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ORIGINAL ARTICLE

Margin of safety of extended-duration transdermal buprenorphine solution following multiple-dose administrations to cats

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Abstract

Transdermal buprenorphine solution (TBS) is approved for the control of postoperative pain in cats where a single preoperative dose provides 4 days of analgesia. It is administered as a unit dose of 8 mg to cats weighing 1.2–3 kg and 20 mg to cats weighing to >3–7.5 kg, which is equivalent to a dosage on a bodyweight basis of 2.7–6.7 mg/kg. In this safety study, the 1X dose was defined as 6.7 mg/kg. Thirty-two cats (16 males and 16 females) were randomly allocated to placebo, 1, 2, and 3X TBS administered topically to the dorsal cervical skin every 4 days for 3 doses. Clinical observations, behavioral scores, mydriasis score (yes/no), and physiological variables were assessed or measured prior to each dose administration (0 h) and at 1, 2, 4, 8, 12, 24, 36, 48, and 72 h following each treatment and prior to euthanasia on Day 12 or 13. Blood samples for clinical pathology were collected on Days - 1, 4, 8, and prior to euthanasia. There was little evidence of respiratory, cardiovascular, or gastrointestinal effects. Respiratory rates were above the reference range in all groups and lower by 10 breaths/min in the 3X group during the third dosing interval compared to placebo. There were no differences in heart rates. Constipation was transiently observed in approximately equal numbers in placebo- and TBS-treated cats. Behavioral scores showed sedation or euphoria was transient in the first dosing interval but became more prolonged with each dosing interval. Mydriasis was prolonged in the first dosing interval and diminished by the third dosing interval consistent with accommodation. Mean body temperatures in TBS-treated cats were up to 0.6°C (1.8°F) greater than placebo-treated cats. There were no clinically relevant changes to serum chemistry, hematology, or urinalysis outcomes nor gross or microscopic observations attributable to TBS. These data demonstrate that TBS is safe and well-tolerated when administered to 16-week-old cats at multiples of the approved dose and duration and supports clinical safety in the event of delayed buprenorphine metabolism, medication errors, or alterations in the dosing regimen.

KEYWORDS
buprenorphine, cat, safety, toxicology, transdermal

1 | INTRODUCTION

Opiates have a long history in medicine as effective analgesics that are associated with well-described side effects including euphoria, respiratory depression, constipation, bradycardia, and histamine release, among others, and the pharmacology was well-understood long before receptor theory (Krueger et al., 1941). These observations triggered the search for opiate analogs that retained effective analgesic qualities without producing detrimental side effects. Thebaine was identified as a major component of opium that had little analgesic action but served as a valuable precursor in the synthesis of other opiate derivatives including buprenorphine, a semi-synthetic opioid of the oripavine chemical class (Gutstein & Akil, 2006). Early studies with buprenorphine demonstrated it was a partial μ-opioid receptor agonist and κ-opioid receptor antagonist (Martin et al., 1976) and systematic animal pharmacology studies showed that it was approximately 20- to 40-fold more potent than morphine as measured in rodent antinociceptive assays, had a ceiling effect on respiratory depression, had little effect on gastrointestinal transit, had an LD50 that was many thousand-fold greater than the ED50 in mice, and had a low potential for physical dependence (Cowan et al., 1977; Cowan et al., 1977).

An approved injectable buprenorphine solution indicated for postoperative pain for use in humans arrived in 1977 in the United Kingdom (UK) and in 1982 in the United States (US) along with supporting evidence of its safety and effectiveness (Harcus et al., 1980). Over time, several formulations for use in humans have been approved that include sublingual tablets, transdermal patches, buccal films, and extended-release injectable solution. Since its introduction in human health, a re-examination of its clinical uses and outcomes as compared to basic pharmacology and animal model studies has led to the consensus that buprenorphine behaves as a full μ-opioid agonist for analgesia with no ceiling effect, but that a ceiling effect is observed for respiratory depression, reducing the likelihood of this potentially fatal adverse event (Pergolizzi et al., 2010).

In early safety studies, evaluation of anesthetized cats using open thorax monitoring techniques showed buprenorphine at dosages of 0.10 and 1 mg/kg IV resulted in little behavioral changes and no major hemodynamic perturbations (Cowan, Doxey, et al., 1977). Later, extra-label use of human approved injectable buprenorphine began to show up in veterinary formularies although controlled clinical studies were lacking (Hansen, 1994). In 1995 in the UK, a low concentration buprenorphine solution (0.3 mg/ml) was approved for use in dogs and cats as an IM and IV injection (0.01–0.02 mg/kg), although it has not been approved in the United States to date. The safety and medical benefits of integrating the low concentration buprenorphine formulation by the injectable or oral transmucosal route (OTM) to cats have been established and the evidence reviewed (Bortolami & Love, 2015; Steagall, Monteiro-Steagall & Taylor, 2014). In 2014, a high-concentration injectable solution (1.8 mg/ml) was approved for use in cats in the United States whereby a single subcutaneous dose (0.24 mg/kg) has a duration of action of approximately 24 h (Doodnaught et al., 2017, 2018; Sramek et al., 2015). The safety of this formulation was examined in a laboratory study in cats which were administered up to 5 times the labeled dosage for 9 days, and other than infrequent hyperactivity, difficulty in handling, disorientation, agitation, and dilated pupils, the product was considered well-tolerated (Sramek et al., 2015).

Parenteral injections or OTM administration of the low concentration buprenorphine formulation to cats has a short duration of action, possibly as short as 4 h, particularly in the absence of NSAIDs (Steagall et al., 2009) and per the label, repeated injections are limited to a single injection 1–2 h following the first. A single subcutaneous dose of the high-concentration formulation has an analgesic duration of approximately 24 h (Doodnaught et al., 2017, 2018; Sramek et al., 2015), and the product cannot be dispensed to owners for home injection, limiting the practicality of extending the duration beyond in-hospital use. Repeat administrations of low- or high-concentration solution by parenteral or OTM routes result in peak-and-trough plasma concentrations, potentially allowing breakthrough pain near the end-of-dosing interval, when cats may be most vulnerable during unobserved periods. Moreover, parenteral injections are potentially painful and difficult to administer, contributing to stress and fear in both cats and medical personnel (Riemer et al., 2021). Finally, although extra-label use of OTM buprenorphine syringes dispensed to owners for repeated administrations at home may extend the analgesic duration and eliminate injection pain and stress, it does not alleviate the legal responsibility of dispensing buprenorphine, a Class III controlled substance in the United States (Kukanich & Papich, 2009).

To overcome the limitations of current buprenorphine formulations for use in cats, a novel, non-aqueous transdermal buprenorphine solution (TBS) with a permeation enhancer intended for topical application has been approved by the US Food and Drug Administration (FDA) (Zorbium™, Elanco US Inc., NADA 141-547). It is available in two strengths in unit dose applicator tubes to deliver 8 mg (0.4 ml) and 20 mg (1 ml) of buprenorphine to smaller (1.2–3 kg) and larger (>3–7.5 kg) cats, respectively, from a 20 mg/ml solution (equivalent to a dosage on a bodyweight basis of 2.7–6.7 mg/kg). A single application has an onset-of-action of 1–2 h and provides analgesia for 4 days. Once applied, buprenorphine depots into the skin resulting in prolonged systemic payout with half-lives of 78.3–91.2 h, typical of flip-flop pharmacokinetics (PK; Freise, Reinemeyer, Warren, et al., 2022). The field effectiveness and safety of the selected dosages were confirmed in two randomized, controlled clinical studies in cats undergoing surgery (Clark et al. 2022a); 2022b). These data resulted in FDA approval but as a part of the approval process, a laboratory study in the target species is required where dosages above the labeled dose and duration are examined to account for possible metabolic differences or medical errors that cannot be accounted for in randomized controlled trials (FDA-CVM, 2009). Therefore, the present study examines dosages of TBS beyond its labeled dose and duration in a placebo-controlled laboratory study in cats conducted according to US Good Laboratory Practices (Title 21 Code of Federal Regulations, Part 58).

2 | MATERIALS AND METHODS

2.1 | Test and control articles

Transdermal buprenorphine solution was the test article formulated to contain 20 mg/ml buprenorphine (calculated as the free base), 5% w/v (50 mg/ml) padimate O as a permeation enhancer, and ethanol. To prepare the formulation, buprenorphine HCl (Spectrum Chemical Mfg. Corp.) was dissolved in a small amount of ethanol (Sigma-Aldrich) and padimate O (Sigma-Aldrich) was added and mixed. The formulation was brought to volume with ethanol and aliquoted into 10 ml amber glass vials sealed with a rubber stopper and aluminum crimp-top until use. The negative control article was placebo transdermal solution containing 5% w/v (50 mg/ml) padimate O in ethanol. Bottles were labeled according to the United States Code of Federal Regulations, Title 21, Part 511.1(a) and stored at controlled room temperature, 20–25°C (68–77°F) in a locked drug storage area.

2.2 | Animals

Prior to study initiation, the study protocol underwent peer review and concurrence by subject matter experts at the FDA and all procedures were approved by the local Institutional Animal Care and Use Committee. Day 0 was defined as the day on which cats were administered the first dose of TBS or placebo. The study included 32 cats (16 males and 16 females) that were housed in standard cages and conditioned at the test facility to feed and management practices for a minimum of seven days prior to Day 0. The inclusion criteria specified cats to be normal, healthy, purpose-bred, intact, domestic shorthaired cats, four months (±2 weeks) of age, weighing between ≥1.2 kg and ≤7.5 kg on Day - 1. A cat was included in the study if it met the test animal description criteria above and had no clinically relevant medical abnormalities detected by physical examination on Day - 1. A cat was excluded if it did not meet the inclusion criteria, was fractious, was administered buprenorphine at any time prior to Day 0, or had an abnormal dose administration site (*i.e.*, the dorsal cervical skin) prior to Day 0.

Cats were individually housed in cages with solid stainless-steel walls on 5 sides (back, bottom, top, and 2 sides). Cage fronts served as the door and were composed of a welded grid of steel dowels. Water was provided *ad libitum*, and a complete dry feline diet was provided in appropriate amounts to assure animal health. An alternating approximate 12-hour light/dark cycle was maintained. Ventilation rates in the animal facility averaged approximately 10 complete air changes per hour with the temperature controlled between 18 and 29°C.

2.3 | Study design

Cats that were included into the study were blocked by gender, and within gender, were randomly allocated to placebo, 1X, 2X, or 3X TBS treatment groups (n = 4 cats/sex/group) (Table 1). On Day 0, 4, and 8, treatments were administered according to Day - 1 body weight. Animals were not fasted prior to treatment administration. The concentration of buprenorphine in TBS was 20 mg/ml, therefore, the volumes administered to the 1X, 2X, and 3X groups were 0.33, 0.67, and 1 ml/kg, respectively. Cats allocated to placebo were administered a dose volume equivalent to that calculated for the 3X TBS group. Transdermal buprenorphine solution was administered topically to the dorsal cervical region (base of the skull) using the tip of a needleless syringe. The syringe tip was placed directly onto the skin at the application site, and the entire dose volume was administered at a single location without moving the syringe. The cats were gently restrained for 2 min post-dosing to prevent the cat from shaking or grooming while the solution dried. All study participants were masked to treatment apart from the single treatment administrator who was responsible for randomization records and treatment administrations.

TABLE 1 Treatment groups and doses administered

Treatment Group[a]	Buprenorphine Dosage	Dosing Interval	Number of Animals
Placebo	0 mg/kg	Days 0, 4, 8	8 (4 M/4 F)
1X TBS	6.7 mg/kg	Days 0, 4, 8	8 (4 M/4 F)
2X TBS	13.3 mg/kg	Days 0, 4, 8	8 (4 M/4 F)
3X TBS	20 mg/kg	Days 0, 4, 8	8 (4 M/4 F)

[a]The FDA-approved dose is 8 mg to cats weighing 1.2 to 3 kg and 20 mg to cats weighing to >3 to 7.5 kg (2.7–6.7 mg/kg). A 1X dose was defined as 6.7 mg/kg, *that is*, the upper limit of the dose on a bodyweight basis.

Complete physical examinations, including body weight measurement, were performed on Day - 1, Day 8 prior to dosing, and on Day 12 or 13 prior to euthanasia and necropsy. Physical examinations were repeated 2 h after dosing on Days 0, 4, and 8. Blood samples for hematology and clinical chemistry were collected from the jugular vein on Days - 1, 4, 8, and 12 or 13 (Appendix I). The samples collected on Day 4 and 8 were prior to treatment. To facilitate blood sample collection, all cats were sedated with 40 μg/kg dexmedetomidine hydrochloride (Dexdomitor®) administered by IM injection. On Days - 1, 4, and 8, sedation was reversed after blood sampling using 0.2 mg/kg atipamezole (Antisedan®) IM. Cats were euthanized after blood collection on Day 12 or 13, so reversal was unnecessary. Urine samples for urinalysis were collected on Day - 1 and again on the day of necropsy (end of study; Appendix I). All urine samples were collected by exchanging the regular absorbent cat litter with polypropylene beads. Cats were provided a clean litter pan containing the beads beginning on the afternoon before the scheduled collection date and continuing on subsequent days until an uncontaminated urine sample was obtained. Once a urine sample had been successfully obtained from each cat, its pan was refilled with the regular, absorbent cat litter. Cystocentesis was not necessary at any collection occasion.

The clinical observations and physiological variables of mucous membrane color, capillary refill time, body temperature, respiratory rate, and heart rate were assessed or measured prior to each dose administration (0 h) and at 1, 2, 4, 8, 12, 24, 36, 48, and 72 h following each treatment and prior to euthanasia on Day 12 or 13. Excursions outside the normal ranges at any observation time point were considered adverse events and included bradycardia (≤90 beats per minute), tachycardia (≥200 beats per minute), hypothermia (≤36.6°C [98°F]), or hyperthermia (≥39.4°C [102.9°F]). At each clinical observation time point, mydriasis was assessed (yes/no) and behavior scored according to the following scale:

- 0 – Normal.
- 1 – Sedated—Subdued and quiet; signs include sleeping, ventral tail curling, and purring; less responsive to human interaction.
- 2 – Euphoric—Exaggerated social and playful behavior; signs include meowing, rolling, kneading with forepaws, play-biting, and rubbing its head and body on cage.
- 3 – Mildly Dysphoric—State of uneasiness and discord; signs include absent staring, hyper-responsiveness, swaying, and/or vocalization, and may be accompanied by increased locomotor activity; no overt signs of fear or disorientation, and no signs of aggression; may initially appear sedated, but then startle suddenly (*i.e.*, hyper-responsive).
- 4 – Dysphoric—State of anxiety or agitation; signs include staring at objects that are not present, hyper-responsiveness, sudden movements, and/or vocalization, and may be accompanied by increased locomotor activity; cats are obviously disoriented or fearful, may become aggressive.

General health observations and observations of the skin at the application site were conducted twice daily, separated by at least 8 h, from Day - 7 through study completion (Day 12 or 13). If there was absence of feces for three or more consecutive observations (*i.e.*, 36 h), then the cat was examined for evidence of constipation. If constipation was found, the animal was administered a laxative. Food intake was scored from Day - 6 through study completion. A score was applied based on food remaining from the previous day's offering. Food intake was scored as all eaten, less than half remaining, or more than half remaining. In addition, any abnormal events noted during routine animal husbandry activities post-treatment were documented.

Samples for buprenorphine plasma analysis were not collected to minimize sampling in light of numerous sampling and handling demands. However, PK has been characterized in another study at the 1X dose used in this study (Freise, Reinemeyer, Warren, et al.). The plasma concentration-time data from Freise et al. (2022) from 12 cats administered the 1X dose were used through 96 h, *that is*, one dosing interval in the present study. In the first dosing interval, these data were multiplied by 2 and 3 to simulate the plasma concentration-time data for the 2 and 3X doses, respectively, because PK is proportional through 3X the approved dose (Freise, Reinemeyer, Warren, et al., 2022). For the second and third dosing interval beginning on day 4 and day 8, the 1, 2, and 3X plasma concentration-time data were added to the plasma concentration remaining at 96 h just prior to the second dose and then to the plasma concentration remaining at 192 h just prior to the third dose. The simulated plasma concentration-time data were plotted. The linear trapezoidal rule was used to calculate the area under the plasma concentration-time curve through each 96-h dosing interval ($AUC_{0\text{-}96}$). The $AUC_{0\text{-}96}$ for each dose within each dosing interval was divided by the $AUC_{0\text{-}96}$ for the 1X dose to determine the relative drug exposure within dose and over time.

2.4 | Study conclusion and necropsy

At the time of allocation to treatment, cats were allocated to necropsy day to be completed on Day 12 or 13. Food was removed on the evening of Days 11 or 12 for cats allocated to necropsy on Days 12 or 13, respectively. On Day 12 or 13 according to randomization, cats were sedated with a combination of xylazine (~1 mg/kg) and acepromazine (~0.05 mg/kg) injected intramuscularly in a quiet room away from other animals. After approximately 15–20 min, the proximal foreleg was clipped to access the cephalic vein, and pentobarbital sodium (Beuthanasia) was injected intravenously at >86 mg/kg and cats were necropsied immediately. The external carcass and the contents of the thoracic, abdominal, cranial, and pelvic cavities were examined for gross lesions. The entire gastrointestinal tract was observed for gross lesions. The organs and tissues listed in Appendix II were collected, weighed, and preserved in 10% buffered, neutral formalin. Eyes were initially placed into Modified Davidson's solution and then transferred to 10% BNF the following day. Skin samples included three collected from the administration site and one from untreated skin anterior to the treatment site. All evaluations were conducted by a board-certified veterinary pathologist.

2.5 | Statistical analysis

The statistical evaluation of safety was on the summaries by treatment group of five categories: physiological measurements, clinical observations, body weights, clinical pathology analytes, and organ weights.

Differences among treatment groups for data of a continuous nature were inferred by one of the three analyses of factor effects. These three were fixed effects models. Model 1 was for gender-specific data collected at a single time point and contained an independent term for treatment. The remaining two models contained the baseline value as a covariate and independent factors for treatment, gender, and gender-by-treatment interaction terms and differed in the frequency of the data collection. Model 2 was appropriate for data collected at a single time point. Model 3 was a repeated measures model and additionally included independent factors for time, time-by-treatment, time-by-gender, and time-by-gender-by-treatment interactions. Statistical significance was

evaluated at the two-side alpha level of 0.05. Pairwise comparisons of each treatment group (*i.e.*, 1X, 2X, and 3X) against the placebo group (*i.e.*, 0X) were performed only when:

1. The 3-way interaction (*i.e.*, time-by-gender-by-treatment) was not significant but either of the 2-way interactions (*i.e.*, gender-by-treatment or time-by-treatment) was significant. The pairwise comparisons were performed within each gender or at each time point; or
2. None of the interaction terms was significant but the main effect of treatment was significant.

If the 3-way interaction was significant, no further inferential analysis was performed due to the complexity of its interpretation.

Physiological variables (*i.e.*, body temperature, heart rate, and respiratory rate) measured over time were analyzed by fitting Model 3 to the data, where time was equal to 1, 2, 4, 8, 12, 24, 36, 48, 72, and ~93 (*i.e.*, prior to subsequent dose administrations or necropsy [Day 12/13]) hours after each dosing. Baseline values (*i.e.*, 0 h, just prior to the first dose administration) were used as a covariate and remained in the model, regardless of significance. The covariance structure in the repeated measures analysis was investigated using two structural assumptions, namely, heterogeneous compound symmetry (CSH) and spatial power [SP(POW)]. The between-subject effect was not included in the random statements. The assumption yielding the minimum Akaike's information criterion (AIC) value was selected in the final analysis.

Quantitative clinical pathology results and body weights measured over time were also analyzed by fitting Model 3 to the data, where time was equal to Day 4, Day 8, and Days 12 or 13. Baseline values (*i.e.*, Day - 1) were used as a covariate and remained in the model, regardless of significance. The covariance structure in the repeated measures analysis was investigated using three structural assumptions, namely, compound symmetry (CS), first-order autoregressive [AR(1)], and heterogeneous first-order autoregressive [ARH(1)]. Animal was identified as the between-subject effect in the random statement for models where the first-order autoregressive covariance [AR(1)] structure or heterogeneous autoregressive [ARH(1)] structure was used. The between-subject effect was not included in the random statements for models utilizing the compound symmetry [CS] covariance structure. The assumption yielding the minimum Akaike's information criterion (AIC) value was selected in the final analysis.

Quantitative urinalysis variables were fitted to the Model 2. Baseline values (*i.e.*, Day - 1) were used as a covariate and remained in the model, regardless of significance. Summaries were calculated for organ weights and their weights expressed as a calculated percentage of the terminal (*i.e.*, Day 12 or 13) body weight for each treatment group, by gender and overall. Model 2 was fitted to the percentages. For male cats, the testes and epididymides were weighed separately, as were the uterus and ovaries for female cats. The percent weight of each organ relative to terminal (*i.e.*, Day 12 or 13) body weight was calculated. Model 1 was fitted to the percentages as described above. The statistical analysis was conducted with SAS for Windows 9.1.3 (Service Pack 4, SAS Institute Inc.) using the MIXED procedure.

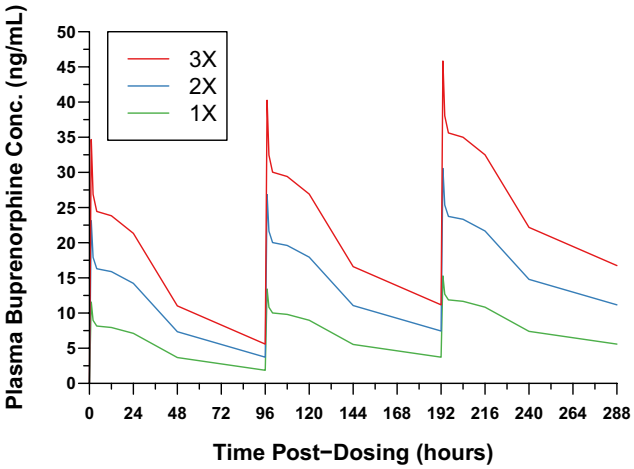

FIGURE 1 Simulated buprenorphine plasma concentration-time data over the study duration. The plasma concentration-time data were simulated from the 1X dose administered to 12 cats sampled through 96 h, *that is*, one dosing interval. From (Freise, Reinemeyer, Warren, et al., 2022)

3 | RESULTS

Thirty-two (32) sexually intact domestic shorthaired cats (16 males and 16 females) ranging in age from 116 to 130 days (~16.6 to 18.6 weeks) and weighing 1.7 to 2.3 kg on Day - 1 were included into the study. No cats were excluded, and all cats completed the study on Day 12 or 13 as scheduled.

The simulated buprenorphine plasma concentration-time data are depicted in Figure 1 and the ratios of $AUC_{0\text{-}96}$ for each dosing interval are summarized in Table 2. There were no instances of sedation, euphoria, dysphoria, or mydriasis prior to the first treatment. Most TBS-treated cats exhibited sedation or euphoria following dosing and were approximately similar in the 1, 2, and 3X groups within each dosing interval (Figure 2). In the first dosing interval, mean scores from the behavior scale were between 1 and 2 (*i.e.*, sedation or euphoria) for 12 h, were less than 1 thereafter, and returned to baseline by 48 h (Figure 2). The duration of sedation and euphoria became more protracted with each dosing interval. In the second dosing interval, mean scores from the behavior scale were between 1 and 2 for 48 h and returned to baseline by approximately 72 h. In the third dosing interval, mean scores from the behavior scale were between 1 and 2 for 72 h and returned to baseline by 96 h.

Few placebo-treated cats displayed mydriasis. A greater proportion of TBS-treated cats displayed mydriasis in the first dosing interval, and for a greater duration, compared to the second and third dosing intervals (Figure 3). In the first dosing interval, all cats in each TBS group had mydriasis within 4 h and returned to baseline by 48 h. In the second dosing interval, the proportion of cats with mydriasis

Buprenorphine Dosage	Day 0–Day4		Day 4–Day8		Day 8–Day12	
	AUC	Ratio[a]	AUC	Ratio[a]	AUC	Ratio[a]
6.7 mg/kg	450	1	629	1.40	807	1.79
13.3 mg/kg	900	2	1257	2.79	1614	3.59
20 mg/kg	1350	3	1886	4.19	2422	5.38

[a]Ratio of the identified dosing interval $AUC_{0\text{-}96}$ divided by the 1X $AUC_{0\text{-}96}$ following the first dose, *that is*, Day 0 to Day 4

TABLE 2 Estimated drug exposure expressed as $AUC_{0\text{-}96}$ within each dosing interval over the study duration. The $AUC_{0\text{-}96}$ results were calculated from 12 cats administered the 1X dose that were sampled through 96 h, *that is*, one dosing interval (Freise, Reinemeyer, Warren, et al., 2022)

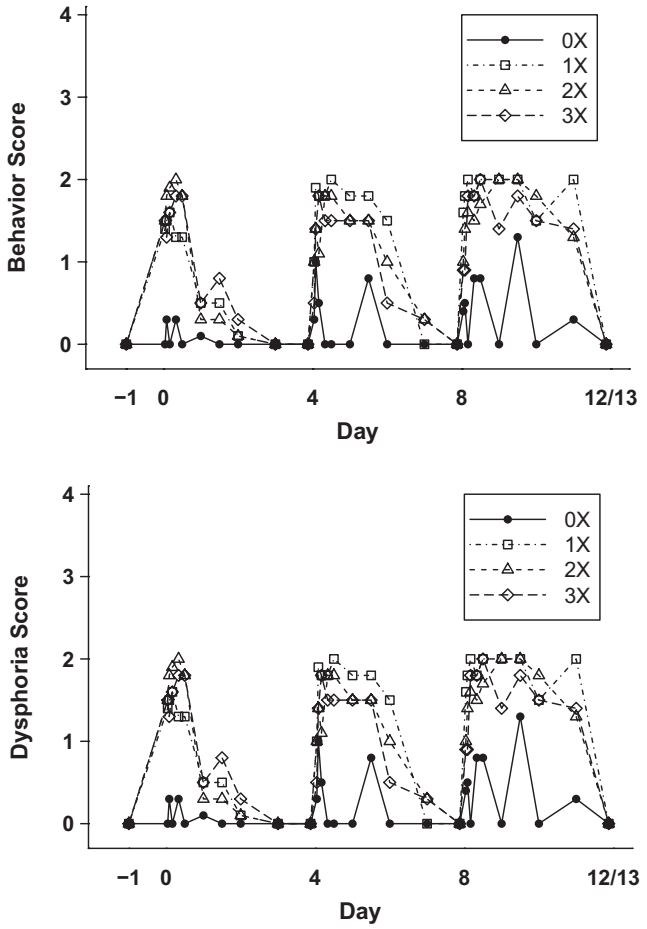

FIGURE 2 Mean behavior score by dose group over the study duration. The behavior score was 0 - normal, 1 - sedated, 2 - euphoric 3- mildly dysphoric, and 4 – dysphoric. Behavior was assessed at baseline (Day - 1), prior to each dose administration (0 h), and at 1, 2, 4, 8, 12, 24, 36, 48, and 72 h following treatment on Day 0, 4, and 8

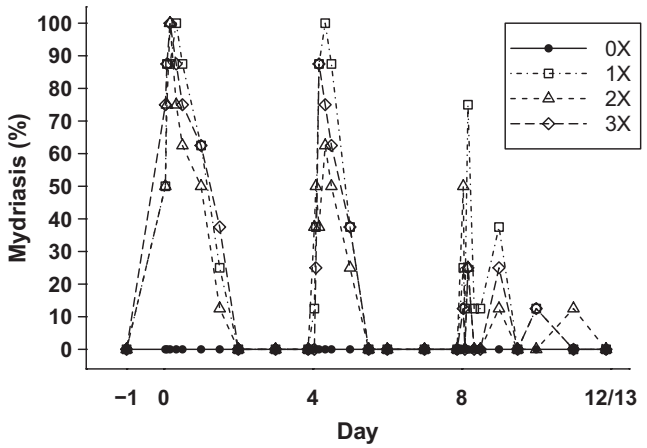

FIGURE 3 Proportion of cats within each dose group with mydriasis over the duration of the study. Mydriasis was assessed as present or absent (yes/no) at baseline (Day - 1), prior to each dose administration (0 h), and at 1, 2, 4, 8, 12, 24, 36, 48, and 72 h following treatment on Day 0, 4, and 8

was less that in the first dosing interval and returned to baseline by 24–36 h. In the third dosing interval, the proportion of cats with mydriasis was lesser in magnitude and duration than in the second dosing interval.

For physiological variables, there were no significant differences in heart rates; however, there were differences in body temperatures in all three dosing intervals and respiratory rates in the third dosing interval only. During the first dosing interval, there was a significant time-by-treatment interaction for body temperature. In the 1X group, body temperatures were significantly higher than placebo through 48 h following dose administration (Figure 4). In the 2X group, body temperatures were significantly higher than placebo at 2, 8, 12, and 36 h, and in the 3X group, body temperatures were significantly higher than placebo through 12 h after treatment. Overall, mean body temperatures in TBS-treated cats were up to 0.6°C (1.8°F) greater than placebo-treated cats. By three days post-dosing, body temperatures in TBS-treated cats were no different than placebo-treated cats. During the second dosing interval, there was a significant gender-by-treatment interaction for body temperature; however, mean body temperatures in placebo-treated cats were below normal and mean body temperatures for both genders of TBS-treated cats were normal. During the third dosing interval, there was a significant treatment effect for body temperature. Body temperatures in all TBS-treated cats were significantly higher compared to placebo-treated cats but similar to the second dosing interval, mean body temperatures of TBS-treated cats were normal while the placebo-treated cats were below normal. During the third dosing interval, there was a significant treatment effect for respiratory rate. In the third dosing interval, respiratory rates were lower by 10 breaths/min in the 3X group compared to placebo but means in all groups over the entire study were above the reference range (24–44 breaths per minute).

There were no significant treatment effects on body weights and weights remained similar over the study duration. Cats ate all

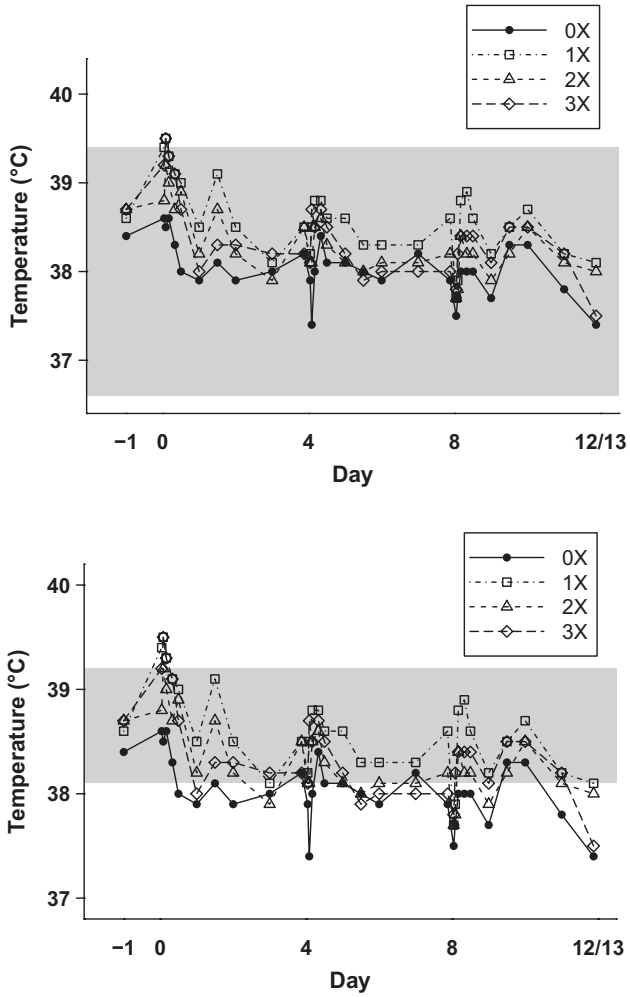

FIGURE 4 Mean body temperature by dose group over the study duration. Body temperature was measured at baseline (Day - 1), prior to each dose administration (0 h), and at 1, 2, 4, 8, 12, 24, 36, 48, and 72 h following treatment on Day 0, 4, and 8. The shaded area represents the normal body temperature range

or more than half of their food each day and food consumption was similar across all treatment groups with the exception of one cat in the 2X group on Day 10 that did not eat. No cats required supportive therapy to encourage food intake. Emesis was not observed in any cats during the study and the presence of urine and feces were similar across all treatment groups. Constipation was defined as at least three consecutive observations (*i.e.*, 36 h) of no feces. Over the study duration, there were 5 placebo-, 5 1X-, 4 2X-, and 6 3X-treated cats that experienced constipation (Table 3). In the second and third dosing intervals, 2 cats in the 1X group were administered a single dose of laxative that was sufficient to resolve constipation along with a single cat in the 3X group during the second dosing interval. Each of these cats was examined and 2 cats in the 1X group (on Day 6 and on Day 10) and 1 cat in the 3X group (on Day 7) were administered a single dose of laxative (Cat Lax®, Dechra). Application site observations were normal with the exception of pruritus observed in 2 placebo-, 1 1X-, 2 2X-, and 1 3X-treated cats on Day 4. All adverse events are summarized in Table 3.

There were no clinically relevant changes to serum chemistry, hematology, or urinalysis outcomes. Most mean clinical pathology values remained within the normal reference intervals although some differences were identified (Table 4). Statistical differences were identified for albumin, amylase, aspartate aminotransferase, chloride, glucose, sodium, total protein (time-x treatment interaction), GGT (gender x treatment interaction), and total bilirubin, and white blood cell count (treatment effect) (Figure 5). Some mean values were above the reference range but were not dose related. Mean aspartate aminotransferase and total bilirubin were above the reference range on Day 4 for placebo-treated cats. At baseline, GGT was not detectable (*i.e.*, <1 U/L) in all treatment groups. Mean GGT was above the reference range on Day 4 for placebo-, 1X-, and 3X-treated cats and on Day 13 for placebo- and 2X-treated cats. Mean glucose concentrations were above the reference range in all treatment groups at each sampling time point.

There were no gross or microscopic changes attributable to TBS. No gross lesions were identified in the pituitary gland, adrenal glands, liver, ovaries, uterus, testes, epididymides, kidneys, brain, pancreas, spinal cord, eyes, skeletal muscle, mammary gland, prostate, gall bladder, thymus, stomach, cecum, colon, thyroid glands, or parathyroid glands in any of the cats. Significant treatment effects were noted for the weights of adrenal glands and pituitary glands, relative to terminal body weight. As a percentage of terminal body weight, adrenal glands were lighter than placebo in the 1X group, and pituitary glands were heavier than placebo in the 2X group. Microscopically, there were minor incidental finding unrelated to treatment. Findings in the application site skin are summarized in Table 5. Bone marrow was evaluated for all 32 cats. The myeloid:erythroid (M:E) ratios, maturation, number of other cells, and megakaryocyte numbers were evaluated in light of contemporaneous CBC findings. A comparison of M:E ratios across groups was performed, and no clinically relevant differences were found (Table 6).

4 | DISCUSSION

These data demonstrate that TBS is safe and well-tolerated when administered to 16-week-old cats at multiples of the approved dose and duration. The 1X dose was defined as 6.7 mg/kg, which is the highest dosage within the approved range. Administration of 1, 2, and 3X the dose every 4 days results in simulated drug exposures of approximately 1.79, 3.59, and 5.38 times the 1X dose, respectively, by the third dosing interval. Despite these high doses and drug accumulation, few safety observations were identified.

Consistent with other studies of buprenorphine in cats, sedation, euphoria, mydriasis, and increased body temperature were observed (Catbagan et al., 2011; Mollenhoff et al., 2005; Murrell et al., 2007; Robertson et al., 2003, 2005; Robertson & Taylor, 2004; Steagall et al., 2006, 2009, 2013). Sedation or euphoria was transient in the first dosing interval but became more prolonged with each dosing interval. In contrast, mydriasis was prolonged in the first dosing interval and diminished by the third dosing interval consistent with

TABLE 3 Summary of adverse events observed within each dose group over the study duration. There were 8 cats per treatment group. The *n* is the number of cats that experienced the adverse event, and the percent is *n*/8×100

	0X		1X		2X		3X	
Adverse Event	**n**	**%**	**n**	**%**	**n**	**%**	**N**	**%**
Constipation[a]	5	62.5	5	62.5	4	50.0	6	75.0
Hyperthermia[b]	0	0.0	8	100.0	4	50.0	7	87.5
Hyperactivity	0	0.0	5	62.5	3	37.5	3	37.5
Tachypnea	3	37.5	2	25.0	3	37.5	1	12.5
Lethargy	3	37.5	0	0.0	3	37.5	2	25.0
Application site pruritus	2	25.0	1	12.5	2	25.0	1	12.5
Euphoria	0	0.0	3	37.5	1	12.5	1	12.5
Conjunctivitis	1	12.5	2	25.0	0	0.0	1	12.5
Aggression	0	0.0	0	0.0	1	12.5	1	12.5
Anxiety	0	0.0	0	0.0	1	12.5	1	12.5
Diarrhea	2	25.0	0	0.0	0	0.0	0	0.0
Distension of abdomen	0	0.0	1	12.5	0	0.0	1	12.5
Hypothermia[b]	2	25.0	0	0.0	0	0.0	0	0.0
Dysphoria	0	0.0	1	12.5	0	0.0	0	0.0
Hypersalivation	0	0.0	0	0.0	1	12.5	0	0.0
Tachycardia[c]	0	0.0	1	12.5	0	0.0	0	0.0

[a]Constipation was defined as any instance of lack of feces for 3 consecutive post-treatment observation periods (*i.e.*, 36 h).

[b]Hypothermia or hyperthermia was defined as any single excursion outside the normal body temperature range (*i.e.*, 36.6–39.4 °C [98–103 °F]) during the study.

[c]Tachycardia was defined as any single excursion ≥200 beats per minute.

accommodation. Increased body temperature above the normal range was transient in the first dosing interval but mean body temperatures did not rise above normal in subsequent dosing intervals. Overall, mean body temperatures in TBS-treated cats were up to 0.6°C (1.8°F) greater than placebo-treated cats and there were no instances where cats were treated for altered body temperature. These results are similar to those observed in laboratory studies and randomized controlled trials with TBS (Clark et al., 2022a; Clark et al., 2022b; Freise, Reinemeyer, Warren, et al., 2022. For example, in a randomized controlled trial, the majority (66.4%) of the postoperative hyperthermia occurred on the day of surgery and mean body temperatures were increased 0.35 (95% CI: [0.20 – 0.50]) °C compared to placebo-treated cats (Clark et al., 2022b). These changes in body temperature were not considered clinically meaningful.

There was little evidence of respiratory, cardiovascular, or gastrointestinal effects of TBS, a feature of buprenorphine, among others, that has given preference to buprenorphine as a frontline opioid analgesic in human health (Davis, 2012). In the present study, respiratory rates were above the reference range in all groups and lower by 10 breaths/min in the 3X group during the third dosing interval compared to placebo. Altered respiratory rates have not been demonstrated with high dose buprenorphine administration (Sramek et al., 2015). There were no differences in heart rates between treatment groups in the present study consistent with other studies. In two randomized controlled trials, TBS was safe when used in conjunction with a variety of anesthetic agents and analgesic rescue drugs (Clark et al., 2022a; Clark et al., 2022b). There were no differences in the intraoperative physiological variables in placebo- or TBS-treated cats except for ET CO2. The mean ET CO2 was increased 2.47 (95% CI: [0.22–4.72]) mm Hg greater than baseline in TBS-treated cats compared to placebo. This small difference was not influenced by the drugs used during anesthesia and was not associated with any incidences of hypercapnia during surgery. There were no differences in respiratory rates and therefore, the small observed difference in ET CO_2 was not considered clinically meaningful. In another clinical study, when physiological variables were compared following buprenorphine/acepromazine premedication to midazolam/ketamine and medetomidine/propofol, pCO2 increased equally in all groups (Akkerdaas et al., 2001). The increased pCO2 was considered acceptable given the depth of anesthesia. However, the study was not placebo-controlled, and therefore, the increased pCO2 could not be causally isolated to any particular drug. In other studies that included buprenorphine, there were no clinically meaningful changes to pCO2 (Bellini et al., 2017; Grint et al., 2009; Ilkiw et al., 2002). In an echocardiographic study of cats examining buprenorphine (0.01 mg/kg IM)/dexmedetomidine (0.04 mg/kg IM) sedation, blood pressure increased and heart rates decreased post-sedation (Johard et al., 2018). Wall thickness and left atrium and aortic diameter were not affected by sedation, but indices of left atrium and left ventricular size increased. The primary concern was the potential for administration of this sedation regimen to cats with hypertrophic cardiomyopathy, and not being placebo-controlled, the effects could not be dissociated from dexmedetomidine administration alone.

TABLE 4 Summary of hematology and clinical chemistry results at baseline and Days 4, 8, and prior to euthanasia on Days 12 or 13 by treatment group

Hematology								
	Baseline		Day 4		Day 8		Day 13	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Erythrocytes (RBC) (10/μl)[2]								
Placebo	8.1	0.3	7.7	0.4	7.5	0.3	8.1	0.5
1X TBS	8.0	0.7	7.4	0.8	7.5	0.6	7.7*	0.7
2X TBS	8.3	0.8	7.5	0.6	7.7	0.5	7.9*	0.6
3X TBS	8.0	0.6	7.1*	0.6	7.5	0.4	7.9	0.3
Hematocrit (%)								
Placebo	30.7	1.8	31.1	3.0	31.2	2.0	34.0	3.9
1X TBS	30.9	2.6	31.1	2.7	31.3	1.7	32.6	2.0
2X TBS	31.3	2.4	31.0	1.0	31.2	0.7	33.0	2.3
3X TBS	31.1	2.0	29.7	2.1	32.5	1.9	35.1	2.0
Hemoglobin (g/dl)								
Placebo	10.8	0.4	10.7	0.7	10.4	0.6	11.1	0.8
1X TBS	10.4	1.0	10.0	0.9	9.9	0.4	10.5	0.5
2X TBS	10.2	1.5	10.2	0.3	10.2	0.3	10.8	0.4
3X TBS	10.0	3.3	8.9	3.2	10.5	0.6	10.9	0.4
Mean Corpuscular Hemoglobin (MCH) (pg)								
Placebo	13.4	0.7	13.8	0.5	13.7	0.5	13.7	0.5
1X TBS	13.0	1.4	13.5	0.7	13.4	0.8	13.6	0.8
2X TBS	12.4	2.0	13.6	1.0	13.4	1.0	13.6	1.0
3X TBS	12.3	4.0	12.6	4.5	14.0	0.7	13.8	0.4
Mean Corpuscular Hemoglobin Concentration (MCHC) (g/dl)								
Placebo	35.1	1.5	34.4	1.3	33.3	1.6	32.9	2.4
1X TBS	33.5	3.0	32.2	1.3	31.8	1.0	32.0	1.3
2X TBS	33.0	6.1	32.9	1.4	32.8	0.9	32.7	1.5
3X TBS	31.7	10.3	29.7	10.4	32.3	1.1	31.0	1.4
Mean Corpuscular Volume (MCV) (fL)								
Placebo	38.1	2.4	40.1	2.4	41.3	2.0	42.0	3.7
1X TBS	38.9	2.6	42.0	3.0	42.0	1.8	42.5	2.3
2X TBS	37.8	3.2	41.2	3.0	41.0	2.9	41.8	4.4
3X TBS	38.8	2.7	41.9	2.8	43.3	1.5	44.6	2.9
Platelets (10/μl)								
Placebo	402.9	183.8	338.0	154.9	441.1	166.0	418.8	212.6
1X TBS	337.6	228.3	341.3	146.8	408.3	171.7	470.1	215.3
2X TBS	300.7	133.5	312.9	108.6	341.9	69.3	367.0	146.1
3X TBS	373.0	179.0	338.3	123.1	412.5	133.2	566.7	118.1
Leukocytes (WBC) (10/μl)[4]								
Placebo	20.5	8.6	20.4	7.9	19.2	8.8	20.5	9.3
1X TBS	15.4	3.8	15.7	3.2	14.8	2.7	14.0	2.9
2X TBS*	19.6	6.2	15.5	5.7	15.6	4.4	15.5	4.4
3X TBS	17.2	5.3	15.1	2.9	16.0	5.0	16.5	4.6

(Continues)

TABLE 4 (Continued)

Hematology	Baseline		Day 4		Day 8		Day 13	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Neutrophils (10/μl)								
Placebo	7.0	2.8	8.4	3.4	6.6	1.8	7.8	3.1
1X TBS	5.4	1.5	7.7	3.0	5.5	2.2	5.4	2.3
2X TBS	9.3	5.7	6.8	3.0	6.4	2.7	6.4	2.5
3X TBS	6.9	2.9	7.0	1.7	8.0	3.3	7.3	3.6
Lymphocytes (10/μl)								
Placebo	12.1	7.1	10.8	7.2	10.9	7.8	11.0	8.5
1X TBS	8.8	3.3	6.8	1.9	8.0	1.8	7.3	1.6
2X TBS	8.9	2.0	7.4	2.9	7.9	2.8	7.6	3.0
3X TBS	8.7	3.1	6.8	1.9	6.8	3.0	7.7	2.2
Monocytes (10/μl)								
Placebo	0.4	0.2	0.3	0.2	0.5	0.3	0.7	0.7
1X TBS	0.2	0.2	0.4	0.1	0.4	0.2	0.3	0.1
2X TBS	0.4	0.2	0.3	0.2	0.3	0.1	0.4	0.2
3X TBS	0.3	0.2	0.3	0.2	0.4	0.4	0.4	0.3
Eosinophils (10/μl)								
Placebo	1.0	0.3	0.9	0.6	1.2	0.4	1.0	0.8
1X TBS	0.8	0.3	0.9	0.3	0.9	0.3	1.0	0.4
2X TBS	1.0	0.3	0.9	0.4	1.0	0.4	1.0	0.2
3X TBS	1.2	0.4	0.9	0.3	0.8	0.4	1.0	0.1
Basophils (10/μl)								
Placebo	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
1X TBS	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
2X TBS	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
3X TBS	0.1	0.1	0.0	0.0	0.0	0.0	0.0	0.0
Clinical Chemistry								
Alanine Aminotransferase (U/L)[1]								
Placebo	57.8	13.9	82.0	50.1	42.0	5.9	53.4	11.6
1X TBS	53.9	11.3	51.9	17.7	41.4	9.3	42.3	7.4
2X TBS	48.9	11.8	47.1	9.7	44.4	8.3	44.9	14.2
3X TBS	54.0	16.2	57.5	26.0	44.6	7.3	42.5	9.4
Albumin (g/dl)[2]								
Placebo	3.2	0.1	3.6	0.3	3.2	0.1	3.3	0.1
1X TBS	3.0	0.2	3.3*	0.2	3.1	0.2	3.1	0.1
2X TBS	3.1	0.1	3.2*	0.1	3.2	0.1	3.2	0.3
3X TBS	3.1	0.2	3.3*	0.2	3.1	0.1	3.1	0.2
Alkaline Phosphatase (U/L)								
Placebo	132.8	18.3	101.8	29.6	109.4	10.7	98.8	19.7
1X TBS	146.5	44.4	108.1	29.4	111.5	34.5	101.8	32.1
2X TBS	161.0	22.8	125.3	28.3	128.0	25.9	107.6	20.6
3X TBS	172.9	59.1	129.1	51.1	135.8	41.7	111.8	34.9
Amylase (U/L)[2]								
Placebo	969.8	142.2	869.1	154.7	973.6	141.7	982.3	161.1
1X TBS	956.5	206.8	991.0*	248.0	905.6	178.3	922.1	178.0
2X TBS	1028.4	266.5	908.6	170.7	974.0	299.2	940.3*	186.9
3X TBS	984.4	199.0	921.9	203.1	878.5*	177.5	875.6*	177.3

TABLE 4 (Continued)

Hematology								
	Baseline		**Day 4**		**Day 8**		**Day 13**	
	Mean	**SD**	**Mean**	**SD**	**Mean**	**SD**	**Mean**	**SD**
Aspartate Aminotransferase (U/L)[2]								
Placebo	55.0	20.1	94.3	70.8	18.6	3.3	32.4	21.3
1X TBS	59.5	32.2	36.5*	32.3	19.3	2.7	22.5	9.1
2X TBS	47.6	35.1	24.5*	10.9	20.4	2.3	24.9	18.2
3X TBS	35.3	13.8	28.9*	22.0	20.0	2.8	20.9	15.3
Blood Urea Nitrogen (mg/dl)								
Placebo	23.8	1.6	24.4	2.9	24.1	2.9	26.5	2.4
1X TBS	22.3	3.5	23.4	4.7	24.0	4.3	25.9	5.5
2X TBS	22.5	2.0	21.8	3.2	20.0	2.7	22.5	3.8
3X TBS	22.4	2.6	21.8	3.8	21.8	3.4	23.5	2.6
Calcium (mg/dl)								
Placebo	10.1	0.3	10.0	0.6	9.9	0.2	10.3	0.2
1X TBS	9.9	0.3	9.9	0.3	9.7	0.1	9.9	0.3
2X TBS	10.1	0.3	10.1	0.3	9.9	0.3	10.1	0.3
3X TBS	10.1	0.2	10.0	0.4	10.1	0.4	10.0	0.3
Chloride (mEq/L)[2]								
Placebo	109.9	4.3	109.0	5.6	116.8	1.7	116.4	2.0
1X TBS	101.9	7.2	116.4*	1.6	116.9	1.1	116.4	1.8
2X TBS	107.9	5.8	116.9*	1.6	116.4	1.8	116.5	0.8
3X TBS	108.6	4.9	116.4*	3.1	117.8	2.5	116.1	1.8
Creatine Kinase (U/L)								
Placebo	3844.5	3881.2	3641.9	6768.2	256.8	135.1	479.9	399.3
1X TBS	6175.6	7051.5	1486.6	2116.4	223.4	108.0	260.9	137.7
2X TBS	4405.1	5201.3	418.4	260.6	257.8	199.3	347.8	270.4
3X TBS	1562.5	1393.8	553.8	304.5	266.6	127.4	240.1	255.5
Creatinine (mg/dl)								
Placebo	0.8	0.1	0.8	0.1	0.7	0.1	0.7	0.1
1X TBS*	0.8	0.1	0.8	0.1	0.8	0.1	0.8	0.2
2X TBS	0.8	0.1	0.8	0.1	0.7	0.1	0.7	0.1
3X TBS	0.8	0.1	0.7	0.1	0.7	0.1	0.7	0.1
Gamma-glutamyltransferase (U/L)[3]								
Placebo	<1		4.1	3.8	1.3	0.5	3.3	2.8
1X TBS	<1		2.6	1.5	1.4	0.5	1.5	0.8
2X TBS	<1		1.9	0.6	1.3	0.5	2.4	2.3
3X TBS	<1		2.6	1.4	1.1	0.4	1.8	2.1
Globulin (g/dl)								
Placebo	3.0	0.2	2.8	0.2	2.6	0.2	2.9	0.2
1X TBS	2.9	0.3	2.7	0.4	2.7	0.2	2.9	0.2
2X TBS	2.9	0.2	2.6	0.2	2.6	0.2	2.7	0.2
3X TBS	2.8	0.2	2.5	0.3	2.5	0.3	2.7	0.3

(Continues)

TABLE 4 (Continued)

Hematology	Baseline		Day 4		Day 8		Day 13	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Glucose (mg/dl)[2]								
Placebo	168.5	44.6	231.5	71.1	165.6	34.4	148.6	34.8
1X TBS	197.8	40.8	161.8*	28.9	146.1*	10.4	160.4	29.8
2X TBS	190.8	36.0	164.3*	46.0	150.8*	32.7	162.6	28.2
3X TBS	195.6	49.6	200.8	63.9	174.5	33.6	164.0	33.3
Phosphorous (mg/dl)								
Placebo	7.6	0.6	8.7	0.7	8.4	0.9	8.9	0.8
1X TBS	7.0	0.5	8.2	1.1	8.3	0.7	8.7	0.9
2X TBS	7.3	0.6	8.2	0.7	7.9	0.7	8.4	0.6
3X TBS	7.1	0.6	8.1	0.7	8.0	0.5	8.1	0.3
Potassium (mEq/L)								
Placebo	4.8	0.5	5.0	0.5	4.8	0.3	4.8	0.6
1X TBS	4.7	0.4	4.9	0.4	4.9	0.5	4.9	0.6
2X TBS	4.8	0.6	4.7	0.4	5.0	0.2	5.0	0.3
3X TBS	4.9	0.4	5.0	0.5	5.1	0.4	4.9	0.4
Sodium (mEq/L)[2]								
Placebo	142.3	2.9	138.1	7.3	146.9	1.7	147.6	1.4
1X TBS	139.1	4.4	145.4*	1.4	147.1	1.6	146.3	4.0
2X TBS	140.4	3.2	146.9*	2.8	147.1	1.4	147.5	2.0
3X TBS	140.1	3.0	145.4*	3.0	147.6	1.4	146.5	3.0
Total Bilirubin (mg/dl)[4]								
Placebo	0.3	0.1	0.5	0.4	0.1	0.0	0.2	0.1
1X TBS*	0.3	0.1	0.2	0.2	0.1	0.0	0.2	0.1
2X TBS*	0.2	0.1	0.2	0.1	0.1	0.0	0.1	0.1
3X TBS*	0.2	0.1	0.2	0.2	0.1	0.0	0.1	0.0
Total Cholesterol (mg/dl)								
Placebo	112.3	14.5	121.6	24.0	105.1	18.3	113.6	17.9
1X TBS	96.5	15.1	93.5	18.0	90.3	8.0	96.6	9.8
2X TBS	110.9	29.8	99.6	20.5	99.6	22.8	105.0	20.5
3X TBS	95.1	14.8	89.5	13.7	87.5	19.6	90.6	22.3
Total Protein (g/dl)[2]								
Placebo	6.2	0.2	6.4	0.3	5.8	0.1	6.2	0.1
1X TBS	5.9	0.4	6.0	0.5	5.8*	0.2	6.0	0.3
2X TBS	6.0	0.3	5.8*	0.2	5.8	0.3	5.9	0.3
3X TBS	5.9	0.3	5.8*	0.3	5.7	0.3	5.8	0.4

Note: Statistical significance of the [1]time x treatment x gender effect; [2]time x treatment effect; [3]gender x treatment effect; or [4]treatment effect.
*Statistical significance of the treatment effect compared to the placebo.

Body weights remained similar over the study duration consistent with normal food intake and growth in these young cats. Food intake was documented, and cats ate all or more than half of their food each day across all treatment groups with the exception of one cat in the 2X group during the third dosing interval. There was no emesis in any cats during the study and the observed presence of urine and feces were similar across all treatment groups. Constipation was transiently observed in approximately equal numbers of cats in placebo- and TBS-treated groups. In the second and third dosing interval which lies outside the labeled use, 2 cats in the 1X group were administered a single dose of laxative that was sufficient to resolve constipation along with a single cat in the 3X group during the second dosing interval. These findings are consistent in study where buprenorphine (0.01 mg/kg IM) was coadministered with acetylpromazine (0.1 mg/

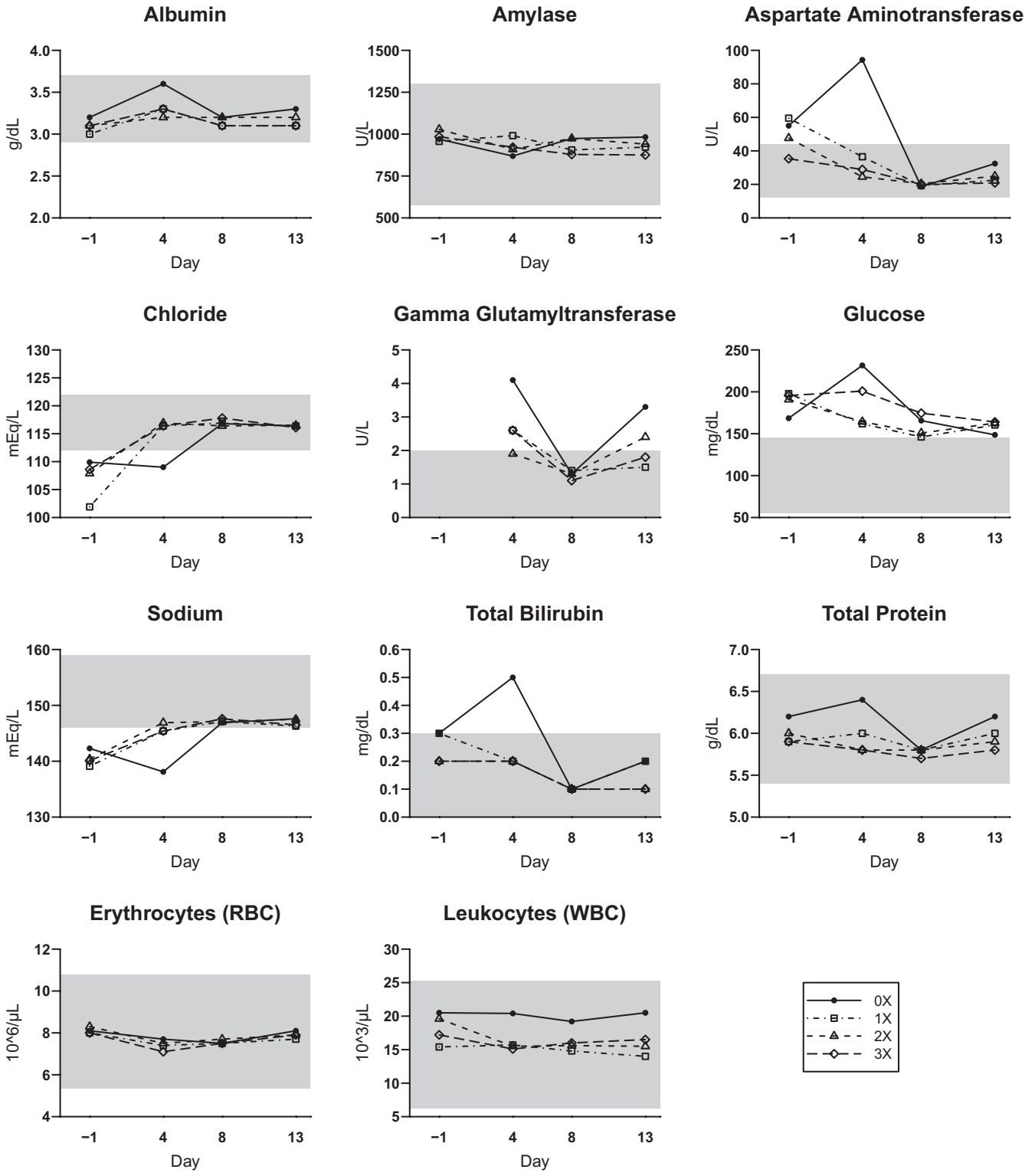

FIGURE 5 Plots of mean clinical pathology values were a time-by-treatment interaction (albumin, amylase, aspartate aminotransferase, chloride, glucose, sodium, and total protein), gender-by-treatment interaction (GGT), and treatment effect (total bilirubin and white blood cell count) was identified. Blood samples for hematology and clinical chemistry were collected on Days - 1, 4, 8, and prior to necropsy at study conclusion. The shaded area represents the reference range

kg IM), and there were no effects on orocaecal transit times (Sparkes et al., 1996).

There were no clinically relevant changes to serum chemistry, hematology, or urinalysis outcomes. Most mean clinical pathology values remained within the normal reference intervals although some differences were identified. Changes were minimal, did not correlate with increasing dose or dose interval, and were likely due to biological variation. At baseline, GGT was not detectable (*i.e.*, <1

TABLE 5 Incidence and magnitude (*i.e.*, increased, minimal, mild) of lymphocytic infiltration in the skin application site at necropsy for each treatment group

Dose	Placebo		1X TBS		2X TBS		3X TBS	
Sex	M	F	M	F	M	F	M	F
N	4	4	4	4	4	4	4	4
Dermal lymphoplasmacytic infiltration								
Increased	0	3	1	2	2	1	3	2
Minimal	0	3	1	1	2	1	2	1
Mild	0	0	0	1	0	0	1	1

Note: M, Male; F, Female.

TABLE 6 Summary of bone marrow myeloid:erythroid ratios by treatment group collected at necropsy. There were no clinically relevant differences

Comparison of Myeloid:Erythroid Ratios				
Groups	Placebo	1X TBS	2X TBS	3X TBS
	1.1	1.4	0.8	0.7
	1.3	1.0	0.9	1.4
	1.5	2.2	1.1	1.1
	1.8	1.1	1.4	1.2
	1.2	1.5	1.4	1.9
	1.3	1.9	1.8	1.7
	1.4	1.7	1.4	1.2
	1.6	0.8	0.9	1.1
Mean	1.40	1.45	1.21	1.29
SD	0.23	0.48	0.34	0.38

U/L) in all treatment groups. Mean GGT was above the reference range on Day 4 for placebo-, 1X-, and 3X-treated cats and on Day 13 for placebo- and 2X-treated cats. Serum GGT is primarily of hepatic origin in health and disease and increases are typically associated with cholestasis (Duncan et al., 1994). It is likely these results are due to biological variation given that it occurred in placebo- and TBS treatment groups and that bilirubin means remained in the reference range with the exception of the placebo group on Day 4. There were no gross or microscopic liver changes on necropsy. Glucose was above the reference range at all time points including baseline. This was likely related to the stress of sample collection or as the result of dexmedetomidine sedation to aid sample collection (Posner & Burns, 2009). Creatine kinase (CK) did not increase with dose as reported in a safety study of the high-concentration injectable formulation (Sramek et al., 2015). In that study, CK increased directly with dose and triglycerides decreased inversely with dose. This observation in cats was raised as a possible concern when interpreting these analytes in humans being treated with buprenorphine (Srinivas, 2016). These changes were not replicated in the present study; although triglycerides were not included in the panel, CK did not increase with TBS dose. Also observed in the high-concentration injectable formulation safety study was higher percent basophils and fibrinogen and lower bile acids. Basophils did not increase in the present study and fibrinogen and bile acids were not assayed. However, in a randomized controlled trial of TBS, fibrinogen increased in a small proportion of both placebo- and TBS-treated cats, likely an outcome of undergoing surgery (Clark et al., 2022b).

Buprenorphine has not been associated with gross or histopathological changes when administered chronically to dogs (Heel et al., 1979; Kleppner et al., 2006) or cats (Sramek et al., 2015). Consistent with these reports, there were no gross pathologic or histopathologic observations attributable to TBS administration. As a percentage of terminal body weight, adrenal glands were lighter than placebo in the 1X group, and pituitary glands were heavier than placebo in the 2X group. These findings were not dose related, were small in magnitude, and were not associated with histological changes. There were no treatment-related changes to the myeloid:erythroid (M:E) ratios, maturation, number of other cells, and megakaryocyte numbers, which were reflected by a lack of changes to hematology outcomes. There were no histological treatment differences in the application site skin. This was consistent with application site observations throughout the study whereby pruritus was observed infrequently and occurred similarly in placebo- and TBS-treated cats. These observations are consistent with those observed in two randomized controlled trials in which no instances of hair loss, pruritus, erythema, edema, pain, or heat at the application site were reported (Clark et al., 2022a; Clark et al., 2022b).

The metabolism of buprenorphine in cats has not been characterized, however, in humans, norbuprenorphine, a cytochrome P450 (CYP)-dependent oxidative dealkylated metabolite has been described (Lutfy & Cowan, 2004). In rats, norbuprenorphine is between 50- and 200-fold less potent than buprenorphine with regard to its respiratory depressant or antinociceptive effects and does not likely play a significant clinical role (Yassen et al., 2007). Norbuprenorphine has been identified in cats but its contribution to thermal threshold antinociception was not identified (Doodnaught et al., 2017). Both buprenorphine and norbuprenorphine are eliminated by a saturable elimination pathway via uridine diphosphate glucuronosyltransferase (UGT) as characterized in human liver microsomes (Rouguieg et al., 2010). There is some evidence of PK variability in cats. Following IV administration, the half-life of buprenorphine in cats ranges from approximately 1–12 h (Freise, Reinemeyer, Warren, et al., 2022; Hedges et al., 2014; Steagall et al., 2013; Taylor et al., 2001). However, the half-life of TBS is 67.7 (90% CI: [59.9–75.6]) hours which reflects absorption rather than elimination, and as a result, a substantial inhibition of elimination pathways would have to occur before drug accumulation would exceed that observed in the present study, *that is*, greater than 5 times drug exposure. Therefore, these data demonstrate that TBS is safe and well-tolerated when administered to 16-week-old cats at multiples of the approved dose and duration and supports clinical safety in the event of delayed buprenorphine metabolism, medication errors, or alterations in the dosing regimen.

5 | CONCLUSIONS

These data demonstrate that TBS is safe and well-tolerated when administered to 16-week-old cats at 1, 2, and 3X the approved dose every 4 days for 3 doses. Few treatment-related effects were observed with the exception of euphoria, mydriasis, and increased body temperature, which are not considered clinically relevant. There were no clinically relevant changes to serum chemistry, hematology, or urinalysis outcomes nor gross or microscopic observations attributable to TBS administration. These results support clinical safety in the event of delayed buprenorphine metabolism, medication errors, or alterations in the dosing regimen.

AUTHOR CONTRIBUTION

TPC contributed to study design, data analysis, and manuscript writing. DDL contributed to protocol development, data analysis, and study execution. KJF was involved in study design, data analysis, and manuscript preparation. CR was involved in study execution and manuscript preparation. KMN led pathology evaluation and interpretation. AA led clinical pathology analysis and interpretation. TL contributed to study design, data analysis, and manuscript preparation. All authors have read and approved the final manuscript.

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ANIMAL WELFARE AND ETHICS STATEMENT

The authors confirm compliance to the ethical policies of the journal, as noted on the journal's author guidelines page. Prior to study initiation, an independent Institutional Animal Care and Use Committee reviewed and approved animal use protocol ETCR-13–0116, confirming compliance to United State Department of Agriculture animal welfare and ethics standards for the protection of animals used for scientific purposes.

CONFLICT OF INTEREST

All authors were paid employees (Freise, Linton, Lin, and Clark) or contractors (Reinemeyer, Newkirk, and Aulbach) of Nexcyon Pharmaceuticals, Inc. at the time the study was conducted. One author (Clark) was a paid consultant of Elanco Animal Health at the time the manuscript was written, and one author (Lin) was a paid consultant of Nexcyon Pharmaceuticals, Inc. at the time the manuscript was written.

DATA AVAILABILITY STATEMENT

Not applicable.

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APPENDIX I

Analytes from blood samples for hematology and clinical chemistry on Days - 1, 4, 8, and study conclusion (Day 12 or 13) or urine samples collected on Day - 1 and study conclusion (Day 12 or 13). The normal reference ranges are within parentheses.

Clinical Chemistry	
Albumin (2.9–3.7 g/dL)	Alkaline phosphatase (52.5–178.8 U/L)
Alanine aminotransferase (27–93 U/L)	Amylase (575–1298 U/L)
Aspartate aminotransferase (12–44 U/L)	Blood urea nitrogen (16–33 mg/dL)
Calcium (9.6–11.5 mg/dL)	Chloride (112–122 mEq/L)
Cholesterol (88–148*mg/dL)	Creatine kinase (≤ 987 U/L)
Creatinine (0.5–1.3 mg/dL)	Gamma-glutamyltransferase (≤ 2 U/L)
Globulin (2.2–3.3 g/dL)	Glucose (55–145 mg/dL)
Phosphorus (6.5–9.7 mg/dL)	Potassium (4.6–7.1 mEq/L)
Sodium (146–159 mEq/L)	Total bilirubin (≤ 0.3 mg/dL)
Total protein (5.4–6.7 g/dL)	
Hematology	
Red blood cell count (5.36–10.78 10^6/μl)	Hemoglobin (7.5–14.4 g/dL)
Hematocrit (22.7–41.5%)	MCV (34.7–46.5 fL)
MCH (12.1–15.1 pg)	MCHC (30.4–37.7 g/dL)
Platelet count (107–725 10^3/μl)	White blood cell count (6.3–25.3 10^3/μl) and differential
Urinalysis	
Volume	Specific gravity (1.048–1.087)
PH (6.0–7.4)	Glucose
Bilirubin	Ketones
Occult blood	Protein
Urobilinogen	Appearance
Color	Casts
Crystals	Amorphous
Epithelial Cells	Leukocytes
Erythrocytes	Sperm
Yeast	Bacteria

APPENDIX II

Organs and tissues collected from all cats, weighed if required, as denoted below, and preserved in 10% buffered, neutral formalin. Eyes were initially placed into Modified Davidson's solution and then transferred to 10% BNF the following day. Skin samples included three collected from the application site and one from untreated skin anterior to the application site.

Organs or tissues collected		
Pituitary gland[a]	Brain[a]	Bone & marrow
Thyroid gland (collected with parathyroid)	Spinal cord	Marrow smear
Parathyroid gland (collected with thyroid)	Eyes	Spleen[a]
Adrenal gland[a]	Lung	Stomach
Pancreas	Muscle (biceps femoris)	Duodenum
Ovaries[a]	Mammary gland	Jejunum
Uterus[a]	Liver[a]	Ileum
Testes[a]	Gall bladder	Colon
Prostate	Kidneys[a]	Cecum
Epididymides[a]	Urinary bladder	Thymus
Heart [a]	Lymph nodes (Retropharyngeal, Cervical, and Mesenteric)	Skin (as above)

[a] Indicates organs weighed; total organ weighed, including both pairs, as applicable.