This study evaluated cage size effects on food consumption and body weight in the presence or absence of tethering. Male Sprague-Dawley rats were assigned to 4 groups of 4 animals each: Non-tethered, Standard Cage; Tethered, Standard Cage; Non-tethered, Small Cage; and Tethered, Small Cage. Clinical observations and food consumption were collected Mon-Fri throughout the study. Rats were weighed weekly. During the first week, rats remained in individual 46x24x20 cm (standard) cages. The following week, rats were tethered and/or moved into small (22x24x20 cm) cages according to assigned group. Subsequently, all rats were moved back into standard cages without tethering. Tethering had a significant effect on both body weight and food consumption in both standard and small cages. The body weight effect was measurable at day 15 and had resolved by day 22 in the animals housed in standard cages, but persisted through day 22 in animals housed in small cages. A significant change in food consumption was measured on days 9 and 10 in both tethered groups. These results are consistent with previous studies demonstrating tethering as a stressor in the rat and indicate that any experiment involving tethered restraint should include acclimation prior to data collection. Further, in experiments where animals are dosed via food, potentialunderstanding of nutritional deficit must be considered. Results also suggest that if tethering is necessary, a larger cage in combination with tethering may be the preferable option and may minimize stress associated with this intervention.

INTRODUCTION
Physical stress of rodents is required during a variety of procedures including serial blood sampling, drug infusion, eeg evaluations, and post-operative exposure to airborne toxicants. It also serves as a model of psychological stress. Tethering is used in a variety of rodent study designs and the stress induced by this procedure must be taken into consideration during these experiments. Additionally, tethering typically involves moving the denchiered animal into smaller cage, which may further exacerbate stress. The objective of this study was to evaluate effects of cage size on food consumption and body weight in the presence or absence of tethered restraint.

MATERIALS AND METHODS
Following a 1-week acclimation period, 16 male Sprague-Dawley rats, 9-12 weeks of age and weighing approximately 300 g, were assigned to 4 groups of 4 animals each: Non-tethered, Standard Cage (Group 1); Tethered, Standard Cage (Group 2); Non-tethered, Small Cage (Group 3); Tethered, Small Cage (Group 4). Each rat was individually using a 12:12 light:dark cycle (6:00 am to 6:00 pm) with enrichment provided. They were fed Teklad 23.5% standard rat Chow Meal ad libitum and had continual access to water. The rats were given a 3-day acclimation period (Study Days 2-7) prior to the protocol diet phase to initiate data collection (Study Day 1). Clinical observations and food consumption measurements were conducted Mon-Fri throughout the study and body weights were obtained weekly (Study Days 1, 8, 16, and 22). During the initial 7 days (Study Days 1-7), animals remained housed in individual standard-sized (46x24x20 cm) cages. The following week (Study Days 8-15), Group 1 animals remained in their current cage size; Group 2 animals were tethered and remained in their current cage size; Group 3 animals were transferred into small (22x24x20 cm) cages, and Group 4 animals were tethered and moved into small cages. On Study Day 16 all rats were transferred back into standard cages. A final body weight collection was conducted on Study Day 22. Body weight and food consumption were assessed by a two-way repeat measure ANOVA (GraphPad Prism 5.03). Post-hoc testing at each time point was performed using Bonferroni’s procedure to correct for multiple comparisons (GraphPad Prism 5.03).

RESULTS
Tethering had a statistically significant effect on both body weight and food consumption in rats housed in both standard size and small cages. The effects were most pronounced in rats housed in small cages (Graph 1, Table 1). A significant decrease in food consumption was measured on Study Days 9 and 10 in both tethered groups (Graph 2, Table 2).