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# The margin of safety of a single application of transdermal fentanyl solution when administered at multiples of the therapeutic dose to laboratory dogs

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Previous studies have demonstrated that a single, topical application of a novel, long-acting transdermal fentanyl solution provides analgesic fentanyl concentrations for at least 4 days. The objective of this study was to describe the margin of safety following application at multiples of the therapeutic dose. Twenty-four laboratory dogs were administered a single placebo or 1×, 3×, or 5× multiple of the dose of 2.6 mg/kg (50 μL/kg) to the ventral abdominal skin and observed for 14 days. Plasma fentanyl concentrations increased in proportion to dose. Adverse reactions in the 1× group were transient and included a low prevalence ( ≤ 33%) of mild sedation, reduced food intake, modest weight loss, and minimal reductions in heart rate and rectal temperature. Moderate to severe sedation emerged in the 3× and 5× groups, which was associated with a dose-limiting reduction in food and water intake, necessitating maintenance fluid replacement for the first 2 days following application. Also observed in the higher-dose groups were an increased prevalence of abnormal stools and transient lens opacities. All abnormal health observations were completely resolved prior to necropsy on day 14, and there were no histological abnormalities identified. These data support the safe use of the 1× dose and describe the outcome of an overdose of up to 5× dose in the absence of opioid reversal.

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## INTRODUCTION

Fentanyl is a selective μ-opioid receptor agonist that was discovered as a meperidine analogue with a potency 100 times that of morphine (Stanley, 1992). The impetus for its discovery was to identify an improved analgesic over morphine, an opioid frequently associated with histamine release, bradycardia, hyper- or hypotension, and prolonged postoperative respiratory depression (Lowenstein, 1971). Early studies in dogs showed that even large intravenous doses of fentanyl in dogs of up to 3 mg/kg resulted in only small reductions in cardiac output, peripheral resistance, and arterial pressure, supporting the idea that fentanyl would be a useful anesthetic agent (Freye, 1974; Liu *et al.*, 1976). As a result, fentanyl citrate was initially introduced for use in humans as an injectable general anesthetic in combination with droperidol and later as a sole injectable agent. The safety pharmacology of fentanyl used within these pharmaceutical formulations has been extensively reviewed (Gutstein & Akil, 2006).

Following the experience in human health, fentanyl has been widely adapted to anesthetic case management in dogs and, because of rapid clearance, has been primarily limited to perioperative single or repeat injections or constant rate infusion (CRI) (Pascoe, 2000). Alternate pharmaceutical forms of fentanyl have been developed in humans to extend the duration of action that include transdermal patches. Although the onset of action is slow, this delivery device extends absorption over 2–3 days, thereby prolonging analgesia in people with a single application. However, this delivery device has introduced new safety warnings. Serious or life-threatening hypoventilation has been documented in humans following fentanyl patch application that, unlike fentanyl citrate injections, specifically contraindicates its use in non-opioid-tolerant patients and for postoperative pain (Janssen Pharmaceutica Products, 2005).

Fentanyl patches have been used off-label in dogs, and safety endpoints have been reported in some laboratory (Kyles *et al.*, 1996; Egger *et al.*, 1998; Robinson *et al.*, 1999; Welch *et al.*, 2002; Gilbert *et al.*, 2003; Pettifer & Hosgood, 2004; Wilson *et al.*, 2006) and clinical (Kyles *et al.*, 1998; Lafuente *et al.*, 2005; Egger *et al.*, 2007) studies. Sustained, steady-state plasma fentanyl concentrations achieved following fentanyl patch application (4.8 μg·kg/h) during experimental diaphragmatic hernia repair in laboratory dogs did not result in postoperative hypoventilation as evidenced by serial blood gas analysis (Welch *et al.*, 2002). Therefore, unlike in humans, respiratory depression has not been a feature of patch-delivered fentanyl in dogs, and therefore, its use in conjunction with anesthesia has been recommended (Hofmeister & Egger, 2004). Studies that have examined the margin of safety by extending the recommended transdermal fentanyl patch dose that include the measurement of numerous safety endpoints have not been conducted.

The use of patches in dogs has revealed several disadvantages that include lack of regulatory approval (Janssen Pharmaceutica Products, 2005), problems associated with maintaining contact of the patch to the skin (Riviere & Papich, 2001), variable fentanyl delivery rate and extent (Kyles *et al.*, 1996; Mills *et al.*, 2004), potential inadvertent fentanyl exposure to the pet or pet owner (Schmiedt & Bjorling, 2007), the possibility of diversion and illicit use when the pet is discharged from the hospital (Carson *et al.*, 2010), as well as the control and disposal of used patches (Marquardt *et al.*, 1995). To overcome these limitations and prolong the therapeutic duration of action, other variations in opioid pharmaceutical delivery have been advanced that include extended release oral tablets (Holt *et al.*, 2007) and liposome-encapsulated injectable opioids (Smith *et al.*, 2004). As a delivery method, the transdermal route has several potential strengths over oral and parenteral administration. These include noninvasive dosing, avoidance of the gastrointestinal tract, lack of first-pass metabolism, steady, continuous drug delivery rather than a peak and trough phenomenon, potential reduction in side effects by elimination of peaks, possible reduction in the lack of effectiveness owing to elimination of troughs, and reduced dose frequency for convenience and increased compliance (Urquhart, 2000).

A novel, long-acting transdermal fentanyl solution (Recuvyra™ transdermal solution, Nexcyon Pharmaceuticals Ltd, London, UK) has recently been developed that potentially mitigates the disadvantages of oral, parenteral, and patch-delivered opioids. A previous study demonstrated that a single topical application of this formulation resulted in dose-proportional plasma fentanyl concentrations (Freise *et al.*, 2012b). A single, small volume dose of 2.6 mg/kg (50 μL/kg) was proposed as a topical application that provided minimally effective analgesic concentrations within 2–4 h of application and an extended duration of at least 4 days (Freise *et al.*, 2012a,b). There were no adverse reactions at the proposed dose, but at 2× dose, adverse reactions included sedation and decreased food and water intake in some dogs (Freise *et al.*, 2012b). To further characterize safety, the objective of this study was to determine the margin of safety of transdermal fentanyl solution following a single topical administration of 0, 1×, 3×, or 5× multiple of the dose in healthy laboratory dogs.

## MATERIALS AND METHODS

### *Investigational drug*

The investigational drug was transdermal fentanyl solution (Recuvyra™ transdermal solution, Nexcyon Pharmaceuticals Ltd, London, UK). It is a clear, colorless to light yellow solution that contains 5% w/v (50 mg/mL) fentanyl base and the skin penetration enhancer octyl salicylate (also known as octisalate and 2-ethylhexyl salicylate) at a concentration of 5% w/v (50 mg/mL) in isopropanol. Placebo was the investigational formulation minus fentanyl.

### *Animals*

Twenty-four healthy purpose-bred laboratory mixed-breed hounds (12 males/12 females) 4–5 months of age weighing 11.0 ± 1.97 (mean ± SD) kg were randomly selected. Dogs were individually housed, fed a commercial dry food formula, and allowed *ad libitum* access to water. All procedures were approved by the animal facility's Institutional Animal Care and Use Committee.

### *Study design*

Day 0 was defined as the day that the animals received the investigational or control (placebo) article. Dogs were randomly allocated to a single application of transdermal fentanyl solution on day 0 to the caudal ventral abdominal skin near the umbilicus at a dose of 0 (placebo), 2.6 (1×), 7.8 (3×), and 13.0 (5×) mg/kg (three males and three females per dose group). Prior to drug administration, the dosing site was clipped and cleaned. The calculated dose was collected into a syringe (average volume of ~ 0.6 mL) and applied by placing the syringe opening against the skin and depressing the syringe plunger while moving the tip in a serpentine manner to spread the small volume of liquid evenly. From prior to dosing on day 0 through day 2, restraint collars (Bite Not®, Bite Not Products, Inc., San Francisco, CA, USA) were applied to the dogs to prevent direct licking of the dosing site on the ventral abdominal area.

Dogs were weighed weekly at days −7, 0, 7, and 14. Food consumption was measured daily from day −7 through day 14 by calculating the difference in the weight of food offered less the weight of remaining food. Physical examinations, including ophthalmic examinations, were conducted on all dogs on days −4, 3, and 13. Ophthalmic examinations were conducted by a board-certified veterinary ophthalmologist (DAW) following pupillary dilation (0.5% tropicamide) and included biomicroscopy (Ziess HSO-10) and indirect ophthalmoscopy (Keeler All Pupil) using a 30-diopter condensing lens. Twice-daily general animal health observations were made throughout the study, and directed clinical observations were conducted three times

daily on days 0, 1, and 3 (at 1, 2.5, and 4 h postdose administration on day 0 and approximately the same time of the day on days 1 and 2), and then daily thereafter. Subjective clinical observations included sedation scoring, dosing site observation, mucous membrane color evaluation, and stool production. Sedation was scored on a 4-point ordinal scale as none, slight, moderate, or severe (score of 0, 1, 2, or 3, respectively). Objective clinical assessment included measurement of rectal body temperature, heart and respiratory rates, and evaluation of electrocardiograms (ECGs) (lead I, II, III, aVR, aVL, and aVF). Electrocardiograms were recorded using a commercially available automated system* that provides individual reports that include selected quantitative electrocardiographic variables. Five-second recordings of ECG leads I, II, and III were reviewed to make an assessment of cardiac rhythm. Study personnel conducting examinations and other observations were not blinded to treatment group.

Following an overnight fast, blood samples were collected for serum chemistry and hematology analysis on days −7, 3, and 14. Serial jugular venous blood samples were collected for fentanyl assay at 0 (predosing), 0.5, 1, 2, 4, 8, 12, 24, 36, 48, 60, and 72 h and then daily thereafter (approximately within 1 h of the initial dosing time) through day 14 into sodium heparin blood collection tubes. Plasma was harvested from the blood samples by centrifugation at 1500 RCF for 10 min at 4 °C and stored at −70 °C until analysis. Samples were assayed for fentanyl according to a previously reported method with a lower limit of quantitation (LLOQ) of 0.025 ng/mL (Freise *et al.*, 2012a).

At the end of the study (day 14), dogs were euthanized and necropsied. Tissues were examined for gross lesions and if found submitted for histopathologic evaluation. Tissues were preserved in neutral-buffered 10% formalin, processed on slides, and stained with hematoxylin and eosin. Other, nonlesioned, tissues that were histologically examined were skin from the dosing site, untreated skin, and eyes. The veterinary pathologist was not blinded to treatment group.

### *Data analysis*

Plasma fentanyl concentrations less than LLOQ were set equal to zero ng/mL for descriptive statistic calculations. Pharmacokinetic parameters were calculated for each subject using noncompartmental PK analysis methods. Plasma samples less than the LLOQ were ignored in the noncompartmental PK analysis, except for the time 0 samples that were replaced by zero ng/mL. The linear up-log down trapezoidal rule was used for the area under the plasma concentration–time curve calculation from time 0 to the time of the last sample at or above the LLOQ ($AUC_{0-LLOQ}$). Log-linear regression was conducted to determine the terminal first-order rate constant in order to calculate the total AUC from time 0 to infinity ($AUC_{0-\infty}$) and the terminal half-life ($t_{½}$). The percentage of $AUC_{0-\infty}$ that was extrapolated ($AUC_{0-\infty}$ extrapolated) beyond the LLOQ was calculated as:

$$AUC_{0-\infty}\text{Extrapolated}(\%) = 100 \cdot (AUC_{0-\infty} - AUC_{0-LLOQ})/AUC_{0-\infty}$$

The maximum observed plasma concentration ($C_{max}$) and time of $C_{max}$ occurrence ($t_{max}$) were taken directly from the data. All PK parameter calculations were conducted using Phoenix WinNonlin® Version 6.1 (Pharsight Corporation, Mountain View, CA, USA).

## RESULTS

All dogs survived to the end of the study. Plasma fentanyl concentrations increased with dose and were detectable (≥0.025 ng/mL) at the first sampling point 30 min postdosing through 14 days in each group (Table 1, Fig. 1). Mean $C_{max}$ were 3.18, 7.27, and 13.5 ng/mL, and $AUC_{0-LLOQ}$ were 323, 824, and 1272 ng·h/mL in the 1×, 3×, and 5× groups, respectively (Table 2). Half-lives in all three dose groups were approximately 70 h. Exposure to fentanyl, as measured by $AUC_{0-LLOQ}$, was dose proportional (Fig. 2).

Sedation was not observed in the placebo group. A few instances of slight sedation were observed in the 1× group over 24 h beginning within 4 h of dosing (Table 3). In the 3× and 5× groups, sedation was evident within 1 h of dosing through days 5 and 4, respectively (Table 3). Moderate to severe sedation was

**Table 1.** Plasma fentanyl concentrations (ng/mL) summary statistics by dose group ($n$ = 6/group)

| | 1× (2.6 mg/kg) | | 3× (7.8 mg/kg) | | 5× (13.0 mg/kg) | |
|---|---|---|---|---|---|---|
| Time (h) | Mean | SD | Mean | SD | Mean | SD |
| 0 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| 0.5 | 0.02 | 0.02 | 0.18 | 0.19 | 0.17 | 0.03 |
| 1 | 0.20 | 0.11 | 0.57 | 0.36 | 1.04 | 0.36 |
| 2 | 0.51 | 0.24 | 1.66 | 0.79 | 2.30 | 0.46 |
| 4 | 1.00 | 0.36 | 3.34 | 1.53 | 4.48 | 1.26 |
| 8 | 1.87 | 0.33 | 4.38 | 1.94 | 8.38 | 3.48 |
| 12 | 3.00 | 0.95 | 6.03 | 2.34 | 12.20 | 5.01 |
| 24 | 2.82 | 0.92 | 6.30 | 0.79 | 12.68 | 3.54 |
| 36 | 2.11 | 0.82 | 4.72 | 1.34 | 11.18 | 3.81 |
| 48 | 1.80 | 0.44 | 5.05 | 1.63 | 8.51 | 3.22 |
| 60 | 2.35 | 0.79 | 5.04 | 1.40 | 9.19 | 1.81 |
| 72 | 2.03 | 0.93 | 4.45 | 2.09 | 7.19 | 2.21 |
| 96 | 1.47 | 0.31 | 3.83 | 2.22 | 5.54 | 2.70 |
| 120 | 0.97 | 0.29 | 3.47 | 2.04 | 3.75 | 1.74 |
| 144 | 1.01 | 0.42 | 2.63 | 1.88 | 3.29 | 2.13 |
| 168 | 0.60 | 0.22 | 1.66 | 0.62 | 1.85 | 0.65 |
| 192 | 0.35 | 0.18 | 1.11 | 0.37 | 1.19 | 0.40 |
| 216 | 0.36 | 0.22 | 1.06 | 0.44 | 1.29 | 0.57 |
| 240 | 0.27 | 0.18 | 1.05 | 0.33 | 1.00 | 0.47 |
| 264 | 0.30 | 0.23 | 0.87 | 0.33 | 1.04 | 0.44 |
| 288 | 0.23 | 0.14 | 0.71 | 0.19 | 0.96 | 0.45 |
| 312 | 0.10 | 0.06 | 0.34 | 0.13 | 0.40 | 0.22 |
| 336 | 0.15 | 0.10 | 0.53 | 0.16 | 0.58 | 0.19 |

SD, standard deviation.

*Computerized ECG by BASi/Vetronics

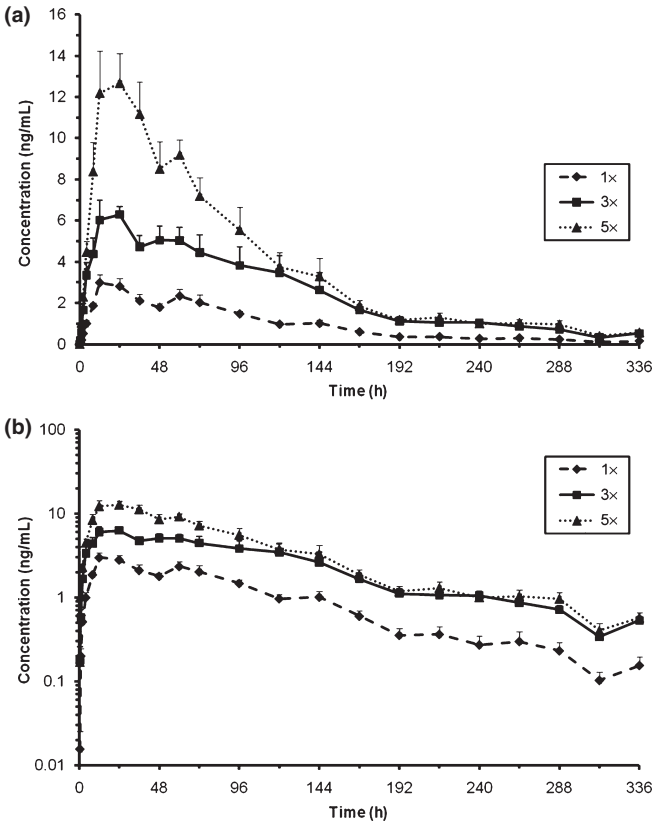


**Fig. 1.** Mean (± standard error) plasma fentanyl concentrations vs. time by dose group on a linear (panel A) and logarithmic (panel B) scale. Bars represent the standard error ($n$ = 6/group).


**Fig. 2.** Mean (± standard error) $AUC_{0–LLOQ}$ by fentanyl dose ($n$ = 6/group). Line represents linear regression with intercept set to zero ($R^2$ = 0.9854).

limited to the 3× and 5× groups, observed from 2.5 h postdose administration until day 3 (Fig. 3). Dogs in the 3× and 5× groups were treated with subcutaneous administration of 40–60 mL·kg/day balanced electrolyte solution (Normosol-R, Hospira, Inc., Lake Forest, IL, USA) on days 0 and 1 because it was determined that they were not consuming maintenance water quantities because of excess sedation.

Mean food consumption decreased in all treated dose groups following dosing (Fig. 4). The greatest decrease in food consumption was in the 5× group where no food was consumed on days 0 through 2. Food consumption had returned to pretreatment amounts by day 4 in the 1× group and day 6 in the 3× and 5× groups. Mean body weights decreased slightly in the 1× group over 7 days and to a slightly greater extent in the 3× and 5× groups (Fig. 5). Over the 14-day study period, there were 4, 1, 4, and 3 vomiting events and 1, 6, 21, and 25 abnormal feces occurrences, including dark or red stools and diarrhea or mucoid feces, in the placebo, 1×, 3×, and 5× groups, respectively. Abnormal feces in the 1× group were limited to days 1–3, whereas in the 3× and 5× groups, abnormal feces were more sporadic beginning at day 4 through the end of the study. Hypersalivation was seen in one dog in the 1× group on days 4 and 5, one dog in the 3× group on days 2 and 3, and three dogs in the 5× group on days 2–3 with one observation on day 10. Lacrimation was seen in one dog in the 3× group on day 2. Miosis was observed in four dogs in the 5× group on days 0 and 1.

Mean heart rates decreased in a dose-dependent manner for 2 days following dose administration and returned to rates similar to that in the placebo group from days 3 through 14 (Fig. 6). The maximal decrease in heart rate was observed in the 5× dose group and was an approximately 50% decrease relative to the placebo controls. Mean respiration rates were more variable than heart rates, but they appeared to decrease slightly in a dose-dependent manner for 2–3 days after dose administration (Fig. 7). However, unlike heart rate, the maximal decrease in the mean respiration rate was similar in both the

**Table 2.** Transdermal fentanyl solution pharmacokinetic parameters by dose group ($n$ = 6/group)

| | 1× (2.6 mg/kg) | | 3× (7.8 mg/kg) | | 5× (13.0 mg/kg) | |
|---|---|---|---|---|---|---|
| Parameter | Mean | SD | Mean | SD | Mean | SD |
| $C_{max}$ (ng/mL) | 3.18 | 0.85 | 7.27 | 1.39 | 13.5 | 4.12 |
| $t_{max}$ (h) | 16 | 6.20 | 28 | 21.0 | 28 | 22.3 |
| $AUC_{0–LLOQ}$ (ng·h/mL) | 323 | 80.5 | 824 | 258 | 1272 | 315 |
| $AUC_{0–\infty}$ (ng·h/mL) | 339 | 88.6 | 883 | 255 | 1333 | 326 |
| $AUC_{0–\infty}$ Extrapolated (%) | 4.74 | 3.10 | 7.13 | 3.14 | 4.63 | 2.03 |
| $t_{½}$ (h) | 71.2 | 15.1 | 75.8 | 11.2 | 72.3 | 15.1 |

SD, standard deviation.

**Table 3.** Mean sedation scores* by dose group ($n$ = 6/group) vs. time

| Time | | | | | |
|---|---|---|---|---|---|
| Day | Hour | Control | 1× (2.6 mg/kg) | 3× (7.8 mg/kg) | 5× (13.0 mg/kg) |
| 0 | 1 | 0 | 0 | 0.50 | 1.00 |
| 0 | 2.5 | 0 | 0 | 1.33 | 2.50 |
| 0 | 4 | 0 | 0.33 | 2.00 | 2.67 |
| 1 | 1 | 0 | 0.33 | 2.17 | 2.83 |
| 1 | 2.5 | 0 | 0.17 | 2.00 | 2.83 |
| 1 | 4 | 0 | 0.17 | 2.00 | 2.83 |
| 2 | 1 | 0 | 0 | 1.33 | 2.33 |
| 2 | 2.5 | 0 | 0 | 1.33 | 2.33 |
| 2 | 4 | 0 | 0 | 1.33 | 2.33 |
| 3 | – | 0 | 0 | 0.33 | 0.50 |
| 4 | – | 0 | 0 | 0.33 | 0.33 |
| 5 | – | 0 | 0 | 0.33 | 0 |
| 6 | – | 0 | 0 | 0 | 0 |
| 7 | – | 0 | 0 | 0 | 0 |
| 8 | – | 0 | 0 | 0 | 0 |
| 9 | – | 0 | 0 | 0 | 0 |
| 10 | – | 0 | 0 | 0 | 0 |
| 11 | – | 0 | 0 | 0 | 0 |
| 12 | – | 0 | 0 | 0 | 0 |
| 13 | – | 0 | 0 | 0 | 0 |
| 14 | – | 0 | 0 | 0 | 0 |

*0 = no sedation, 1 = slight, 2 = moderate, 3 = severe

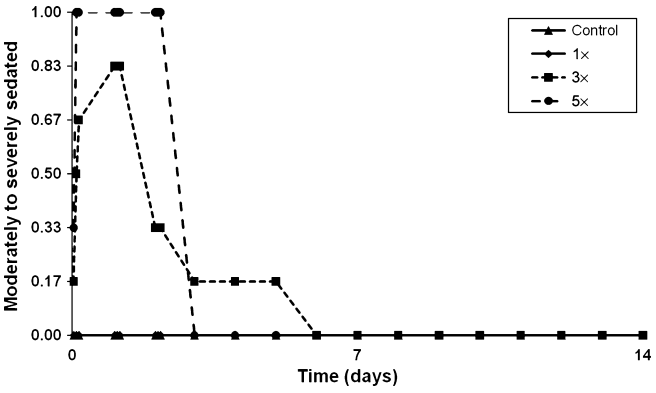


**Fig. 3.** Proportion of dogs moderately to severely sedated by dose group ($n$ = 6/group).

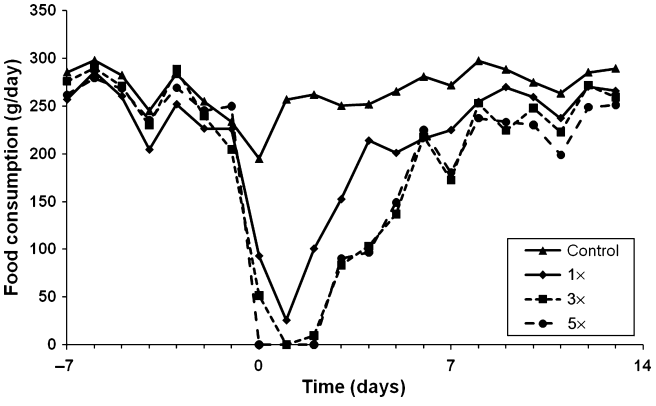


**Fig. 4.** Mean daily food consumption by dose group ($n$ = 6/group).

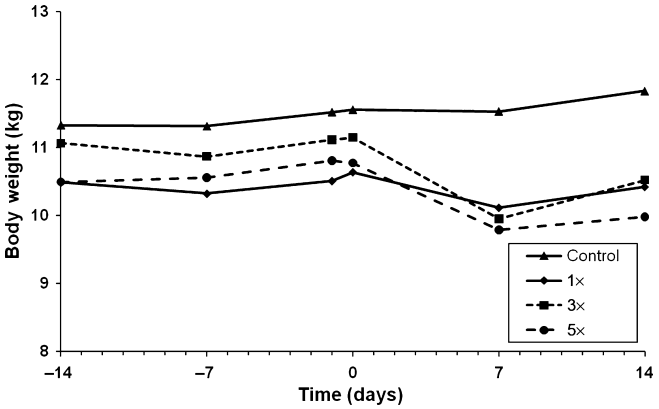


**Fig. 5.** Mean body weight by dose group ($n$ = 6/group).

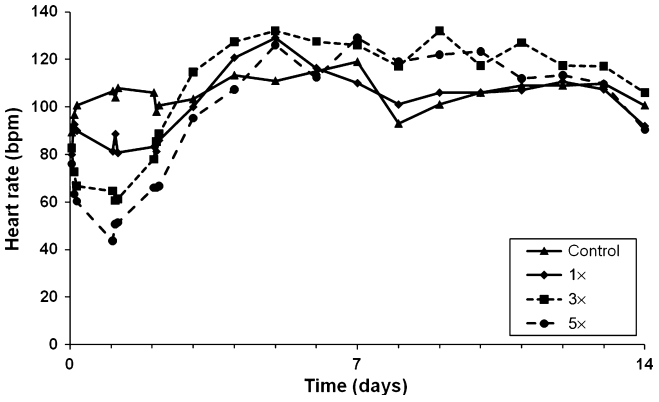


**Fig. 6.** Mean heart rate by dose group ($n$ = 6/group).

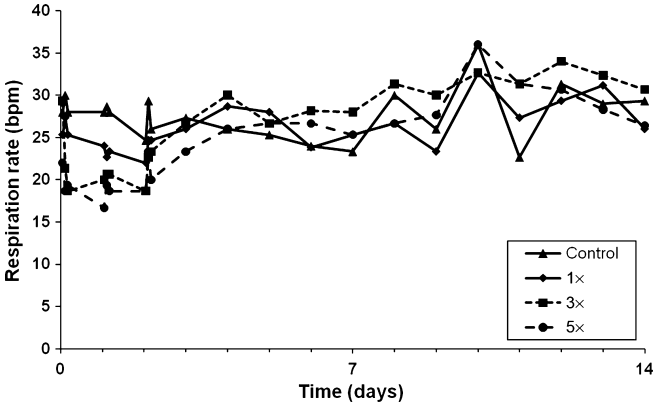


**Fig. 7.** Mean respiration rate by dose group ($n$ = 6/group).

3× and 5× dose groups at approximately 30%. The mean rectal body temperatures decreased in a dose-dependent manner and remained below the placebo control in all treated dose groups from 1 h postdose administration through day 3 or 4 (Fig. 8). The maximum drop in body temperature was approximately 2, 3, and 4 °C on day 1 in the 1×, 3×, and 5× groups, respectively.

Diffuse, bilateral ocular lens opacities were reported on day 3 in one 3× dog and three 5× dogs by the attending veterinarian.

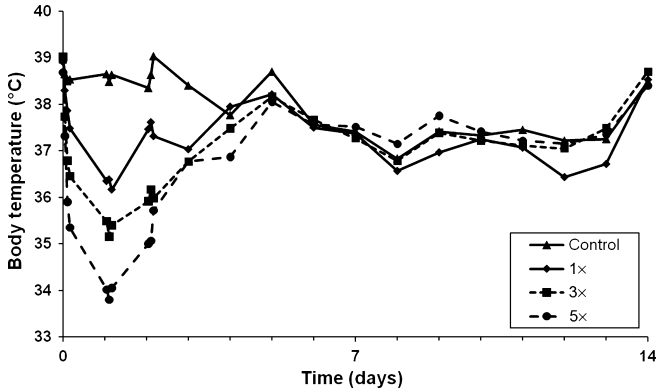


**Fig. 8.** Mean rectal body temperature by dose group ($n$ = 6/group).

A follow-up ophthalmic examination was conducted on day 7 by the attending veterinarian, and the ocular opacities were limited to one 3× group dog. A board-certified veterinary ophthalmologist was consulted to examine the dogs on day 8, and the day 7 findings were confirmed by biomicroscopy following pharmacologic mydriasis. The opacities were observed to involve the tips of the posterior lens sutures and were considered to be focal and incipient. By day 13, the lens opacities were not observed in the single 3× dog as confirmed by a veterinary ophthalmologist.

Prior to drug administration, negative P-waves that reflected physiologic, autonomically mediated variation in atrial activation or possibly, a brief paroxysm of idiojunctional tachycardia, were recorded from one study subject. Evaluation of all other ECG recordings disclosed sinus rhythm or sinus arrhythmia; pathologic arrhythmias were not identified from any study subject on day 3 or 13. A single PR interval that marginally exceeded the upper limit of the reference interval (133 msec, ref: 60–130 msec) was recorded prior to drug administration. All other recorded PR intervals were within the reference interval. All recorded heart rates and QT intervals were within reference intervals.

All mean hematology and serum chemistry results remained within the normal range. The mean blood urea nitrogen (BUN) on day 3 in both the 3× and 5× groups increased to 14.2 and 13.2 mg/dL, respectively, but remained within the normal range. By day 14, the BUN in both the 3× and 5× group was similar to day −7 values. There were no necropsy or histopathology findings considered to be related to fentanyl treatment. There were no gross or microscopic evidence of abnormalities at the skin application site. There were no gross or microscopic evidence of abnormalities in the eyes of the four dogs in the 3× and 5× groups that were observed with lens opacities over the first 7 days of the study.

## DISCUSSION

The results from this study demonstrate the margin of safety of transdermal fentanyl solution in healthy, laboratory dogs when administered at multiples of the therapeutic dose. Adverse reactions in the 1× group were infrequent and consistent with those associated with the therapeutic use of opioids and included a low incidence of mild sedation, reduced food intake, modest weight loss, and minimal reductions in heart rate and rectal temperature. All observations were transient and were primarily limited to the first 48 h following application of transdermal fentanyl solution. These effects increased in magnitude and duration in the 3× and 5× groups. Moderate to severe sedation emerged that was associated with a marked but temporary reduction in food and water intake necessitating maintenance fluid replacement for the first 2 days following application. Also observed in the higher-dose groups were an increased incidence of abnormal stools and transient posterior lens suture tip opacities. All abnormal health observations were completely resolved prior to necropsy on day 14. These data support the safe use of the 1× dose and describe the outcome of an overdose of up to 5× dose in the absence of opioid reversal.

The onset and duration of action of transdermal fentanyl solution is further supported by the results of this study. In the 1× group, mean plasma fentanyl concentrations achieved concentrations considered to be analgesic in humans of 0.2–1.2 ng/mL (Gourlay *et al.*, 1988) within approximately 2–4 h of application and were maintained at or above these concentrations for at least 4 days. Associated within this time frame were markers of opioid physiological effects that include mild sedation, reduced heart rate, and rectal temperature as well as diminished food intake. The pharmacokinetics of transdermal fentanyl solution were consistent with that previously reported to justify the dose of 1× (Freise *et al.*, 2012a,b). Drug exposure increased in proportion to dose as did the magnitude of opioid physiological effects. Systemic fentanyl exposure was ensured to be via transdermal absorption by the placement of collars to eliminate oral access to the application site.

Sedation is an expected extension of the pharmacological effect of opioids (Gutstein & Akil, 2006). Mild sedation was observed sporadically in some dogs in the 1× dose group over 48 h and with a greater magnitude and duration in the 3× and 5× groups. These observations are consistent with previous reports where sedation increased with plasma fentanyl concentrations when parenterally administered (Bailey *et al.*, 1987; Hendrix & Hansen, 2000). Sedation has been reported in dogs following fentanyl transdermal patch application as well when used at the recommended dose of 4 $\mu$g·kg/h (Hofmeister & Egger, 2004). At the higher doses of 3× and 5× used in this study, sedation was a dose-limiting adverse event in that it resulted in lack of food and water intake requiring maintenance fluid replacement over 2 days. This was unlikely the result of nausea as the emesis rate was no different in placebo- and fentanyl-treated dogs. In the 5× group, food intake was eliminated altogether over 48 h with a gradual return to baseline over 7 days. Food intake was sufficiently suppressed to cause a modest decrease in mean body weight. Inappetence has been described with fentanyl administration (Hofmeister & Egger, 2004), and the reduction in food intake may be a direct result of the drug, independent of sedation. A surgical standard of care is that dogs are typically fasted prior to surgery and are gradually offered increasing quantities of food over time postoperatively depending on the disease that necessitated surgery. Fentanyl-

induced reduced food intake may be superimposed on these postoperative care practices resulting in an under-awareness of this outcome.

The reversible bilateral lens opacities observed in one 3× and three 5× group dogs on days 3 and 7 were likely due to corneal drying caused by prolonged moderate to severe sedation. Although not reported in dogs, this change has been observed in rats that were anesthetized or that had corneal drying for any reason (Calderone *et al.*, 1986; personal communication with veterinary pathologist Dr. K. Regan, 2006). These changes are not cataracts because they are reversible. However, the changes can become irreversible if the condition(s) causing corneal drying are left unchecked. It was therefore considered likely that the extended sedation associated with 3× and 5× dosing in the present study resulted in transient corneal and lens drying, which in turn caused the reversible lens opacities. These conclusions are also supported by the lack of histopathologic findings in the lens. Although not observed in the 1× group, a prudent clinical practice is to use eye lubrication for a period of time until the normal palpebral reflex has been established following anesthesia.

Hypoventilation and respiratory depression were not dose-limiting adverse reactions in this study. This adverse event has been described in humans in association with patch-delivered fentanyl that has resulted in acute death. As a result, transdermal patches are contraindicated for use in conjunction with surgery and necessitate prior tolerance to opioids (Janssen Pharmaceutica Products, 2005). This is clearly not the case with transdermal fentanyl solution in dogs. Reductions in respiratory rates were transient and marginally dose dependent with a maximum reduction in rate of approximately 30% in the 3× and 5× groups over the first 48 h (Fig. 7). These observations are further supported by fentanyl data in dogs following injection and patch transdermal delivery. Plasma fentanyl concentrations as high as approximately 80 ng/mL reduce the respiratory rate by only approximately 11 breaths/min (50%) in spontaneously breathing dogs (Arndt *et al.*, 1984). Additionally, respiratory rate, oxygen consumption, and blood gases ($pCO_2$, $pO_2$, and pH) do not change further as concentrations increase above 100 ng/mL. Sustained, steady-state plasma fentanyl concentrations of approximately 2 ng/mL as delivered by a patch over 48 h do not cause postoperative hypoventilation as confirmed by blood gas analysis (Welch *et al.*, 2002). When taken together, there are no data to support the necessity of prior opioid tolerance or contraindication with anesthesia for transdermal fentanyl solution in dogs.

Mean heart rates decreased in a dose-dependent manner following transdermal fentanyl solution administration. Reduced heart rates have been reported with both parenteral and patch-delivered fentanyl. At plasma fentanyl concentrations of 15 ng/mL, heart rates decreased by approximately 35 beats/min (50%), and further decreases in heart rate were not observed when plasma fentanyl concentrations exceeded 15 ng/mL (Arndt *et al.*, 1984). The mean $C_{max}$ in the 5× group was 13.5 ng/mL, and the maximum drop in heart rate was approximately 50% at 24 h following dose application. Consistent with previous reports, pathologic arrhythmias were not associated with drug administration during the present study (Gardocki and Yelnosky, 1964).

Although reduced rectal temperature is discussed in general terms in the opioid literature for dogs, body temperature outcomes in conscious dogs over time have not been reported following fentanyl administration. Opioids appear to alter the equilibrium point of the hypothalamic heat regulatory mechanism resulting in reduced body temperature (Gutstein & Akil, 2006). The mean rectal body temperatures in this study decreased in a dose-dependent manner. In the 1× dose, a transient decrease in body temperature was observed with a maximum drop of 2 °C at 24 h following dosing. In anesthetized dogs, mean rectal temperatures decreased 0.9 °C at 60 min into mastectomy with an administered 30 $\mu$g·kg/h CRI of fentanyl throughout surgery (Steagall *et al.*, 2006), an infusion rate that is more than threefold the 1× dose (Freise *et al.*, 2012a), and was no different than placebo.

The appearance of abnormal feces that included discolored stools (dark or red), diarrhea, or mucoid feces increased with the dose of transdermal fentanyl solution over the 14-day study period. Abnormal feces in the 1× group were infrequent and limited to days 1–3, whereas in the 3× and 5× groups, abnormal feces were more sporadic beginning at day 4 through the end of the study. Opioids have been reported to diminish small intestinal secretions and decrease colonic propulsive peristaltic waves resulting in reduced, desiccated feces (Gutstein & Akil, 2006). The appearance of loose, mucoid, or dark stools later in the study in the 3× and 5× groups may be related to this phenomenon or may be related to a return to feeding after marked food reduction.

All mean clinical pathology results remained within the normal range throughout the study although the mean BUN on day 3 in both the 3× and 5× groups increased slightly from baseline. Creatinine did not show an increase in parallel with BUN, and there were no gross or histological lesions in the kidneys. This was most likely associated with reduced water intake secondary to sedation that necessitated fluid replacement. Simultaneous urine samples were not collected to confirm a prerenal association. Alternate possibilities are reduced urine output secondary to the release of antidiuretic hormone (ADH). High doses of fentanyl have been shown to have antidiuretic properties in the dog and are likely related to the release of ADH (Biswai *et al.*, 1976).

An important consideration in this laboratory evaluation in dogs is that there was no pain present. The presence of pain as stimulus is important in the evaluation of opioid safety in that the clinical safety of opioids is greater in the presence of pain. In human health, the idea that patients with more severe pain may tolerate 3–4 times greater doses of opioids is based in part on the observation of greater adverse events (e.g., respiratory depression) in nonpainful humans (Gutstein & Akil, 2006). Opioid adverse events in humans can be antagonized by pain, particularly decreased respirations (Eckenhoff & Oech, 1960). Similarly, in veterinary medicine, it has been suggested that clinical doses of opioids can be used safely in some species,

particularly when pain is present (Hall *et al.*, 2001). Taken together, this suggests that proper, safe use of opioids is predicated on administration to patients with sufficiently painful stimuli. Therefore, the full evaluation of the safety of transdermal fentanyl solution will depend on the outcome of well-controlled clinical studies in dogs experiencing pain.

## CONCLUSION

The results from this study demonstrate the margin of safety of transdermal fentanyl solution in healthy, laboratory dogs when administered at 1×, 3×, and 5× therapeutic dose of 2.6 mg/kg (50 μL/kg). Adverse reactions in the 1× group included a low incidence of mild sedation, reduced food intake, modest weight loss, and minimal reductions in heart rate and rectal temperature. These effects increased in magnitude and duration in the 3× and 5× groups resulting in the dose-limiting effects of sedation and decreased food and water intake. Moderate to severe sedation emerged that was associated with a marked but temporary reduction in food and water intake, necessitating maintenance fluid replacement for the first 2 days following application. Also observed in the higher dose groups were an increased incidence of abnormal stools and transient lens opacities. All abnormal health observations were completely resolved prior to necropsy on day 14, and there were no histological abnormalities identified. These data support the safe use of 1× dose of 2.6 mg/kg (50 μL/kg) and describe the outcome of an overdose of up to 5× dose where observations were consistent with known opioid effects in the absence of opioid reversal.

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## CONFLICTS OF INTEREST

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

## REFERENCES

Arndt, J.O., Mikat, M. & Parasher, C. (1984) Fentanyl's analgesic, respiratory, and cardiovascular actions in relation to dose and plasma concentration in unanesthetized dogs. *Anesthesiology*, **61,** 355–361.

Bailey, P.L., Port, J.D., McJames, S., Reinersman, L. & Stanley, T.H. (1987) Is fentanyl an anesthetic in the dog? *Anesthesia and Analgesia*, **66,** 542–548.

Biswai, A.V., Liu, W.S., Stanley, T.H., Bidwai, V., Loeser, E.A. & Shaw, C.L. (1976) The effects of large doses of fentanyl and fentanyl with nitrous oxide on renal function in the dog. *Canadian Anaesthetists' Society Journal*, **23,** 296–302.

Calderone, L., Grimes, P. & Shalev, M. (1986) Acute reversible cataract induced by xylazine and by ketamine-xylazine anesthesia in rats and mice. *Experimental Eye Research*, **42,** 331–337.

Carson, H.J., Knight, L.D., Dudley, M.H. & Garg, U. (2010) A fatality involving an unusual route of fentanyl delivery: chewing and aspirating the transdermal patch. *Legal Medicine (Tokyo)*, **12,** 157–159.

Eckenhoff, J.E., Oech, S.R. (1960) The effect of narcotics and antagonists upon respiration and circulation in man: a review. *Clinical Pharmacology and Therapeutics*, **1,** 483–524.

Egger, C.M., Duke, T., Archer, J. & Cribb, P.H. (1998) Comparison of plasma fentanyl concentrations by using three transdermal fentanyl patch sizes in dogs. *Veterinary Surgery*, **27,** 159–166.

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., Tudan, C., Clark, T.P. (2012a) 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. (2012b) 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.

Freye, E. (1974) Effect of high doses of fentanyl on myocardial infarction and cardiogenic shock in the dog. *Resuscitation*, **3,** 105–113.

Gardocki, J.F. & Yelnosky, J. (1964) A study of some of the pharmacologic actions of fentanyl citrate. *Toxicol Appl Pharmacol*, **6,** 48–62.

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.

Gourlay, G.K., Kowalski, S.R., Plummer, J.L., Cousins, M.J. & Armstrong, P.J. (1988) Fentanyl blood concentration-analgesic response relationship in the treatment of postoperative pain. *Anesthesia and Analgesia*, **67,** 329–337.

Gutstein, H.B. & Akil, H. (2006) Opioid analgesics. In *Goodman & Gilman's The Pharmacological Basis of Therapeutics*, 11th edn. Eds Brunton, L.L., Lazo, J.S. & Parker, K.L., pp. 547–590. McGraw-Hill, New York, NY.

Hall, L.W., Clarke, K.W. & Trim, C.M. (2001). *Veterinary Anaesthesia*. W.B Saunders, London, Edinburgh, New York, Philadelphia, St. Louis, Sydney, Toronto.

Hendrix, P.K. & Hansen, B. (2000) Acute pain management. In *Kirk's Current Veterinary Therapy XIII Small Animal Practice*. Ed Bonagura, J.D., pp. 57–61. W.B. Saunders Company, Philadelphia, PA.

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

Holt, D.V., Viscusi, E.R. & Wordell, C.J. (2007) Extended-duration agents for perioperative pain management. *Current Pain and Headache Reports*, **11,** 33–37.

Janssen Pharmaceutica Products, L.P. (2005). *Duragesic (Fentanyl Transdermal System) Full Prescribing Information*. Janssen Pharmaceutica Products, L.P., Titusville, 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.

Lafuente, M.P., Franch, J., Durall, I., Diaz-Bertrana, M.C. & Marquez, R.M. (2005) Comparison between meloxicam and transdermally administered fentanyl for treatment of postoperative pain in dogs undergoing osteotomy of the tibia and fibula and placement of a uniplanar external distraction device. *Journal of the American Veterinary Medical Association*, **227,** 1768–1774.

Liu, W., Bidwai, A.V., Stanley, T.H. & Isern-Amaral, J. (1976) Cardiovascular dynamics after large doses of fentanyl and fentanyl plus N2O in the dog. *Anesthesia and Analgesia*, **55,** 168–172.

Lowenstein, E. (1971) Morphine ''anesthesia'' – a perspective. *Anesthesiology*, **35,** 563–565.

Marquardt, K.A., Tharratt, R.S. & Musallam, N.A. (1995) Fentanyl remaining in a transdermal system following three days of continuous use. *Annals of Pharmacotherapy*, **29,** 969–971.

Mills, P.C., Magnusson, B.M. & Cross, S.E. (2004) Investigation of in vitro transdermal absorption of fentanyl from patches placed on skin samples obtained from various anatomic regions of dogs. *American Journal of Veterinary Research*, **65,** 1697–1700.

Pascoe, P.J. (2000) Opioid analgesics. *The Veterinary clinics of North America. Small animal practice*, **30,** 757–772.

Pettifer, G.R. & Hosgood, G. (2004) The effect of inhalant anesthetic and body temperature on peri-anesthetic serum concentrations of transdermally administered fentanyl in dogs. *Veterinary Anaesthesia and Analgesia*, **31,** 109–120.

Riviere, J.E. & Papich, M.G. (2001) Potential and problems of developing transdermal patches for veterinary applications. *Advanced Drug Delivery Reviews*, **50,** 175–203.

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.

Schmiedt, C.W. & Bjorling, D.E. (2007) Accidental prehension and suspected transmucosal or oral absorption of fentanyl from a transdermal patch in a dog. *Veterinary Anaesthesia and Analgesia*, **34,** 70–73.

Smith, L.J., Krugner-Higby, L., Trepanier, L.A., Flaska, D.E., Joers, V. & Heath, T.D. (2004) Sedative effects and serum drug concentrations of oxymorphone and metabolites after subcutaneous administration of a liposome-encapsulated formulation in dogs. *Journal of Veterinary Pharmacology and Therapeutics*, **27,** 369–372.

Stanley, T.H. (1992) The history and development of the fentanyl series. *Journal of Pain and Symptom Management*, **7,** S3–S7.

Steagall, P.V., Teixeira Neto, F.J., Minto, B.W., Campagnol, D. & Correa, M.A. (2006) Evaluation of the isoflurane-sparing effects of lidocaine and fentanyl during surgery in dogs. *Journal of the American Veterinary Medical Association*, **229,** 522–527.

Urquhart, J. (2000) INTERNAL MEDICINE IN THE 21ST CENTURY: controlled drug delivery: therapeutic and pharmacological aspects. *Journal of Internal Medicine*, **248,** 357–376.

Welch, J., Wohl, J. & Wright, J. (2002) Evaluation of postoperative respiratory function by serial blood gas analysis in dogs treated with transdermal fentanyl. *Journal of Veterinary Emergency and Critical Care*, **12,** 81–87.

Wilson, D., Pettifer, G.R. & Hosgood, G. (2006) Effect of transdermally administered fentanyl on minimum alveolar concentration of isoflurane in normothermic and hypothermic dogs. *Journal of the American Veterinary Medical Association*, **228,** 1042–1046.