optimization have not been established. Specifically, it is not known whether (i) testing duration or (ii) time of day effects influence movement patterns. First, it was theorized that a total testing period of 10 min would be adequate. Second, given their crepuscular nature, it was hypothesized that these guinea pigs would display periods of heightened activity during early and late testing periods. To assess these variables, 10 5-mo-old male Dunkin Hartley guinea pigs were used. Animals were housed in filter top caging on corncob bedding with a 12:12 h light:dark cycle. No treatments were administered. Voluntary movement in a 4 ft diameter, walled enclosure containing a red hut shelter was allowed for 14 min. Monitoring software was calibrated to divide the total testing duration into 2-min intervals, allowing incremental but uninterrupted detection of activity patterns. Overhead monitoring was performed at 4 different times of day (0530-0700, 0930-1100, 1115-1330, 1515-1700). Key quantitative mobility measures included total distance traveled, total time mobile, average speed while mobile, and total time spent in the hut shelter. Statistical analyses (repeated measures one-way ANOVA) demonstrated: (i) no significant differences within 2-min testing intervals across the 14-min protocol; and (ii) when compared to other time points, animals in the 0530-0700 testing interval spent significantly more time mobile (142 s vs 40 s, $P < 0.03$) and traveled a significantly farther distance (15.5 m vs 4.5 m, $P < 0.05$); mobility measures for other time points did not differ from one another. Given our findings, a trial period greater than 10 min is likely unnecessary. Notably, the impact of time of day on mobility measures should be accounted and controlled for when possible.

P324 Effects of Different Grades of Carbon Dioxide (CO_2) on Euthanasia of Mice

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Many animal research studies involve euthanasia to relieve pain and/or as an experimental endpoint for the study. Euthanasia literally means "good death" and should include ways to minimize or eliminate any pain or distress due to the process of euthanasia. Guidelines for the euthanasia of animals have been established by the American Veterinary Medical Association (AVMA), and include the use of carbon dioxide (CO_2) as an acceptable means for euthanasia of many laboratory species including mice. Euthanasia research to date has focused on the flow rate of CO_2 into the euthanasia chamber or mixtures of CO_2 with other gases. However, possible effects of different grades of CO_2 on euthanasia are not clear or published. In a 2012 Office of Laboratory Animal Welfare (OLAW) webinar, OLAW, United States Department of Agriculture (USDA), and AAALAC International asserted that different grades of CO_2 including non-pharmaceutical industrial grade were acceptable as they all provide a minimum purity of CO_2 of 99.0%. There are currently 3 grades of CO_2 being used commonly: USP medical (>99.2% CO_2), Bone Dry (>99.9% CO_2) and industrial (>99.0% CO_2). Animal care programs source and use all 3 grades of CO_2 depending on availability. For this study, the inclusion criteria consisted of: mice already slated for euthanasia and age >10 d old. Age, strain, and gender were blocked, and then mice were randomized to each of the 3 grades for euthanasia. Based on the pilot data, time to recumbency and time to cessation of respiration were on average 35-38 s and 132-149 s, respectively. Bone Dry was the quickest and medical grade the longest to effect, although no significant differences were detected. Tissues from a subset of animals were submitted for histopathology to assess the effects on the respiratory tract including the nasal epithelium and lungs. This data confirmed that the grade does not compromise animal welfare. Therefore, standardizing the use of industrial grade CO_2 would provide approximately a $300 per month per average facility cost savings to the program, and all grades may serve as alternative options in disaster management.

P325 Buprenorphine Does Not Attenuate Postoperative Hypersensitivity in NSG Mice

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Buprenorphine is one of the most commonly prescribed analgesics for management of postoperative pain in mice. Although various forms of buprenorphine have proven to be effective for commonly used immunocompetent mouse strains, very little is known about the effectiveness of buprenorphine in immunodeficient mice. This study aimed to evaluate the efficacy of 3 different buprenorphine formulations for attenuating postoperative hypersensitivity in the immunocompromised NSG mouse using a plantar incisional pain model. We hypothesized that regular, sustained-release, and extended-release buprenorphine would attenuate postoperative mechanical and thermal hypersensitivity for NSG mice using this model. Male and female NSG mice (n=40) were randomly allocated to 1 of 4 treatment groups: 1) Saline (0.9% NaCl, 1 ml/kg, SC; once); 2) bup-HCl (buprenorphine HCl, 0.1 mg/kg, SC, BID for 48h); 3) SR (sustained-release buprenorphine, 1 mg/kg, SC, once); 4) XR (extended-release buprenorphine, 3.25mg/kg, SC, once). Mechanical and thermal hypersensitivity assessments were conducted at 24 h presurgery and then at 4, 8, 24, 48, and 72 h postsurgery before euthanasia and necropsy. For mechanical hypersensitivity: 1) Saline, SR, and XR groups did not exhibit mechanical hypersensitivity at any time point; 2) Bup-HCl group exhibited mechanical hypersensitivity at all time points. For thermal hypersensitivity: 1) Saline group exhibited thermal hypersensitivity at 4, 8, 24, and 48 h; 2) Bup-HCl, SR, and XR groups exhibited thermal hypersensitivity at 4, 8, 24, 48, and 72 h. No abnormal clinical observations or gross pathologic findings were seen in any groups. Results indicate that regular, sustained released, and extended-release buprenorphine do not attenuate postoperative hypersensitivity for NSG mice in this model.

P326 An Investigation of Bupivacaine for Euthanasia in African Clawed Frogs (*Xenopus laevis*)

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The 2020 AVMA Euthanasia Guidelines accept the use of MS-222 immersion (5 g/L; minimum 1 h) for euthanasia of African clawed frogs (*Xenopus laevis*). Pilot studies using bupivacaine at 1.5 g/L indicated a similar effect on heart rate as MS-222 revealing a potential use as a euthanasia agent for *Xenopus laevis*. The aim of this project was to investigate whether bupivacaine overdose of African Clawed frogs via immersion ceases heart function similar to MS-222. In the first part of the study, frogs (n = 10/group) were randomly immersed for 1 h in 1 of 3 treatment groups: 1) MS-5 (MS-222, 5g/L); 2) MS-10 (MS-222, 10 g/L); or 3) Bupi-1.5 (0.5% Bupivacaine, 1.5 g/L). Frogs were then removed from solutions, rinsed with system water, and placed into a recovery cage. Heart rate was evaluated audibly via doppler ultrasound over 1 min at immediate removal (T1) and at 2 (T2), and 3 (T3) h in the recovery cage. In the second part of the study, frogs (n = 10/group) were randomly immersed for 5 h in either MS-5 or Bupi-1 (0.5% Bupivacaine, 1 g/L). Heart rate was assessed twice at 2.5 (T2.5) and 5 (T5) h. Righting reflex and withdrawal reflex of the hindlimb were tested during both experiments at 1 h of immersion. For part 1, neither MS-5, MS-10, or Bupi-1.5 ceased heart rate at T1. Heart rate in Bupi-1.5 was significantly lower than that in MS-5 at T2 and T3. In the part 2 results, neither MS-5 nor Bupi-1 ceased heart rate at T2.5 or T5. Bupi-1 animal heart rates were significantly lower at T5 than at T2.5. No groups exhibited righting reflex or withdrawal reflex. These data suggest that immersion of African clawed frogs in an overdose of