

PHARMACOKINETICS OF A SINGLE SUBCUTANEOUS DOSE OF SUSTAINED RELEASE BUPRENORPHINE IN NORTHERN ELEPHANT SEALS (*MIROUNGA ANGUSTIROSTRIS*)

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Abstract: Information regarding analgesics in pinnipeds is limited. This study aimed to establish the pharmacokinetic parameters of a single subcutaneous dose of sustained release buprenorphine (*Buprenorphine SR*[®]) in juvenile northern elephant seals (*Mirounga angustirostris*) with regard to its potential to provide long-lasting analgesia that requires infrequent dosing. Seals ($n = 26$) were administered a single dose of sustained release buprenorphine at 0.12 mg/kg s.c. Blood samples were collected from the extradural intervertebral vein at 0 hr and at three or four of the following time points: 0.5, 1, 2, 6, 12, 24, 36, 48, 60, 96, 120, and 144 hr. Seals were examined daily for systemic and local adverse reactions. Plasma was analyzed by liquid chromatography tandem-mass spectrometry for buprenorphine and norbuprenorphine concentrations. A noncompartmental analysis for pharmacokinetic parameters was calculated using standard methods and equations. An average maximum concentration of 1.21 ng/ml (0.3–2.9 ng/ml) was detected 12 hr postadministration. Concentrations were quantifiable up to 144 hr postadministration but were below those expected to provide analgesia in some other species. No systemic adverse effects were noted in healthy seals receiving sustained release buprenorphine. Cellulitis or abscesses at the injection site were observed in 6/26 (23%) seals between 24 and 168 hr postadministration. Adverse local effects suggest that this drug should be used with caution in northern elephant seals.

Key words: Analgesia, marine mammal, *Mirounga angustirostris*, northern elephant seal, opioid, sustained release buprenorphine.

INTRODUCTION

Little information exists regarding analgesics in pinnipeds. However, seals, sea lions, and walrus may experience potentially painful clinical conditions, such as soft tissue and orthopedic trauma; surgical and postoperative pain; ocular discomfort associated with corneal disease and glaucoma; integumentary lesions, including branding and ulcerative northern elephant seal skin disease; and neoplasia, including urogenital carcinoma.^{3,9,10,15,19,23,24,30,48–50} Pinnipeds exposed to oil may also experience pain due to intramyelinic edema,

axonal swelling and degeneration, severe conjunctivitis, corneal ulcers, and abrasions.^{14,16}

Opioids are commonly used as analgesics in domestic dogs and cats and are used empirically in pinnipeds.¹¹ Very little information exists regarding the pharmacokinetics or pharmacodynamics of opioids as primary analgesics in any pinniped species, and what information exists is limited to preliminary pharmacokinetic studies evaluating butorphanol in northern elephant seals (*Mirounga angustirostris*) and tramadol in California sea lions (*Zalophus californianus*).^{4,36} Despite this paucity of information, butorphanol, a μ -antagonist and κ -agonist, remains the most common opioid analgesic used at The Marine Mammal Center, a rehabilitation facility in Sausalito, California, that admitted more than 500 pinnipeds in 2012.⁴⁶ At this institution, butorphanol is administered at dosages ranging between 0.1 to 0.2 mg/kg i.m. two to four times daily in California sea lions and 0.05 mg/kg i.m. two to four times daily in northern elephant seals. Repeated restraint for injections is stressful for the animals and labor intensive for staff and volunteers. Although there are no pharmacodynamic studies evaluating its analgesic efficacy in pinnipeds, butorphanol is considered weakly

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analgesic in domestic dogs and cats, with a relatively short duration of effect spanning a few hours.^{7,29,37,40}

The opioid buprenorphine, a partial μ -agonist, is known to have anti-nociceptive effects in multiple species, including domestic dogs and cats, at various dosages and routes of administration.^{18,26,39–42,44,45} A plasma concentration of 1.0 ng/ml or greater is expected to produce analgesia in multiple species, although the minimum plasma concentration at which anti-nociceptive effects exist varies between species and by evaluation methodology.^{1,6,12,13,26,33,35,39,43,51–53} In domestic dogs and cats, buprenorphine provides relatively long-lasting analgesia, with few adverse effects reported.²⁸ Given the potential to provide pain relief in domestic carnivores, it is reasonable to consider its use as a primary analgesic in aquatic carnivores, including pinnipeds.

A compounded sustained release buprenorphine (*Buprenorphine SR*[®], Wildlife Pharmaceuticals, Windsor, Colorado 80550, USA) containing a proprietary biodegradable liquid polymer matrix is commercially available.⁵³ Although this product is not approved by the Food and Drug Administration (FDA), it has been evaluated in several studies and is anecdotally reported to be successful in some nondomestic species.^{8,13,35} Recently, the FDA approved a formulation of sustained release buprenorphine for injectable use in domestic cats (*Simbadol*, NADA 141–434, Abbott Laboratories, Abbott Park, Illinois 60064, USA); however, this product is not yet commercially available.³² In laboratory rats, sustained release buprenorphine administered at 1.2 mg/kg s.c. provided analgesia for 2–3 days with plasma concentrations of greater than 1.0 ng/ml.¹³ A study in domestic cats undergoing ovariohysterectomy compared buprenorphine administered every 12 hr oral-transmucosally at 0.02 mg/kg and sustained release buprenorphine administered at 0.12 mg/kg s.c. administered once. These two methods produced similar analgesia and adverse effect profiles.⁸ In macaques, sustained release buprenorphine at 0.2 mg/kg s.c. resulted in plasma concentrations believed to be analgesic for a 5-day duration.³⁵

Initial studies on this drug are promising and represent a potentially long-lasting, effective analgesic drug choice for pinnipeds that requires infrequent dosing. The goals of this study were to establish the pharmacokinetic parameters of a single subcutaneous dose of sustained release buprenorphine in juvenile northern elephant seals, evaluate clinical safety, and compare

achieved plasma concentrations to those of other species in which analgesic plasma concentrations are described.

MATERIALS AND METHODS

Study design

This project was approved by The Marine Mammal Center Animal Care and Use Committee (2013-001). Twenty-six healthy juvenile, weaned male ($n = 12$) and female ($n = 14$) northern elephant seals undergoing rehabilitation at The Marine Mammal Center (Sausalito, California, USA) were included in this study. Seals were identified as healthy based on observations of normal behavior, physical examination, complete blood count, and serum biochemistry profile using in-house reference ranges. All seals were co-housed with age-matched conspecifics with access to a saltwater pool and concrete deck area. Herring was meal fed two to three times daily, with fresh water offered ad libitum. Seals continued to receive a multivitamin supplement (Pinnivites, Mazuri, Richmond, Indiana 47374, USA), but they received no other medications during or within 1 wk prior to the study period.

A single dose of sustained release buprenorphine at 0.12 mg/kg s.c. (10 mg/ml, *Buprenorphine SRR*, Wildlife Pharmaceuticals) was calculated based on body weight obtained within 7 days of administration (mean body weight = 57.6 kg \pm 5.9 kg). The drug was stored refrigerated, as per label instructions, and was drawn up using a 20-ga, 1-inch needle (Kendall, Mansfield, Massachusetts 02048, USA) on a 1-ml Luer lock syringe (Terumo Medical Corporation, Somerset, New Jersey 08870, USA) after warming to room temperature. Seals were manually restrained for drug administration. The skin over the caudal right dorsal pelvis was sprayed with betadine prior to injection, and the skin was tented as the needle was fully inserted under the skin. Care was taken to pinch the skin over the injection site while removing the needle after administration. Although the drug is labeled for a subcutaneous interscapular injection, this was not deemed possible given the method of manual restraint, with a restrainer positioned over the thorax and interscapular space while straddling the thoracic flippers and controlling the head of the seal.

Seals were manually restrained for venipuncture of the extradural intervertebral vein for each assigned collection time point.²³ A 20-ga, 1.5-inch needle (Kendall) attached to a BD Vacutainer[®] holder (Becton, Dickinson and Company, Frank-

lin Lakes, New Jersey 07317, USA) was used to collect blood into 7.5% ethylenediamine tetraacetic acid tubes (Monoject, Kendall). Less than 1% of body weight was collected in total from each seal over the duration of the study. Collection times for each seal included a baseline ($t = 0$) sample that was collected up to 7 days prior to sustained release buprenorphine administration. Seals were randomly assigned three or four predetermined collection time points according to a population pharmacokinetic matrix, which was based on a previous pinniped population pharmacokinetic study.²⁷ The original collection schedule allowed for collection for up to 72 hr but was extended based on pilot data to the following collection times: 0.5, 1, 2, 6, 12, 24, 36, 48, 60, 72, 96, 120, and 144 hr. A total of six complete data sets were generated, with six to eight individual samples included at each time point.

Blood samples were centrifuged at 1,378 *g* for 10 min utilizing a Rotofix 32A centrifuge (Hettich Lab Technology, Beverly, Massachusetts 01915, USA), and plasma was transferred via disposable plastic pipettes into individual cryovials (Fisher Scientific, Pittsburgh, Pennsylvania 15275, USA) within 1 hr of collection. Plasma samples were stored at -80°C until processing.

Seals were assessed for systemic and local adverse effects daily for the duration of the study period. Seals demonstrating systemic adverse effects, including but not limited to sedation, lethargy, euphoria, or anorexia, were antagonized with naloxone at 0.04 mg/kg *i.m.*, as needed (0.4 mg/ml; Hospira, Inc., Lake Forrest, Illinois 60045, USA) and excluded from the study. Seals demonstrating local adverse effects at the injection site were treated at the discretion of The Marine Mammal Center's attending veterinarian, but they were not excluded from the study unless antagonized with naloxone or treated with other drugs, such as antibiotics.

Measurement of plasma buprenorphine and norbuprenorphine

Buprenorphine and norbuprenorphine (Cerilliant Corporation, Round Rock, Texas 78665, USA) working solutions were prepared by dilution of the 1 mg/ml stock solutions with methanol (Fisher Scientific) to concentrations of 0.001, 0.01, 0.1, 1, and 10 ng/ μl . Plasma calibrators were prepared by dilution of the working standard solutions with drug-free northern elephant seal plasma to concentrations of 0.1, 0.25, 0.5, 1, 2, 4, 7, and 10 ng/ml. Calibration curves and negative control samples were prepared fresh for each

quantitative assay. In addition, quality control samples (plasma fortified with different analyte working solutions at two concentrations within the standard curve) were included with each sample set as an additional check of accuracy. Prior to analysis, 200 μl of plasma was diluted with 2.5 ml of 0.1 M, pH 6 phosphate buffer and 100 μl of water containing *d*₄-buprenorphine or *d*₃-norbuprenorphine internal standard (Cerilliant Corporation) at 40 ng/ml.

The samples were vortexed briefly to mix and were subjected to solid phase extraction using CUC18 3-ml, 200-mg Clean-Up Extraction Columns (United Chemical Technologies, Inc., Bristol, Pennsylvania 19007, USA). In brief, the columns were rinsed with 2.5 ml of methanol and then 3 ml of water; samples were loaded slowly onto the columns and then washed with 2 ml of 50% methanol in water (Burdick and Jackson, Muskegon, Michigan 49442, USA) prior to elution with 2.5 ml of 100% methanol. Samples were dried under nitrogen and dissolved in 120 μl of 10% acetonitrile (ACN; Burdick and Jackson) in water, both with 0.2% formic acid (Alfa Aesar, Ward Hill, Massachusetts 01835, USA), with vortexing and brief sonication. Forty microliters were injected into the liquid chromatography-mass spectrometry (LC-MS) system.

The concentration of buprenorphine and norbuprenorphine was measured in plasma by liquid chromatography tandem-mass spectrometry (LC-MS/MS) using positive electrospray ionization [ESI(+)]. Quantitative analysis of plasma was performed on a Quantum Ultra triple quadrupole mass spectrometer (Thermo Scientific, San Jose, California 95134, USA) with a 1100 series liquid chromatography system (Agilent Technologies, Palo Alto, California 94306, USA). The spray voltage was 4,000 V. The sheath and auxiliary gas were 40 and 10, respectively (arbitrary units). Product masses and collision energies of each analyte were optimized by infusing the standards into the mass spectrometer. Chromatography employed an ACE 3 C18 10-cm by 2.1-mm column (Mac-Mod Analytical, Chadds Ford, Pennsylvania 19317, USA) and a linear gradient of ACN in water, with a constant 0.2% formic acid at a flow rate of 0.40 ml/min. The initial ACN concentration was held at 10% for 0.2 min, ramped to 95% over the course of 4.3 min, then held at that concentration for 0.20 min, before re-equilibrating for 2.8 min at initial conditions.

Detection and quantification were conducted using Selective Reaction Monitoring of initial precursor ion for buprenorphine (mass to charge

ratio [$m : z$] 468.3), norbuprenorphine ([$m : z$] 414.2), and the internal standards d_4 -buprenorphine ([$m : z$] 472.3) and d_3 -norbuprenorphine ([$m : z$] 417.3). The response for the product ions for buprenorphine ([$m : z$] 414.2, 396.2) and the internal standards d_4 -buprenorphine ([$m : z$] 400.2, 415.3) and d_3 -norbuprenorphine ([$m : z$] 210.9, 237.0) were plotted, and peaks at the proper retention time were integrated using Quanbrowser software (Thermo Scientific). Quanbrowser software was used to generate calibration curves and to quantitate buprenorphine and norbuprenorphine in all samples by linear regression analysis. A weighting factor of $1/X$ was used for all calibration curves.

The response for buprenorphine and norbuprenorphine was linear and gave correlation coefficients [R^2 values] of 0.99 or better. The intraday, interday, analyst-to-analyst precision and accuracy of the assay were determined by assaying quality control samples in replicates ($n = 6$) for buprenorphine and norbuprenorphine. Accuracy was reported as percent nominal concentration, and precision was reported as percent relative standard deviation. The technique was optimized to provide a limit of quantitation of 0.1 ng/ml and a limit of detection of approximately 0.05 ng/ml for both buprenorphine and norbuprenorphine.

Pharmacokinetic analysis

Naïve pooling of datum points was used to combine data from different elephant seals at each time point.¹⁷ Nonlinear least squares regression was performed on plasma buprenorphine concentration at each time point using commercially available software (Phoenix WinNonlin Version 6.0, Pharsight, Cary, North Carolina 27518, USA). Noncompartmental analysis for sparse data was used for determination of the pharmacokinetic parameters.

RESULTS

Buprenorphine and norbuprenorphine were not detected in any baseline samples. Quantifiable plasma buprenorphine concentrations were measured up to 144 hr postadministration. Mean buprenorphine plasma concentrations increased to 1.0 ng/ml (0.2–2.8 ng/ml) at 2 hr but then decreased to 0.8 ng/ml (0.2–2.0 ng/ml) at 6 hr before peaking at 12 hr. The average maximum concentration of buprenorphine, 1.21 ng/ml (0.3–2.9 ng/ml), was detected 12 hr postadministration. Mean plasma concentrations decreased to less than 1.0 ng/ml (0.2–2.1 ng/ml) in most seals

after 24 hr. Two seals had plasma concentrations of greater than 1.0 ng/ml at 48 hr postadministration, but no plasma concentrations greater than 1.0 ng/ml were detected after that point. A high degree of individual variation in plasma concentration was noted at all time points, with more variation noted between 0.5 and 48 hr and less variation noted between 60 and 144 hr (Fig. 1; Table 1). Norbuprenorphine plasma concentrations remained below the limit of quantification at all time points sampled.

Systemic adverse effects were noted in one seal, which showed subtle but progressive signs of sedation, lethargy, and anorexia noted within 1 hr of sustained release buprenorphine administration. This seal was monitored for several hours and then administered a single dose of naloxone at 0.04 mg/kg i.m. 9 hr after sustained release buprenorphine injection. This animal was excluded from the study. Blood was collected at 1 hr post-sustained release buprenorphine injection, prior to observation of progressive clinical signs. This sample was analyzed and the buprenorphine plasma concentration was 0.4 ng/ml, which is within the range of concentrations achieved by other seals. No other blood samples were collected from this animal for this study. This animal was euthanized within 24 hr of naloxone administration and was found to have an *Otostrongylus circumlitus* infestation on gross postmortem examination. No other systemic adverse effects were noted in any seals.

Local adverse effects were noted in 6/26 (23%) seals between 24 and 168 hr post-sustained release buprenorphine administration. These included cellulitis or abscess formation at the injection site. The sites were evaluated by fine needle aspiration and cytology, which revealed variably nonproductive to serohemorrhagic or purulent material. Cellulitis was monitored and abscesses were lanced and flushed with a combination of betadine and sterile saline, occasionally requiring multiple cytologic evaluations and treatments. Naloxone and systemic antibiotics were not deemed necessary, and no seals were excluded from the study as a result of local reactions. Vials of sustained release buprenorphine used in the affected seals were cultured for aerobic and anaerobic bacteria and were found to be sterile. An injection site abscess from one seal, but from no others, was cultured for aerobic and anaerobic bacteria, which grew *Psychrobacter phenylpyruvicus*. No seals demonstrated adverse effects in relation to multiple venipuncture events.

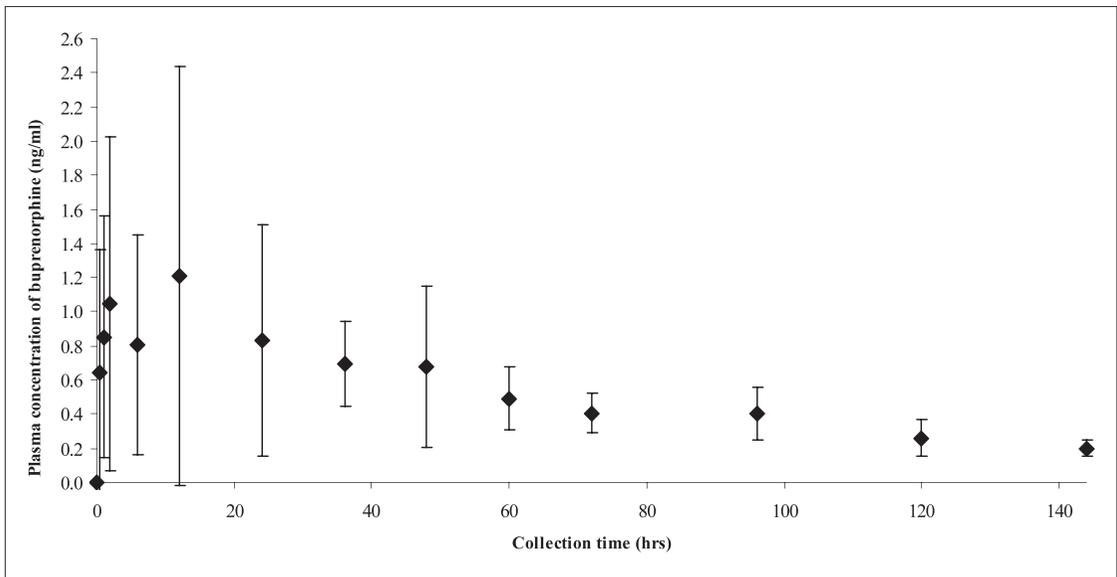


Figure 1. Mean population concentrations of buprenorphine following a single dose of sustained release buprenorphine administered at 0.12 mg/kg s.c. in juvenile northern elephant seals. Each time point represents the average of at least six individual seals, and bars represent the standard deviation. The standard deviation is wide because of a high degree of individual variation in plasma concentration.

All seals included in the study were clinically normal after the conclusion of the sampling period and injection site treatments, though 3/6 (50%) with local reactions retained small, firm, nonproductive nodules presumed to be fibrous tissue. These seals were released from rehabilitation following The Marine Mammal Center's standard protocols.

DISCUSSION

Information on analgesics in pinnipeds is scarce and is limited to a few studies, despite recognition of multiple potentially painful conditions. A previous study evaluated the effects of nonsteroidal anti-inflammatory drugs (NSAIDs) in Steller sea lions (*Eumetopias jubatus*) by treating pain associated with abdominal surgery with single doses of either flunixin meglumine 1 mg/kg or

carprofen at 4.4 mg/kg i.m. administered once, and then by mouth every 24 hr for three more doses. The study⁴⁹ suggested that neither drug at the administered dose provided effective analgesia based on behavioral observations. Opioids previously studied include butorphanol at 0.055 mg/kg i.m. in northern elephant seals and tramadol at 2 mg/kg p.o. in California sea lions.^{4,36} Both drugs were found to have a short duration of detectable plasma concentrations, lasting 5 and 24 hr, respectively.^{4,36} No study has evaluated the pharmacodynamics of any analgesic in a pinniped species. However, behavior, heart rate, and respiratory rate have been assessed in response to abdominal surgery and iron branding in Steller sea lions.^{49,50} Therefore, dosages for both NSAIDs and opioids in clinical practice are empirical or based on anecdotal evidence. The sustained release buprenorphine dosage of 0.12 mg/kg s.c. was chosen for elephant seals based on the compounder's recommendation for the domestic cat and the low end of the domestic dog dosage range, as published at the start of this study.⁵² Initially, the recommended dosage for domestic dogs was 0.12–0.27 mg/kg s.c. This has been revised to a lower dosage of 0.03–0.06 mg/kg s.c. to avoid the depression seen at higher doses.⁵³ The current recommended dosage for domestic cats is 0.12 mg/kg s.c.⁵³ Other current recommended

Table 1. Mean population pharmacokinetic parameters of buprenorphine following a single dose of sustained release buprenorphine administered at 0.12 mg/kg s.c. in juvenile northern elephant seals ($n = 26$).

Pharmacokinetic parameter	Buprenorphine
T_{max} (hr)	12
C_{max} (ng/ml)	1.21
C_{last} (ng/ml)	0.2
AUC_{0-inf} (hr \times ng/l)	93.8

compounder dosages are 1.0–1.2 mg/kg s.c. for rats and 0.5–1.0 mg/kg s.c. for mice.⁵³

Sustained release buprenorphine has been evaluated in several other species. In laboratory rats, a single 1.2 mg/kg s.c. dosage resulted in plasma concentrations of 1.0 ng/ml or greater for 72 hr and correlated with 2–3 days of analgesia.¹³ In domestic cats undergoing ovariohysterectomy, a single 0.12 mg/kg s.c. dose resulted in a similar analgesic efficacy and adverse effect profile to 0.02 mg/kg oral-transmucosal doses administered every 12 hr for 72 hr.⁸ In domestic dogs, a pilot study⁵² found that a single dose of sustained release buprenorphine at 0.27 mg/kg s.c. resulted in plasma concentrations of 1.0 ng/ml or greater for a variable duration up to 72 hr. In two species of macaque (*Macaca mulatta* and *Macaca fascicularis*), a single 0.2 mg/kg s.c. dose resulted in plasma concentrations of greater than 0.1 ng/ml for a duration of 5 days.³⁵ No study has been published evaluating multiple doses of sustained release buprenorphine.

No pharmacodynamic parameters were tested in this or any other study for northern elephant seals or any pinniped species. Analgesic plasma concentrations have been published for various species based on anti-nociceptive testing and analgesic demand studies. In humans a minimal plasma concentration of buprenorphine of 0.1 ng/ml with a targeted concentration of 0.5–0.7 ng/ml is expected to provide analgesia.^{6,12,51} Macaques are presumed to be similar to humans and are expected to have a similar minimum analgesic plasma concentration of 0.1 ng/ml.³⁵ In laboratory rats a plasma concentration of 1.0 ng/ml or greater provided analgesia.¹³ In domestic cats a plasma concentration of 0.5–1.0 ng/ml is expected to provide analgesia based on prospective and pilot data, compared to a range of 0.1 to 1.0 ng/ml in domestic dogs.^{1,26,39,43,52} In domestic sheep a plasma concentration of 0.7–1.0 ng/ml is expected to provide analgesia.³³ However, these values must be interpreted with great caution, as there is individual variation in analgesia and analgesic plasma concentrations based on degree and type of pain, as well as differences in analysis methodology between the studies. Based on studies in other species it may be reasonable to suspect that seals experience analgesia at plasma concentrations above 1.0 ng/ml. In this study, mean maximum plasma concentrations reached 1.21 ng/ml by 12 hr and then declined below that concentration for most individuals by 24 hr, with a high degree of variation. Although plasma concentrations were below 1.0 ng/ml for the majority

of the study, the pharmacodynamics of this drug were not assessed, and it is possible that some seals experienced analgesia at lower plasma concentrations as well. It is important to note that no definitive interpretations on analgesic efficacy can be made based on this study at any time point. Future studies evaluating the direct anti-nociceptive properties of buprenorphine in northern elephant seals are needed.

Variation in plasma concentrations detected may be due to the unique anatomy and physiology of phocid seals evolved for diving. Phocid integument includes an epidermis, dermis, and hypodermis or subcutaneous tissue, which includes a thick layer of adipose or blubber.²¹ All of these tissues are highly vascular and include arteriovenous anastomoses, which play a role in thermoregulation.^{21,31} Injections of sustained release buprenorphine were given in the subcutaneous tissue or blubber layer. Given the high degree of vascularity of this tissue, sequestration of drug in blubber is considered unlikely. However, it is speculated that the rate of absorption into the circulation could be altered secondary to body temperature, ambient environment, whether the seal remained on land or water postinjection, and peripheral vasoconstriction, as the seal regulated core body temperature.^{21,31} Correlations between these factors and plasma concentrations were beyond the scope of this study. The exact venous drainage route from the injection site is unknown, but it is presumed that drainage from the blubber layer would eventually enter the extradural intervertebral vein via a vascular network and not directly.³⁸ Therefore, injection site location is considered an unlikely factor in resultant plasma concentrations. The extradural intervertebral vein has a vertical intralaminar septum and is located dorsal to the spinal cord and inside, with close association to the walls of the vertebrae composing the spinal canal.³⁴ It is a common site for venipuncture in phocids and is accessed between caudal lumbar vertebrae. The vessel is joined by other primarily valveless veins and connects with the hepatic sinus, dorsal spinal muscle veins, posterior vena cava, renal venous plexus, anterior vena cava, thoracic vena cava, and the associated connecting veins.³⁸ The extradural intervertebral vein has highly variable blood velocity and flow, which are presumed to serve as adaptations for diving, transporting large volumes of blood rapidly, maintaining cardiac output, and regulating blood pressure.^{34,38} Although blood flow decreases during diving, overall changes in velocity and blood flow direction are difficult to predict

before, after, and during diving.³⁴ Blood is anecdotally reported to pool with little active circulation, which has clinical implications in terms of evaluating blood work or administering drugs intravenously into this vessel.⁴⁷ It is possible that buprenorphine metabolites stagnated within this vein, resulting in an artificially elevated measurement of concentration compared to peripheral circulation in some seals. Conversely, it is possible that buprenorphine metabolites were shunted away from the venipuncture site, resulting in artificially low measurements of concentration compared to peripheral circulation. This may account for some of the analogously high concentrations and variation observed between individual seals at some collection time points. However, without comparing buprenorphine concentrations from blood collected from the extradural intervertebral vein and a peripheral vein simultaneously, any effect of the extradural intervertebral vein is speculative. Other venipuncture sites available for sampling in phocids under manual restraint include the plantar interdigital veins of the hind flippers, but these may yield an arterial-venous mixture, require prolonged manual pressure post-venipuncture to prevent hemorrhage, are challenging to collect large volumes from, and were considered impractical for the purposes of this study.²³

One seal demonstrated adverse systemic effects following sustained release buprenorphine administration. This seal was diagnosed with an *Otostrongylus circumlitus* infestation that can result in a rapid decline in condition and death.²² Despite antagonism with naloxone, the animal continued to demonstrate sedation, which the authors believe is a factor of the disease process and not the sustained release buprenorphine administration, as no other seals were sedate during the study. However, incomplete or short duration of antagonism with naloxone cannot be ruled out. Naloxone is an opioid antagonist that has a short duration of effect, generally lasting less than 1 hr when administered intravenously.²⁸ Moreover, buprenorphine has a high affinity for and slow dissociation from μ -opioid receptors, which makes it difficult to antagonize.²⁸ The protocol for this study allowed for multiple doses of naloxone, as clinically indicated, though this seal received only a single dose prior to euthanasia. Although the animal appeared clinically normal prior to drug injection, the potential sedative effect of the drug that was unappreciated in healthy seals may have unmasked more subtle clinical signs associated with the disease process.

In other studies^{8,13,34} evaluating sustained release buprenorphine in domestic cats, macaques, and laboratory rats no systemic adverse effects were noted.

Multiple seals 6/26 (23%) developed adverse local reactions at the sustained release buprenorphine injection site in the form of cellulitis and abscesses. In domestic cats, 1/11 (9%) developed an injection site reaction in the form of a small scab 1 wk after administration; this scab resolved without intervention.⁸ In macaques, 4/10 (40%) developed injection site reactions ranging from mild erythema to a raised pink plaque, which resolved without intervention.³⁵ In laboratory rats, when sustained release buprenorphine was administered a small portion of the drug seeped out of the injection site upon withdrawal of the needle. This resulted in erythema and scabbing of the skin within 24 hr. After this was noted modifications to the drug administration technique in rats were made to mitigate adverse reactions, including skin tenting for the entire duration of the injection, slow needle withdrawal, and pinching of the skin for approximately 15 sec after needle withdrawal.¹³ These strategies were applied to the injection technique used for all seals in this study. However, injection site reactions still developed. These reactions may have been due to an inherent species predisposition to abscessation at subcutaneous injection sites, to subcutaneous injections being truly intrablubber injections, to the high viscosity of the drug, or to a reaction to the proprietary matrix. The only cultured abscess grew *P. phenylpyruvicus*, a gram-negative bacteria (formerly *Moraxella phenylpyruvicus*) previously reported to be found in orthinogenic soils in Antarctica, rivers, and freshwater fish.^{5,20} *Psychrobacter phenylpyruvicus* has been isolated on vaginal and preputial cultures from California sea lions previously, including rehabilitated adults at The Marine Mammal Center, free-ranging adult females and variably aged juveniles from a rookery in California, USA, and free-ranging adult males in Washington, USA, in a study investigating associations between aerobic bacteria and urogenital carcinoma. No association between this bacteria and urogenital carcinoma was determined.²⁵ This bacteria has also been isolated on cultures from Antarctic fur seals (*Arctocephalus gazella*) in fighting-induced wounds, pneumonia-affected lungs, and healthy tissue, including lung, pleura, spleen, and bladder.² Review of historic records at The Marine Mammal Center indicated 29 instances of *P.*

phenylpyruvicus, of which six were cultured antemortem and 23 were cultured postmortem. Antemortem *P. phenylpyruvicus* isolates were identified from abscesses in two northern elephant seals (including the individual from this study), an abscess from one harbor seal (*Phoca vitulina*), the prepuce of one California sea lion, and the ocular structures from one harbor seal, and one isolate did not specify location or species. The significance of *P. phenylpyruvicus* in the only cultured abscess in this study is unknown but is subjectively thought to be of little clinical importance as a result of its common nature and presence in healthy tissue, or it may represent a possible environmental contaminant.

Overall, buprenorphine plasma concentrations for most individuals were less than 1.0 ng/ml after 24 hr, with a large degree of individual seal variation and concentrations lower than that reported as analgesic in some other species at most time points. Local adverse effects suggest that this drug should be used with caution in northern elephant seals.

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