Association of nausea with buprenorphine analgesia for rats

To the editor:

Long-acting suspensions of buprenorphine may combine management and medical approaches to reduce adverse effects associated with buprenorphine analgesia. Extended-release drugs reduce the animal's exposure to cycles of hyper- and hypo-therapeutic drug levels. They also reduce the opportunity for iatrogenic injuries associated with efforts to secure small animals for repeated drug injections. A single injection of these drugs provides the animal with two to three days of analgesic therapy, and can be given when the animal is still under post-surgical anesthesia support[1–4]. However, the effects of long-acting buprenorphine analgesics on the risks of nausea in rats remain uncertain.

We previously conducted Target Animal Safety (TAS) tests of a lipid-bound, extended-release buprenorphine suspension in Fischer rats[2]. Safety trials for new veterinary pharmaceutical products require clinical and histopathology studies of male and female animals given up to tenfold excess doses of the drug. Our tests included trials using a single injection with increasing concentrations of buprenorphine, and three repeat injections at four-day intervals with increasing concentrations of buprenorphine. The tests included blinded behavioral observations to monitor unexpected signs of stress and pain. Because we avoided the use of hardwood bedding, nausea-induced gastric distress and pica were not expected. We did not find significant weight losses. However, we observed nausea-related behaviors consisting of excessive self-licking and gnawing in both the single and repeat dose-escalation trials.

The objective of this study was to measure the incidence, duration, and characteristics of nausea behavior in rats treated with an extended-release buprenorphine analgesic and maintained on soft fiber bedding. We monitored signs of nausea in a trial using 18 female Fischer F344/NTac rats weighing 170–180 g obtained from Taconic Farms (Hudson, NY). The study used three groups of six rats: two groups of drug-treated rats and one group with the drug-free vehicle as a control to investigate the effects of the drug on nausea-related behavioral signs. Guidelines for TAS studies specify a minimum number of three animals per group. Four rats were used in TAS studies to account for potential morbidity during jugular vein phlebotomy. Because there is limited information about the side effects of nausea associated with extended-release buprenorphine, we used six rats per group in the present tests to increase the potential to observe side effects.

Rats were housed in an environmentally-controlled room maintained at 68 to 79° F, a relative humidity of 30 to 70% and with a 12-h light/12-h dark cycle. Rats were housed two to three per cage during the quarantine and acclimation period in ventilated micro isolator cages; they were housed two per cage during the study period. Cages were changed daily for the first seven days of the study to prevent re-dosing by coprophagy. Carefresh Natural bedding (Ferndale, WA) was used to absorb liquids. The rats were provided *ad libitum* access to drinking water (Baltimore City Water System, Baltimore, MD) and Harlan TEKLAD Certified Global Rodent Diet 2016C (Harlan TEKLAD, Indianapolis, IN). Health surveillance was conducted by a soiled-bedding sentinel system. Rats were considered negative for pneumonia virus of mice, reovirus, Sendai virus, lymphocytic choriomeningitis virus, rat coronavirus, sialodacryoadenitis virus, rat parvovirus, Kilham rat virus, Toolan H1 parvovirus, rat theilovirus, cilia-associated respiratory bacillus, *Pneumocysti carinii, Mycoplasma pulmonis* and pinworms. The rats were quarantined and acclimated for six days before dosing. A clinical veterinarian approved the animals for study use and all rats appeared normal with no signs of disease. Animals were weighed before assignment to the dose groups to assure the weight of each rat was within 10% of the group average. The order of dosing started with the 1.3 mg/kg group and ended with the vehicle control group.

TABLE 1 | Cumulative incidence of nausea per dose, female F344/NTac rats

Buprenorphine dose (mg per kg body weight)	Number of rats	Day 1	Day 2	Day 3	Day 4	Day 5-7
0	6 female	0	0	0	0	0
0.65	6 female	1	1	1	0	0
1.3	6 female	1	2	2	2	0

Rats were given a vehicle control, 0.0 mg/kg buprenorphine, the intended dose of 0.65 mg/kg of buprenorphine, and a twofold excess dose of 1.3 mg/kg buprenorphine. The control suspension consisted of cholesterol and glycerol tristearate (96:4) suspended in a medium-chain triglyceride oil (8 mg/100 uL). The drug suspension consisting of buprenorphine, cholesterol, and glycerol tristearate, trade name Animalgesics for Mice, was supplied by Animalgesics Labs (Millersville, MD). Procedures were done in a ventilated hood in the animal room containing the rat cages. Because of its ease of use and history of safety in our laboratories, we anesthetized rats with 0.65 mL of an intraperitoneal (IP) solution containing 25 mg/mL ketamine hydrochloride, 2.5 mg/mL xylazine, and 14.25% ethyl alcohol in saline. At approximately 7:00 a.m. we then injected the rats once subcutaneously with the designated dose of test article or buprenorphine-free control suspension in the mid-dorsal area using a 22 G needle attached to a 1 mL syringe. Rats were anesthetized and dosed in approximately 45 min. Following dose administration, we transferred the rats to a recovery cage on a heating pad until recovered. Once the animal regained consciousness, demonstrated normal movement and the absence of signs of distress, we returned it to its home cage. The tail of each animal was marked with a sharpie pen to denote the animal number in the group.

During the first week of this study, animals were observed at the cage level once daily at approximately 9:00 a.m. for morbidity, mortality, signs of toxicity (abnormal respiration, tremors, ocular discharge, facial signs, posture, and movement) and overall appearance. Rats received "hands-on" detailed clinical observations once daily after 2:00 p.m. for abnormal clinical signs (ocular discharge, motor activity, signs of pain or distress, etc.). Paw gnawing and excess grooming were scored on a yes-no basis. Rats were observed weekly thereafter. The observer, a female veterinarian, was blind to the treatment group. The experimental unit was the single animal.

To extend the baseline data on nausea-related behavior associated with buprenorphine analgesia, we compared the data from this study with the results from the 64 rats in the previous two TAS trials[2]. The plasma drug concentrations reported here were collected in the TAS

TABLE 2 | Cumulative incidence of nausea in TAS trials

TAS Trial 1					
Buprenorphine dose (mg per kg body weight)	**Number of rats**	**Day 1**	**Day 2**	**Day 3**	**Day 4**
0	8 (4 male, 4 female)	0	0	0	**
1.3	8 (4 male, 4 female)	0	1 female	3 (2 female, 1 male)	**
3.9	8 (4 male, 4 female)	1 male	2 (1 male, 1 female)	1 female	**
6.5	8 (4 male, 4 female)	0	0	2 (1 male, 1 female)	**
TAS Trial 2					
Buprenorphine dose (mg per kg body weight)	**Number of rats**	**Day 1**	**Day 2**	**Day 3**	**Day 4**
0	8 (4 male, 4 female)	0	0	0	0
1.3	8 (4 male, 4 female)	1 male	1 male	1 male	3 (2 male, 1 female)
3.9	8 (4 male, 4 female)	1 female	1 female	1 female	0
6.5	8 (4 male, 4 female)	0	0	0	0

**Rats in TAS Trial 1 were removed on Day 4 without clinical observations.

study[2]. Rat blood samples were obtained from technician-restrained, un-anesthetized animals by jugular vein phlebotomy. Samples were collected at 6, 24, 48, 72, and 96 h after the drug injection. The zero time samples were collected 24 h before the rats were dosed with drug. The plasma samples were analyzed by a Shimadzu LC-20AD mass spectrometer (Columbia MD). The sensitivity of the assay was 0.5 ng/mL[1].

We did not observe pica in the present study. All rats survived until the pre-determined endpoint of the study at one year with no observed abnormalities. The majority of rats in both treatment groups did not exhibit behavior indicative of nausea during either the morning or afternoon observation periods (**Table 1**). We did find that 1 of 6 (17%) rats dosed with 0.65 mg/kg of lipid-bound buprenorphine and 2 of 6 rats (34%) dosed with 1.3 mg/kg of the drug showed signs of nausea, including excess grooming and self-gnawing. The time of nausea onset was between 24 and 48 h and lasted three to four days. By day 4, the behavior of the single affected female in the intended dose group was unremarkable. By day five, the behavior of the two affected rats in the higher dose group also was within normal limits.

Blood level concentrations of the drug poorly correlate with the appearance of the nausea signs. Following a single subcutaneous (SC) dose of 0.65 mg/kg, the plasma concentrations of buprenorphine ranged from 1 ng/mL at 6 h to 0.7 ng/mL at day three. Drug levels in the group dosed with 1.3 mg/kg ranged from 5 ng/mL at 6 h post injection to 7 ng/mL at day three. Opioid binding properties of buprenorphine may account for lack of coordination between nausea signs and blood concentrations of drug. Buprenorphine plasma concentrations greater than 0.75 ng/mL have demonstrated clinically relevant analgesia in laboratory animals and humans[5,6]. Despite having applicable blood concentrations of drug for at least three days, plasma levels of buprenorphine were not closely coupled with the appearance or the duration of nausea-related behavior.

These data are consonant with the results of our TAS study, which showed excessive grooming and self-gnawing of the forepaw in 6 of 24 (25%) rats in a single injection trial. Nausea-related behavior was seen in 4 of 24 (17%) rats in the second trial of the extended-release buprenorphine suspension[2]. The incidence of the behavior was highest in animals receiving 1.3 mg/kg, twice the intended dose. The onset of the behavior in all studies was generally within the first day of dosing. Yet delayed onset commonly was observed. In the present study, one rat exhibited the behavior on day 2. In the first TAS Trial, 5 of 24 (21%) drug-treated rats did not exhibit signs until day two or three. In the second TAS trial, 2 of 24 (8%) of the drug-treated rats had latencies through day three (**Table 2**).

Since the early reports by Clark *et al.*[7] and Bender[8] describing lethal consequences of buprenorphine-induced pica in rats, buprenorphine analgesia has been regarded with caution in research with laboratory rat models of human disease. Subsequent reports that the dangers of pica can be managed with appropriate husbandry are confirmed in the present study. Although further studies are needed regarding the safe use of buprenorphine analgesics in other strains of rat, the data in this study extend data reviewed by Foley[6] and Guarnieri *et al.*[5] demonstrate a high safety index for buprenorphine in laboratory animal medicine. More work also is needed regarding the incidence and severity side effects from buprenorphine analgesia delivered as a food ingredient, and in polymer and gel-bound suspensions.

The consensus is that rats can be treated safely with acute doses and extended-release buprenorphine analgesia provided the rats are allowed to recover on paper or soft bedding. Nonetheless, because hardwood bedding is a standard in many facilities, more information is needed about the duration of nausea with different strains, sex, analgesics, and procedures to allow the safe return of the rats to such standard husbandry condition[9–11].

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COMPETING FINANCIAL INTERESTS

M. Guarnieri owns a significant financial interest in Animalgesic Labs.

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Adverse events at research facilities

To the editor:

The Office of Laboratory Animal Welfare (OLAW) provides guidance and interpretation of the Public Health Service (PHS) Policy on Humane Care and Use of Laboratory Animals (Policy) to ensure oversight of humane care and use of animal models in biomedical research in the US. On behalf of the PHS, OLAW oversees compliance with the PHS Policy by institutions using live vertebrate animals for research, training, or testing activities that are funded by the PHS agencies: National Institutes of Health (NIH), the Centers for Disease Control and Prevention (CDC) and the Food and Drug Administration (FDA). OLAW also oversees activities funded by the National Science Foundation (NSF), the National Aeronautics and Space Administration (NASA), the Biomedical Advanced Research and Development Authority (BARDA), and the Veterans Administration (VA) under separate memoranda of understanding.

In their oversight of animal welfare in biomedical research, OLAW has encountered events that endanger the health and well-being of research animals. In this paper, we share OLAW's experience to encourage institutions to proactively plan appropriate measures to avoid or mitigate adverse advents. OLAW defines adverse events as those unexpected incidents that lead to harm, or endanger the well-being of animals and humans at a research facility. This article also provides information to help institutions maintain optimal care for their research animal population during adverse events while complying with the federal regulations and guidelines. Many adverse events are preventable, but because they are unanticipated, steps for prevention and mitigation may not always be well developed. In this article we list, categorize, and describe serious adverse events that have been documented by OLAW while overseeing biomedical research. Identifying the various events that can endanger animal and human lives and lead to loss and damage of property is essential in planning efficient measures for prevention and mitigation.

Adverse events and reportable events

NIH Grants Policy Statement[1] requires institutions to negotiate an Animal Welfare Assurance (Assurance) with OLAW to receive PHS support for the conduct of animal activities. OLAW approves the Assurance for domestic institutions on the basis of compliance with the PHS Policy, the *Guide for the Care and Use of Laboratory Animals* (*Guide*), and the Animal Welfare Act (AWA) Regulations[2–5]. In the Assurance, the institution commits to promptly report non-compliance or reportable situations to OLAW. The PHS defines noncompliance as serious or continuing noncompliance with the PHS Policy, serious deviations from the provisions of the *Guide*, or any suspension of a protocol by the Institutional Animal Care and Use Committee (IACUC)[3]. Reportable events include conditions that jeopardize the health or well-being of animals, including natural disasters, accidents, and mechanical failures, resulting in actual harm or death to animals[3].

Assured institutions are advised to submit a preliminary report of a reportable event promptly, prior to the completion of a full investigation and implementation of a corrective plan. OLAW will provide guidance to the institution as they take corrective actions and institute corrective measures to prevent recurrence. Institutions are usually able to address noncompliant and reportable incidents appropriately and institute suitable actions to prevent recurrence. Institutions must provide a final report signed by the Institutional Official that includes a detailed explanation of the circumstances and corrective actions taken. Institutions are always given the opportunity to take corrective action, and only rarely is a grant or an award suspended or terminated due to failure of implementing corrective action[6].

Risk management, prevention, and planning

Effective risk management typically requires assessment of two factors: the likelihood the risk will occur (probability) and the magnitude of the consequences if it does occur (impact). Although the probability of a serious adverse event at a research facility may be low, the impact can be very high. In addition to animal welfare consequences and the loss of data and research animal(s), the institution may suffer negative media attention.

OLAW assures a variety of domestic and foreign institutions, including colleges, universities, government agencies, small businesses, pharmaceuticals research, hospitals, contract and non-profit research organizations. The care and use of animals in research, testing, and training at these institutions includes live vertebrate animals, namely, laboratory rats and mice, birds, reptiles, amphibians, fish, ungulates (sheep, cattle, and pigs), non-human primates, and other vertebrate animals. The domestic entities Assured by OLAW (**Fig. 1**), broken down into percentages[7], and the species involved in reportable events from these institutions are presented in **Fig.2**.

Occurrence of adverse events

OLAW received 6,575 case reports of non-compliance from various domestic institutions from 2009 to 2016. Of these, 765 were new cases that were reported in 2016. While many of the reportable concerns fall under animal study protocol issues (32%) or institutional policy issues (15%), a significant number can be categorized as part of the various adverse events listed below. Adverse events caused by human error, accident, neglect, abuse, crime, training failure, equipment failure and natural disaster comprised about 17% of all non-compliance cases reported to OLAW between 2009 to 2016 (**Fig. 3**).