Journal of Veterinary Pharmacology and Therapeutics*, **35**(Suppl. 2), 21–26.

Gilbert, D.B., Motzel, S.L. & Das, S.R. (2003) Postoperative pain management using fentanyl patches in dogs. *Contemporary Topics in Laboratory Animal Science*, **42,** 21–26.

Girard, P. & Mentre, F. (2005) *A Comparison of Estimation Methods in Nonlinear Mixed Effects Models Using a Blind Analysis*. Population Approach Group in Europe (PAGE) Conference, Pamplona.

Hofmeister, E.H. & Egger, C.M. (2004) Transdermal fentanyl patches in small animals. *Journal of the American Animal Hospital Association*, **40,** 468–478.

Holton, L., Reid, J., Scott, E.M., Pawson, P. & Nolan, A. (2001) Development of a behaviour-based scale to measure acute pain in dogs. *Veterinary Record*, **148,** 525–531.

Jonsson, E.N., Karlsson, M.O. & Milligan, P.A. (2007) *Graphical Displays for Modeling Population Data*. In *Pharmacometrics the Science of Quantitative Pharmacology*. Eds Ette, E.I. & Williams, P.J., pp. 183–222. P.J. John Wiley & Sons Inc., NJ.

Kyles, A.E., Papich, M. & Hardie, E.M. (1996) Disposition of transdermally administered fentanyl in dogs. *American Journal of Veterinary Research*, **57,** 715–719.

Kyles, A.E., Hardie, E.M., Hansen, B.D. & Papich, M.G. (1998) Comparison of transdermal fentanyl and intramuscular oxymorphone on postoperative behaviour after ovariohysterectomy in dogs. *Research in Veterinary Science*, **65,** 245–251.

Linton, D.D., Wilson, M.G., Newbound, G.C., Freise, K.J. & Clark, T.P. (2012) The effectiveness of a long-acting transdermal fentanyl solution compared to buprenorphine for the control of postoperative pain in dogs in a a randomized, multicentered clinical study. *Journal of Veterinary Pharmacology and Therapeutics*, **35**(Suppl. 2), 53–64.

Muir, W.W., Hubbell, J.A.E., Skarda, R.T. & Bednarski, R.M. (1995) *Handbook of Veterinary Anesthesia*. Mosby, St. Louis, MO.

Murphy, M.R., Hug, C.C. Jr & McClain, D.A. (1983) Dose-independent pharmacokinetics of fentanyl. *Anesthesiology*, **59,** 537–540.

Ohtsuka, H., Kobayashi, H., Shigeoka, T., Torii, S., Shimomura, H. & Nemoto, H. (2001) Absorption, metabolism and excretion of 3H fentanyl after intravenous and subcutaneous administration in dogs. *Japan Pharmacology and Therapeutics*, **29,** 877–886.

Robinson, T.M., Kruse-Elliott, K.T., Markel, M.D., Pluhar, G.E., Massa, K. & Bjorling, D.E. (1999) A comparison of transdermal fentanyl versus epidural morphine for analgesia in dogs undergoing major orthopedic surgery. *Journal of the American Animal Hospital Association*, **35,** 95–100.

Sano, T., Nishimura, R., Kanazawa, H., Igarashi, E., Nagata, Y., Mochizuki, M. & Sasaki, N. (2006) Pharmacokinetics of fentanyl after single intravenous injection and constant rate infusion in dogs. *Veterinary Anaesthesia and Analgesia*, **33,** 266–273.

Savic, R.M. & Karlsson, M.O. (2009) Importance of shrinkage in empirical bayes estimates for diagnostics: problems and solutions. *The AAPS Journal*, **11,** 558–569.

Savides, M.C., Pohland, R.C., Wilkie, D.A., Abbott, J.A., Newbound, G.C., Freise, K.J. & Clark, T.P. (2012) The margin of safety of a single application of transdermal fentanyl solution when administered at multiples of the therapeutic dose to laboratory dogs. *Journal of Veterinary Pharmacology and Therapeutics*, **35**(Suppl. 2), 35–43.

Wahlby, U., Jonsson, E.N. & Karlsson, M.O. (2002) Comparison of stepwise covariate model building strategies in population pharmacokinetic-pharmacodynamic analysis. *AAPS PharmScience*, **4,** E27.

APPENDIX 1

Table A1. Volume of TFS applied to the dorsal scapular area

Dosing table

Body weight		
Lbs	Kgs	Dose (mL)
6.0–9.3	3.0–4.2	0.2
9.4–13.4	4.3–6.1	0.3
13.5–17.6	6.2–8.0	0.4
17.7–21.8	8.1–9.9	0.5
21.9–25.9	10.0–11.7	0.6
26.0–30.1	11.8–13.6	0.7
30.2–34.3	13.7–15.5	0.8
34.4–38.4	15.6–17.4	0.9
38.5–42.6	17.5–19.3	1.0
42.7–46.8	19.4–21.2	1.1
46.9–50.9	21.3–23.1	1.2
51.0–55.1	23.2–25.0	1.3
55.2–59.3	25.1–26.9	1.4
59.4–63.4	27.0–28.8	1.5
63.5–67.6	28.9–30.6	1.6
67.7–71.8	30.7–32.5	1.7
71.9–75.9	32.6–34.4	1.8
76.0–80.1	34.5–36.3	1.9
80.2–84.3	36.4–38.2	2.0
84.4–88.4	38.3–40.1	2.1
88.5–92.6	40.2–42.0	2.2
92.7–96.8	42.1–43.9	2.3
96.9–100.9	44.0–45.8	2.4
101.0–105.1	45.9–47.7	2.5
105.2–109.3	47.8–49.6	2.6
109.4–113.4	49.7–51.4	2.7
113.5–117.6	51.5–53.3	2.8
117.7–121.8	53.4–55.2	2.9
121.9–125.0	55.3–57.0	3.0