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ABSTRACTS OF SCIENTIFIC **POSTERS**

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Abstracts of Scientific Sessions—Poster Sessions

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Contents

Animal Welfare, Training, and 3Rs

Clinical

Innovations

Laboratory Investigations

Poster Sessions

P1 Welfare Assessment of Genetically Altered Mice in a French Multisite Phenogenomics Infrastructure

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Since the adoption of the European Directive 2010/063 EU, many specific provisions had to be implemented to improve protection and welfare of animals used for scientific purposes. Among them, welfare assessment of newly created genetically altered (GA) mice have to be considered, as their use in research is significant. We provide a comprehensive set of specialized services related to generating GA mice on a large scale with a high-throughput phenotypic analysis. We present here how we've implemented welfare assessment. A working group has been set up in order to establish a common process of welfare assessment on our new mouse lines. For that, we have followed the recommendations from the European Working Group on severity assessment specifying that welfare assessment should be performed when the line is established (from F2 onwards); at 3 key time-points (after birth, around weaning, and following sexual maturity); on 7 animals per gender and per genotype, and from 2 different litters as a minimum. We have created a tool for caretakers to record their daily observations easily in a macros-enabled spreadsheet. A set of criteria dedicated to neonates and to grown-up animals from high-level categories, such as appearance, behavior, clinical signs, and relative size has been used. Mendelian ratios and fertility are also followed. A scoring sheet is used to interpret those observations, score the phenotype, and compile data in a kind of passport, a document which is to follow mice when the lines are distributed worldwide. This passport will ensure that specific information related to animal welfare is accessible to whoever will care for these lines. This assessment framework provides an improvement of welfare by minimizing the potential for pain, suffering, and distress. The other benefits include improved communication with a real connection between animal caretakers and researchers, new skills acquired for our animal caretakers as this welfare assessment was seen as a first step in our mouse phenotyping pipeline, and the creation of comprehensive scientific information on GA mice.

P2 Evaluation of Stress Associated with Warming and Restraint Methods for Blood Collection from the Lateral Tail Vein in Sprague Dawley Rats

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Blood collection from conscious animals avoids the physiologic and pharmacologic impact of anesthetic drugs. However, restraint is a well-described stressor for many species. At our company, blood collection from the lateral tail vein in conscious rats is commonly performed for pharmacokinetic studies. As a standard, groups of rats are held in tall plastic buckets to facilitate warming for vasodilation and then restrained in a perforated stainless steel restrainer for blood collection. A question arose during an IACUC semiannual facility inspection about whether these conditions may be stressful to the animals. We sought to compare standard conditions with alternate practices to determine the least stressful holding and restraint conditions for lateral tail vein blood collection. A crossover design was used to evaluate 3 holding conditions (bucket, home cage, and home cage with supplemental heat) and 3 restraint devices (stainless steel, plexiglass, and another rodent restraint device). Six groups (n=5) of Sprague Dawley rats were used in each investigation, with serum corticosterone and blood glucose measured for each individual, and frequency of positive (60 kHz) and negative (22 kHz) ultrasonic vocalizations calculated for each group. A linear mixed model minimally including sex and sequence was used to evaluate the data. Comparison of holding conditions showed that blood glucose was significantly increased ($P = 0.0005$, <0.0001) and ratio of positive: negative vocalizations was significantly decreased ($P = 0.02$, 0.004) for the bucket compared to the other 2 holding conditions, suggesting that holding in the bucket increases stress. When restraint devices were compared, differences in corticosterone were variable, with no significant differences for blood glucose or vocalization frequency, which may indicate that any variation in stress due to type of restraint device is eclipsed by the stress inherent to the experience of being restrained. This data demonstrates the value of ultrasonic vocalization in assessing welfare in rats and is helping us to refine practices for blood collection from conscious rats, improving animal welfare at our institution.

P3 Establishment of a Swine Training Lab Program

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The training of medical professionals on live animal models is a necessity to ensure proper techniques and patient safety. These training scenarios allow trainees to perform surgical interventions, follow up on the systemic responses, practice and have their skills evaluated in a live patient, and experience new procedures and medical equipment without risking human life. Most labs require IACUC protocols, ordering animals, performing operating room (OR) set-up, and coordinating between departments. These details can be time consuming and challenging to organize. We sought to streamline the process by creating a swine training protocol, coordinated and supervised by a faculty veterinarian, and a simplified billing system. We began with the IACUC protocol in which the PI is an experienced clinical veterinarian. The protocol allows for swine to be anesthetized using a variety of drugs to address a specific study focus (for example, cardiothoracic labs). Once the animal has reached a surgical plane of anesthesia, it is prepped for the day's lab, then taken to the OR and set up for anesthetic monitoring. The protocol is written to allow for any procedure as long as the animal remains under a surgical plane of anesthesia, has veterinary supervision, and is euthanized at the end of the procedure. A veterinarian and/or veterinary technician is present for the entire duration of the lab (typically 3-8 h) to monitor the animal and aid in any other procedures such as vascular cut downs. Basic supplies such as surgical tools are available and MDs and companies are encouraged to bring their own specialty equipment in as needed. We use a flat-rate billing system so that all the lab coordinator needs to do is contact the vet to request a time, the number and size of the animals, and any special equipment. The addition of this training protocol has been praised for its streamlined approach to training labs using live animals.

P4 Turkey (Meleagris gallopavo) Handling and Acclimation for a Nonweight Bearing Sling

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Digit amputation is a debilitating injury when not properly repaired. A surgeon's experience with digit replantation positively affects the success rate, which publications show to be declining. The turkey's (*Meleagris gallopavo*) third toe possesses similar anatomic structures and healing properties in relation to human fingers. Our pilot study of 6 adult tom turkeys investigated if the turkey is an appropriate in vivo surgical training model for digit replantation. To be clinical relevant the turkeys had to be nonweight bearing during the healing process. It is crucial to properly acclimate the turkeys to being housed in a nonweight bearing sling. Unlike quadrupeds, turkeys cannot have a jacket placed to prevent weight bearing on a surgical appendage. Therefore, a nonweight bearing sling was developed. The sling was designed to socially house 2 turkeys together and to be an independent structure providing feed, water, and enrichment. A week prior to surgery the turkeys were acclimated in pairs starting with 1 h and ending with 8 h in the sling. Tightly restraining the wings had a calming effect; we secured tight cotton sleeves over the turkeys' wings prior to placing them in the sling. With proper handling placing them in the sling went smoothly after the first few introductions. The higher the turkeys' breast sat the calmer the turkey appeared, relaxing their snood and turning pale in color. Turkeys were observed eating, drinking, and defecating while housed in the sling. If they became agitated simply holding their wings put them back in a tranquil state. Acclimation to the sling slowly was successful. By the last day they only became aroused by unanticipated noises. Completion of this pilot study encourages us that the turkey could be a suitable model for digit replantation training. Further studies are needed to validating long term housing in the sling.

P5 Implementation of Automated Blood Sampling during Physiological Monitoring of Telemeterized Beagle Dogs

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Accurate data collection is imperative for cardiovascular physiological monitoring of telemeterized beagle dogs when evaluating novel pharmacologic compounds. Exposure assessments for cardiovascular telemetry dog studies were limited due to the disruption of cardiovascular signals when entering the room. Historically, blood was collected at 3 and 16 h postdose to minimize room disruption and the negative impact of the cardiovascular data. To construct a full pharmacokinetic-pharmacodynamic (PK-PD) profile for each compound, a separate pharmacokinetic (PK) study using nontelemetrized dogs would be conducted. Collaboration of the Integrative Pharmacology and Comparative Medicine departments resulted in the successful incorporation of automated blood sampling (ABS) into the telemetry dog cardiovascular studies. Implementation of ABS allows for uninterrupted cardiovascular data collection with simultaneous blood collection. Over the last year, modifications to caging, apparel, and samplers were performed allowing us to streamline the implementation of ABS within the telemetry dog colony. The impact of this collaboration between the 2 teams attributes to a 50% reduction of animal use. The pharmacokinetic assessment and cardiovascular data can be achieved within 1 study rather than 2 separate studies. The generation of exposure-response data (PK-PD) in 1 study allows for less compound requirements due to fewer animals dosed, and a shorter timeline to achieve overall results.

P6 The Design and Refinement of a Solid-Bottom Caging Rat Infusion Bank Model

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Our IACUC began requiring justification for housing rats on wire-mesh bottom caging. As a result, a commitment was made to house all rodents in solid-bottom caging by the end of 2015. This presented the challenge of designing an alternative to the wire-mesh bottom infusion caging used for tethered rat infusion studies. Ease of access to individual animals, adequate visibility of both the animals and infusion pumps, and efficient use of space were essential design requirements for the refined infusion bank. To maximize equipment use, the wire-mesh caging banks no longer being used to house rat were modified for solid-bottom caging, therefore saving costs associated with procurement of new cage banks and enabling the old caging to be repurposed. All the wire bottom cages were replaced with solid-bottom litter boxes with wire lids. Gimbel mounts were attached to the wire lids to anchor the tethered infusion system. Custom automated water lines were designed that maximized the number of litter boxes per bank and enabled proper weight distribution of both caging and infusion equipment. Initial bank modifications positioned infusion pumps hanging in front of the animal cages. This design proved to be cumbersome and difficult to work with. The final design incorporated shelves on both sides of the bank that could accommodate infusion pumps positioned in from of up to 6 litter boxes per row, up to 36 animals per bank. This bank design also facilitates the conduct of rat reproduction studies with littering dams. The modified banks have been used for over a year and provide efficient, cost-effective use of repurposed caging, resulting in an improved and refined rat infusion model. This modified design meets our commitment to improved/appropriate rat housing and operational requirements for improved animal and infusion equipment access while also minimizing overall caging footprint and maximizing animal room space.

P7 Use of Vascular Access Ports in Sprague Dawley Rats: A Refinement for Long-Trm, Repeat Intravenous Bolus Injections in Socially Housed Rats

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External equipment such as jackets/tethers, quick-connect harness systems, tail-cuffs, miniature access ports, and/or skin buttons used for long term daily IV bolus dosing in rats often present challenges associated with animal growth, equipment fit/size, access system maintenance/repair, and the potential for infection. Further, most external equipment does not allow for the animals to be socially housed. Vascular access ports (VAPs) were evaluated to eliminate use of external equipment in an effort to improve animal welfare and to allow for social housing. VAP maintenance methods were evaluated to assess multiple doses of heparinized saline used to lock the VAPs following repeated port access. Thirty Sprague Dawley Rats were surgically implanted with either a

top-access style VAP or a smaller, lower profile all silicone port. Rats were housed 2 to 3 per cage approximately 10 d postoperatively. The skin over the VAP was aseptically prepared prior to each IV bolus administration and the port was accessed using a Huber needle. The rats were dosed with 0.5 mL of saline, 5-7x per week for up to 90 d. Ten of the rat VAPs were locked using a heparinized saline lock solution (5 IU/mL) to prevent loss of catheter patency. An attempt was made to draw off the heparinized saline lock prior to each dose. The remaining 20 rat VAPs were not locked with a heparinized saline solution following the saline dose to evaluate this maintenance regimen's effect on catheter patency. Body weights and clinical observations were collected weekly and clinical pathology parameters were evaluated approximately monthly. The ports remained patent regardless of the use of a heparinized saline lock solution. Clinal pathology parameters were not affected in rats that had VAPs locked with heparinized saline versus the ones that did not. Use of subcutaneously implanted VAPs provides an alternative to rat IV bolus models that require external equipment to maintain vascular access and allow the animals to be socially housed with no impact on the vascular access system. This refinement of standard IV administration method allows for improved animal welfare and an alternative for long-term daily IV access.

P8 Improving Methods of Pharmacokinetic/Bile Duct Study: Automated Blood Sampling and Access Button Compared to Manual Sampling and Exteriorized Catheters

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Bile sampling combined with blood and urine collection can be stressful for the animal. Under our current procedure, Sprague-Dawley rats are received with exteriorized catheters (jugular and recirculating bile duct) from an approved vendor. Upon receipt, animals are individually housed to protect the exposed cannulas. After the appropriate acclimation period, the rats are placed in metabolism cages with a harness and tether for bile collection. Blood samples are taken manually by cannula throughout the day at specific time points. Our goal was to develop a method which not only decreased the stress for the animal but also lessened the labor required. The improved method uses a protected access button allowing for dual housing of the animals upon arrival. Animals are placed into a response movement caging system which allows for simultaneous bile, urine, and automated blood collections when on study. This decreases handling and increases the integrity of the sample lines with less manipulation. Studies were conducted using our standard inhouse study design for pharmacokinetic studies with multiple blood sample collection. All dosing and blood sample collections were performed in accordance with an IACUC-approved animal use protocol. Corticosterone levels through blood sampling, patency, sample quality, and animal welfare (bodyweights, general examination of surgical site) were measured and observed throughout the study. For the purpose of this study, a total of 8 rats were used, 4 with exteriorized catheters and 4 with access buttons. By improving the methods we were able to demonstrate that stress levels were decreased due to the combination of less manipulation of the animal and catheters, as well as the ability to pair house while not on study.

P9 The Use of Food-Grade Dye for Training in Preparation of Tumors Prior to In Vivo Implantation

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When implanting tumors into mice for in vivo research purposes, it is imperative that the resulting tumors be similar across all animals. Minimal variability across tumors creates a more uniform model which allows for the most accurate data to be gathered. Therefore thorough training is required so that technicians can properly engraft tumors with accuracy and consistency. Technicians are trained using real tumors from donor mice. However, tumors are often similar in color to skin and muscle, making it difficult to assess whether the tumor material is the right shape, size, or even in the right location at the time of implantation. When homogenizing a tumor, it is required that the tumor is a consistent mass, with no large pieces in the mixture. When the tumor is minced, it can become difficult to determine if large pieces remain and whether more mincing is required. All of these challenges can be minimized by using food-grade dye during training. When dye is mixed in with the tumor material, visibility of this material increases drastically allowing a technician to see exactly where and how the tumor is being implanted. Furthermore, the consistency of the mixed tumor is more easily observed due to the dye highlighting larger pieces of tumor. Food-grade dye also aids in the detection of any remaining tumor tissue on instruments. With this direct, visible feedback, it is easy for technicians to judge the shape and size of their implanted tumors. It is also easier to visualize a dirty needle, which can lead to unwanted trace tumors, thus reaffirming the importance of cleanliness and sterility for the technician. Therefore, the use of food-grade dye when training on the implantation of tumors provides a clear advantage to better the technician's technique and sterility.

P10 Start-Up Meetings Improve Lab Compliance

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Successfully communicating the start of a research project between the investigator and husbandry staff is challenging if multiple offices are involved. Start-up meetings, in conjunction with husbandry, safety, and the veterinary staff, were implemented to improve personnel safety and help fulfill animal research training expectations. Start-up meetings are conducted with investigators and their staff, the Animal Resource Center, the Office of Animal Research Support, and Environmental Health and Safety whenever a high-risk activity is approved on a protocol. Such activities could include new investigators, hazardous agents, rodent pathogens, surgery involving USDA species, or any nonhuman primate protocol. These meetings include a detailed discussion of the research and the requirements for labeling and handling of the animals; cage and waste for hazards; or anesthesia monitoring, postoperative care, and evaluating potential pain for surgery cases. By providing training prior to the start of the experiment, we are able to improve investigator compliance, as well as reduce risk to the researchers, husbandry staff, and animal wellbeing. Meetings are required following IACUC approval of the protocol or modification and conducted prior to the start of the experiment. Hazardous agent SOPs, IACUC policies, and veterinary services are all reviewed at the start-up meeting, making changes and adjustments as needed. Investigator feedback is positive, with the general sentiment being that the start-up meetings are a useful service provided by the Office of Animal Research Support. Investigator buy-in to the process is vitally important, as this will be translated into improved compliance and better animal welfare. Since the start of a more thorough hazardous agent start-up meeting process in 2009, safety-related incidents have been reduced. Further reduction was demonstrated with the implementation of the start-up meeting matrix in 2015. The matrix standardized the process further by ensuring that all associated parties are included in the process. Prior to the start of start-up meetings in 2010, hazard-related incidents ranged from 8-17 per year. Following the start of the process in late 2009, incidents were reduced to 3-10 per year. With implementation of the start-up meeting matrix, incidents have been reduced further to 4 per year.

P11 Expanding Welfare Checks by Simultaneous Recording of Home-Cage Rodent Activity for up to 96 Cages

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Daily laboratory animal welfare checks take less than 5 min per cage, according to scheduling around cage changes and other institutional duties. Any transient observations may not represent the full scope of activities and behaviors, especially those during the opposite light cycle. One solution is to record animal behavior in a recording chamber, which requires relocation of animals from the home cage or modifying the caging. This solution removes the animal from its normal environment on the cage rack, stressing the animal, and possibly modifying its behavior. In order to observe normal behavior, the animals need to be recorded in their home-cage on the cage rack. This can involve expensive, technical equipment that is difficult to set up, easily displaced, and limited in number of cameras and storage capacity. To record and evaluate laboratory animal home cage behavior, we assembled an inexpensive multicamera array that used surveillance cameras (low light, red light, UV light capable) and digital video recorders (DVR). Each camera (~$45) was secured inside a disposable, independently ventilated cage (IVC) to record from the rear of the opposite cage on the rack (across the divide). Each modified camera cages could be

moved quickly to record from different housing cages, without changing alignment, the settings, or refocusing, as well as minimizing the equipment footprint in animal rooms. Up to 16 camera feeds were recorded on a single DVR simultaneously, using video compression format that allowed a 1TB hard drive (HHD) to hold 2,400 h of recordings (150 h per cage). The advantages of this surveillance camera-based system include: (1) camera cages are unobtrusive to normal animal behavior, (2) equipment was housed within existing cage racks, which (3) ensure that prefocused cameras line up exactly with the rear of the cage on the opposite side of the IVC rack, and (4) moving cameras to record from other cages requires no additional focusing or set-up. A single 16-camera system cost ~$1470, and permits longer term animal welfare and behavior assessment.

P12 The Effect of Pair-Housing on Response to Construction Noise in Rhesus Macaques (Macaca mulatta)

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Improving and ensuring the welfare of nonhuman primates are top priorities for caretakers and biomedical institutions. One of the best ways to promote welfare for captive macaques is through social housing. Studies have shown multiple benefits of social housing, including promoting species-normative behaviors and decreasing abnormal behaviors. Further, having a social partner can help mitigate response to stressful events, a phenomenon known as social buffering. While social buffering is known to reduce stress in a variety of species, there are relatively few studies that have specifically examined it in macaques. In a preliminary study, we examined whether having a social partner reduced response to construction noise, an unpredictable environmental stressor, in 22 indoor-housed adult (5-19-y-old) male rhesus macaques (14 pair-housed, 8 single-housed). A trained observer took 5 min focal observations 2 to 3 times a week and recorded the occurrence of stress-related behaviors (for example, stereotypy and anxiety behaviors such as scratching, yawning, shaking) every 30 s. We compared behavior between days in which there was construction or no construction using repeated measures ANOVA, with housing type as a grouping variable. Single housed animals showed more anxiety ($F_{(1,20)}$= 5.3, P = 0.03) and stereotypical behavior ($F_{(1,20)}$= 4.7, P = 0.04) than paired monkeys regardless of whether construction was occurring. Interestingly, while both single housed and paired monkeys showed more stereotypy on days in which there was construction than days without ($F_{(1,20)}$= 13.9, P = 0.001), this increase was significantly more pronounced for the single-housed animals ($F_{(1,20)}$= 7.9, P = 0.01), suggesting that the construction may have been more stressful for these animals. While we have a relatively low sample size, our results support the benefits of pair housing by way of social buffering in rhesus macaques faced with unpredictable environmental stressors.

P13 Refinement of Surgical Treatments: Defining a Standard of Care

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Our In Vivo Sciences department is responsible for providing survival surgeries and postoperative care for research animals. There are about 10 different types of survival surgeries that are included in 23 active IACUC protocols. Historically, the treatments described for each type of surgery varied across protocols. As the In Vivo Sciences staff provide assistance on multiple protocols every day, this variability led to confusion and instances of noncompliance. To ensure consistent and standardized treatment descriptions across all protocols, a standard of care dosing protocol was developed. For each treatment, the dosing protocol defines a dosage range, acceptable volume, route of administration, and the standard regimen for treatment. To further refine our practices, the different types of surgical procedures were classified into 4 categories. For each category, dosing protocols were combined to form surgery-specific care modules. The categorization of surgeries and development of the care module has helped to define the type of care expected for each surgical category, while reducing variability across protocols. The dosing protocols and care modules are reviewed and approved by the IACUC and can easily be referenced by an investigator when writing a protocol. Since implementation of the standard of care dosing protocol, we have seen a reduction of noncompliance events, while also reducing the back-and-forth time between support staff and research staff deciphering requests. We have seen no adverse effects on the health of the animals in standardizing the treatment regimen during and after surgery. By implementing this standard of care through dosing protocols and care modules, we hope to reduce variability in research outcomes, while staying in line with the 3Rs to minimize pain and distress.

P14 Learning the Art of Training: Perspectives on Becoming an Effective Trainer

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There is a strong emphasis in the laboratory animal profession on the importance of ensuring proper training of research personnel on animal handling and techniques. Yet, there is little emphasis placed on how to properly train new trainers to conduct such training. Individuals are often selected as new trainers because of their proficiency in performing common animal procedures; however, possessing the expertise to perform a procedure proficiently does not automatically equip someone to teach others how to perform that procedure. New trainers may be surprised to learn that the procedures they perform quite naturally can actually be the most difficult procedures for them to teach to others. New trainers often need to overcome many misconceptions about being an effective trainer, and they may face several challenges during the process of learning how to train others. Embracing the dynamic process of becoming a trainer and formulating the right approach to this process will have profound impact on beginner trainers and their future trainees. We offer a novel perspective on the trainer in training process. Several common misconceptions about training are addressed, as are the qualities of an effective trainer. We discuss tips for training new trainers, tips for learning to train, and encouragement for individuals who are interested in embarking on the rewarding journey to becoming an effective trainer.

P15 Are Callipers Obsolete? A Novel 3D Scanning Technology to Measure Subcutaneous Tumor Volume

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Most preclinical oncology studies (xenograft, PDX, GEMMS) involve monitoring tumor growth rates, measuring them with calipers, and calculating the volume. Volume is calculated from the width and the length to estimate a 3D volume and is directly used to assess treatment efficacy. Although this technique is useful, it is unable to accurately assess nonuniformly shaped or small tumors and introduces a systematic bias by assuming that tumors present with spheroid shape. We describe the development and validation of a 3D scanner as an alternative method to calipers to monitor tumor progression in rodents. The resulting 3D scanner has the potential to deliver significant 3Rs benefits, identified as reduction of animals via improved data accuracy allowing reduction in group sizes or the ability to include irregularly shaped tumors to test. In addition, the scanner system described will make it possible to record tumor measurements in a rapid, minimally invasive, morphology-independent, and human bias-free way, removing interoperator variability. We describe the development and validation of the scanner system within our laboratories, evaluating the finalprototype hardware. Using the 3D scanner alongside tumor calipers to monitor tumor growth of oncology tumorstudies, we demonstrate that we can measure tumor size parameters (length, width, and volume), in multiple mouse strains and across a range of tumor models, with accuracy and precision comparable to tumor measures generated from calipers. If successful the introduction of this system to replace tumor calipers could have a large impact for groups running oncology in vivo tumor studies. It operates with different sized aperture holes to accommodate different rodents and tumors. The process of taking a measurement includes taking the rodent by the scruff (in the same way as with calipers), placing the rodents tumor in the center of the scanning window, and then capturing the 3D data by pushing the scan button on the device or in the software. The software then segments the tumor for size and shape data which is then exported for analysis over time. Mouse strains tested internally include immunocompromised athymic nude, SCID and NSG mice, and immunocompetent Balb/C and C57BL6 mice.

P16 Dermal Dose Administration—Refinements of Procedures Used on Toxicology Studies in Minipigs

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The minipig is the preferred species for many toxicology studies requiring dermal applications. The commonly used dose site for dermal application on the minipig is across the dorsal area and onto the flanks of the animal, totaling approximately 10% of the total body surface area based on bodyweight. Semi-occluded dermal administration is a commonly selected method for dermal application and requires a semi-breathable covering that prevents ingestion of the dose material, minimizes cross contamination, and protects the animal's skin. Thus, the dressing must be comfortable for the animal but secure enough to prevent dislodging during routine movement of the animal. By applying a commercially available dressing made up of a hypoallergenic, latex-free adhesive that is both gentle to the skin and breathable and by covering it with a modified jacket designed specifically for animal use on dermal studies, we have successfully covered dermal dose sites and assured adequate exposure of over 100 minipigs on 4 separate toxicity studies without interference by the test animal while still being able to easily access the animal's dose area.

P17 The 3Rs in Training: Creating and Implementing a Hand-Crafted, Inanimate Training Tool for Hands-On Mouse Training

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Both the National Research Council (NRC) and the Candian Councill on Animal Care (CCAC) provide guidelines on the training of personnel working with animals in research. These general guidelines include that "all personnel working with animals in science must be knowledgeable about the principles of humane experimental science and ethical issues associated with the use of those animals, including the 3Rs. We strongly advocate for the implementation of the 3Rs during hands-on training sessions to improve the quality of teaching and to demonstrate alternative methods to our trainees. Handling and restraint of mice is a primary component in our Introduction to Rodents class, and is the building block for all other techniques that we teach. However, handling live mice can be quite stressful for the inexperienced student, and this stress can transfer from the trainee to the training animals. To manage these concerns, we revised our hands-on training methods, initially by identifying several key learning issues (KLIs) that challenged trainees during the process of learning mouse handling and restraint. With a goal to implement the 3Rs, we then initiated the use of a variety of commercially available stuffed mice to teach proper handling and restraint. While the stuffed mice aided in achieving some of our training goals, the use of these inanimate mice did not adequately address all of the identified KLIs. Therefore we created an effective, multiuse inanimate training tool from readily available and affordable materials which provided a means for trainees to master the KLIs related to mouse handling. The tool is one of several hands-on tools created as part of our training concept: Translational Training Tools. We describe the KLIs identified during hands-on training of mouse handling and restraint. We also elaborate on how we used our hand-crafted training tool to address a variety of training goals, while providing better implementation and demonstration of the application of the 3Rs.

P18 Murine Mammary Tumor Detection and Measuring Techniques in Genetically Modified Mouse Models for Breast Cancer Research

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Genetically modified mouse models created for the study of various types of breast cancers have become a valuable tool to study tumor development, growth rates, and the possible remedies to combat tumorigenesis. There are a variety of challenges that arise when studying a genetically modified mouse model for breast cancer research. Depending on the model, only a certain number of animals are expected to develop tumors at a certain age. In our particular model, preliminary studies are required to determine the average age for onset of tumor development. Knowing this information allows for the sufficient planning of animal production so an adequate cohort of tumor bearing animals are produced and experiments can be run efficiently and with statistical significance. A method must be developed to ensure that tumors are reliably detected and consistently measured so a direct comparison can be made between animals and against existing data. First, it is crucial to have an excellent understanding of the complete murine mammary gland anatomy so one can be familiarized with the areas where the tumors can develop. The next step is incorporating a combination of full body visual screening followed by a physical palpation approach to tumor detection which allows for the early discovery of new tumors. This ensures the ability to collect more tumor growth rate data points resulting in a more detailed view of the tumor growth rate over time. Finally, when a tumor is detected, the animal is anesthetized and the fur over the tumor bearing area is shaved to obtain unhindered access to the tumor for measurement. Calipers are then adjusted around the tumor until they are in contact with the perimeter of the tumor. The calipers are then moved up and down perpendicularly to the plane being measured to check for proper clearance and ensure there was no compression of the tumor by the calipers during the initial adjustment, possibly resulting in smaller measurements than actual size. This ordered operation of caliper measurement is utilized so tumor measurements are accurately recorded. Employing all of these methods and techniques will ensure consistent, reproducible tumor detection and measurement results.

P19 The Use of Air-Activated Thermal Devices as Postsurgical Thermal Support in Mice

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Laboratory mice (*Mus musculus)* are susceptible to periods of hypothermia especially during anesthetic events, disease states, and environmental stressors. Thermal support devices for small mammals are numerous, but often require a power source and may be expensive or impractical to use for cages on a rack. Air activated thermal devices (AATD) are mixtures of chemicals that, when exposed to air, cause an exothermic reaction. Previous studies have shown that these devices along with supplemental equipment are effective at warming the cage, however, have not demonstrated an increase in body temperature of a mouse or efficacy without auxiliary equipment. We examined the in vitro effects of AATDs on internal cage temperature without the use of additional equipment as well as the in vivo effects of AATDs as postoperative thermal support in mice. For in vitro experiments, temperatures measured inside the cage and directly above the AATD peaked at 101 +/- 2.2°F (SE) (25°F higher than control cages). In addition, we demonstrated that the heat amount and temporal distribution provided by an AATD is dependent on cage and rack type, with a static cage on a plastic surface or static cage on metal surface measuring, at its peak, 86.3 +/- 2.4°F and 73.4 +/- 0.9°F, respectively. After application of AATD, the temperature in IVC cages peaked sharply at 60 min whereas temperatures in static cages on plastic and metal plateaued at 50 and 100 min, respectively. For in vivo experiments, mice were surgically implanted with an intraperitoneal temperature telemetry device. Between mice with and without an AATD, there were no significant differences between recovery times and final body temperature at 6 h postoperatively. In the 0-3 h postoperatively after returning to their cage, a remarkable drop in body temperature (average = 11°F) was noted in mice without AATDs and absent in mice with AATDs. Based on this result the in vivo results of our study support that AATDs can be useful in providing extended thermal support for mice to help maintain body temperature post surgically. In vitro results of our studies demonstrated that AATDs provide local thermal support for 4-6 h depending on cage set-up.

P20 Conscious Urine Sampling and Quantification from Indwelling Catheterization of Female Yucatan Swine

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Aside from routine diagnostic testing of urine for health status, data from urine collection is often necessary for support in various nutritional, metabolic, biochemical, and physiologic studies. Collection methods can be

stressful to the animal (manual restraint), may require sedation (cystocentesis in larger animals), or may become contaminated (metabolism cages or free catch samples). With most methods, the ability to quantify total urine output over a number of hours is lost, and currently, published options in swine are limited. To quantify urine output and obtain serial samples, foley catheters were placed in 4 anesthetized 50-60 kg female Göttingen minipigs *(Sus scofa)* on 2 separate occasions (n=8). A closed system urine drainage bag was then attached and the catheter secured to the ventral abdomen of the pigs using stay sutures and a custom jacket to avoid displacement once conscious. The Foley catheters were successfully maintained over a 72-h period while urine was quantified and samples were obtained. During and after the sampling, there were no clinical signs of inflammation or infection. Animals did not show any overt signs of stress or agitation from the presence of the indwelling catheters. Prior and continuous positive reinforcement clicker training contributed to the ease and success of sample collection, as well as conscious catheter and suture removal. One of 8 indwelling catheters in our study was misplaced resulting in failure of sample collections, and 2 of 8 pigs experienced momentary stress requiring manual restraint for foley catheter removal. Proficiency in placing urinary catheters in female pigs with minimal irritation was attainable after training on terminal surgical pig models under the supervision of experienced personnel. The combination of using modified stay sutures, a custom jacket to hold the urine collection bag, and animal training allowed us to successfully obtain conscious, serial, quantifiable urine samples with minimal stress to the animals and handlers. This method resulted in a decreased number of animals per study by increasing the number of samples obtained per animal. Additionally, this method eliminated the stress, potential risk, and confounding factors involved in serial sedation for sample collections.

P21 Thioacetamide Administration via Drinking Water Yields Equivalent Liver Fibrosis Compared to Traditional Chronic Carbon Tetrachloride Injections in C57BL/6 Mice

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Animal models for liver fibrosis aim to increase our critical understanding of human liver disorders that encompass the initiation, progression, and resolution of fibrosis. Elucidation of these mechanisms can drive potential therapeutic targets to develop effective antifibrotic treatments. Hepatotoxins are often used to model acute and chronic liver injury in rodents. Two commonly employed hepatotoxins are carbon tetrachloride (CCl_4) and thioacetamide (TAA). CCl_4ultimately alters and damages hepatocyte intracellular and plasma membranes, resulting in cell death. Alternatively, bioactivation of TAA results in unstable metabolites that covalently bind to proteins and lipids, causing necrosis. Due to its highly reversible fibrosis, repeated injections of CCl_4 are needed to induce chronic liver injury. To minimize animal handling and invasiveness necessary in this technique, we evaluated liver fibrosis induced by administration of TAA in drinking water compared to the widely used method of chronic injections of CCl_4 in female C57BL/6 mice. At 8 wk of age, the experimental mice were injected with 1.5% CCl_4 at 1 mL/kg 3 times a week for 6 wk intraperitoneally, with respective control mice. These mice were compared to age-matched mice exposed to 0.025% TAA in their water bottles for 7 wk. Longitudinal body weights were collected, and study termination resulted in left lateral liver lobe collection for histology and serum collection for ALP, AST, and ALT assays. Histological analyses of CCl_4- and TAA- treated mice demonstrated minimal to mild, and moderate liver injury in the centrilobular zones of the hepatic acinus, respectively. TAA-treated mice had significantly elevated levels of AST and ALP when compared to CCl_4-treated mice, further indicating liver injury. Notably, TAA-treated mice developed clinical signs associated with disease progression by 7 wk, more rapidly than literature for 16-24 wk in mice. In summary, thioacetamide administered via drinking water was able to yield a similar degree of liver fibrosis compared to repeated injections of carbon tetrachloride with minimal handling and invasiveness.

P22 Food Reward Preference in Turkeys (Meleagris gallopavo)

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Reducing stress and creating a positive, cooperative relationship with animals used in biomedical research is a focus in laboratory animal science. Positive interaction and reduced stress can affect both research data and animal welfare. A method for developing this level of care and cooperation is through positive reward following aversive procedures. There is little in the literature which addresses reward preferences in poultry. This study focused on treat reward preferences in farm turkeys (*Meleagris gallopavo*) being housed long-term for repeated blood collection events. Based on the foraging characteristics and ethogram of wild turkeys, 5 different treat items were selected and offered to farm turkeys on a feeding tray with 5 wells. The 5 items presented on the tray in a randomized order included carrots, broccoli, mealworms, dried corn, and red grapes. The order and completeness of consumption were recorded during 5 min individual trials. A total of 11 turkeys participated in these trials. Based on the data collected, turkeys anticipate and readily consume food rewards. They also display individual treat preferences. The results can be used to develop reward strategies for laboratory housed poultry, and inform enrichment decisions.

P23 Journal Support of the ARRIVE Guidelines Has Not Resulted in Improved Reporting Standards in Animal Welfare, Anesthesia, and Analgesia

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Poor research reporting impedes experimental reproducibility, unnecessarily increasing financial and animal resources. The Animal Research: Reporting of In Vivo Experiments (ARRIVE) guidelines were developed to improve reporting in animal research. We hypothesized that papers published in veterinary journals supporting the ARRIVE guidelines would show improved reporting compared to nonsupporting journals. Journals were designated as ARRIVE-supporting (SUPP: 5 journals) or nonsupporting (nonSUPP: 2 journals) based on referencing the ARRIVE guidelines in author instructions. Comparisons were made between journal types and between pre (2009) and postARRIVE (2015). Prospective studies with a focus on animal welfare, anesthesia, and analgesia were included. Relevant studies were identified by manual search of tables of contents (title, abstract, and keywords). Adherence to the ARRIVE checklist of 20 items was independently assessed by 2 authors. Items were identified as fully, partially, or not reported. Scores were compared and differences resolved by consensus. An unequal variance t-test was used to compare mean percentages of items fully reported by each paper. A total of 236 papers were included: 120 from 2009 (SUPP; n=52, nonSUPP; n=68) and 116 from 2015 (SUPP; n=61, nonSUPP; n=55). The percentage of fully reported items was similar between journal types in 2009 (SUPP; 55.3±11.5%, nonSUPP; 51.8±9.0%, p=0.07) and 2015 (SUPP; 60.5±11.2%, nonSUPP; 60.2±10.0%, p=0.89). Between 2009 to 2015, reporting improved for each journal type (both $P < 0.05$), but the percentage of improvement was similar between SUPP (5.2%) and nonSUPP (8.4%, 95%CI -0.54 - 4.3%, $P = 0.09$). No paper fully reported 100% of items on the ARRIVE checklist. Full reporting of several items was consistently low: study design (< 30%), sample size justification (< 15%), allocation to experimental groups (< 30%), housing and husbandry details (< 20%), and experimental animal details (< 25%). Supporting the ARRIVE guidelines did not lead to improved reporting standards in this sample. Journal support alone is insufficient to ensure adherence to the ARRIVE guidelines.

P24 Improved Animal Welfare During a 99-Day Continuous Tethered Infusion Study in 36 Cynomolgus Macaques (Macaca fascicularis) through New Equipment Implementation.

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Continuous tethered infusions in any animal model pose a unique set of problems; many are amplified when nonhuman primates are needed. Starting from an already proven methodology and system we set out to refine procedures and develop hardware to decrease the stress on the animals, improve workflow for technicians, and insure the success of the study. Most of this effort focused on decreasing stress on the animals and improving their comfort. Several new designs of infusion jackets were tested that employed modifications to improve fit and to add adjustability and padding. The catheter was also updated to a model that incorpo-

rated elements to decrease irritation to the vein, provide more secure placement, increase the robustness of the overall system, and improve workflow. Procedures were also examined and improved based on the new equipment. Changes were made to the electronic data capture system (EDCS) to allow the majority of data to be collected electronically. The combined impact of the changes to the system was significant. The new jackets improved fit and decreased irritation to the skin. The jacket and catheter design allowed more procedures to be performed without sedating the animals. The technicians found the system and the animals much easier to work with and incidents of the animal being able to damage the catheter were eliminated. Wear and tear on the system decreased, including the infusion jacket, catheter line, and extension lines which had considerably less damage caused by the animals. Overall improvements to the tethered infusion system increased the level of animal wellbeing, decreased man hours needed per dose, and provided data that was easier to interpret.

P25 Application of the 3Rs in Animal User Training Programs: Implementation of an Inanimate Training Tool during Hands-On Training of Intracardiac Blood Collection and Injections

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In providing high-quality training to personnel working with animals, we must consider the needs of the trainee, the procedures being taught, and various guidelines from governing agencies, which include the concept of the three Rs. The Candian Council on Animal Care (CCAC) states that "animals are used in teaching in order to communicate scientific concepts, and they are used in training to develop manual skills and expertise in specific techniques (for example, animal handing or surgical skills). However, in some cases nonanimal teaching and training alternatives exist." In consideration of overall training objectives and in compliance with regulatory guidelines, we have created a training method that uses inanimate training tools for training of various nonsurgical procedures in rodents. Intracardiac (IC) blood collection and injection is a technique that was essential to include in this training method. For the IC procedure training, we set a goal to develop an effective and affordable tool to address the key learning issues (KLIs) that most challenged our trainees during hands-on training of the procedures. Further, we were motivated to replace the use of live animals with an inanimate tool during the early stages of training; thus, allowing trainees' to refine their skills on the tools before they move towards proficiency of the IC procedures on live animals. By accomplishing our goals, our belief was that trainees would be better equipped to translate their skills they learned on the tool to live animal practice; thus successfully reducing the number of animals required to master the skills needed to achieve proficiency in IC procedures. We provide an overview of the IC inanimate tool development and concept, describe the KLIs addressed with this training tool, and discuss the impact of the tool on the implementation of the 3Rs during hands-on training of the IC procedures.

P26 Modified Hebb-Williams Maze to Assess Affective State in Swiss Webster Mice

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Assessment of animal wellbeing includes evaluation of overall health, natural behavior, and affective state (for example, optimism versus pessimism). For mice, evaluations of health and natural behavior are relatively well qualified. However, assessment of the affective state has remained elusive. We evaluated a modification of the Hebb-Williams test to measure learning as a proxy for affective state. In this modified Hebb-Williams maze, the testing paradigm is simplified by assessing their performance on 2 days with 5 consistent maze configurations. Two groups of Swiss Webster mice were tested. One group was handled normally, while the other group was exposed to mild daily stressors at random times over 3 d. At the beginning of the study, all mice were acclimated to the testing apparatus with a single wall maze. Following acclimation, the stressors (for example, mild heat stress or brief forced swim) were initiated for this group. On day 5, the mice were tested with 3 mazes, with increasing complexity. Latency times to complete the maze were compared between groups. A terminal blood sample was collected, and the neutrophil:lymphocyte ratio and levels of serum corticosterone were assessed. There were no significant differences between the treatment groups of females, but the stressed males demonstrated significant increases in serum corticosterone, neutrophil:lymphocyte ratio, and latency time to complete maze 3. These findings suggest that the modified Hebb-Williams maze may be able to be used to identify negative affective states in male mice.

P27 Hydrodynamic Tail Vein Injection Refinements Resulted in Improved Animal Welfare

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Hydrodynamic tail vein injection is a rapid DNA administration in a high-volume diluent matching blood osmolarity, dosed at 10% body weight, and results in cellular expression of DNA due to extravasation. Homeostasis is restored as the body adapts over time to the volume overload following the injection but undesirable physiological side effects are seen in animals. The typical side effects are prolonged cessation of movement and shallow, rapid breathing. However, these side effects can escalate to include seizures, moribundity, and even morbidity. Multiple factors can lead to side effects including strain sensitivity, large body mass, and repeated hydrodynamic tail vein injections. Multiple strains (Balb/c, C57BL/6, SCID Beige, and CD-1) were evaluated to determine if the incorporation of isoflurane gas anesthesia would lessen physiological side effects. Strains were divided into 2 groups with the first group injected while conscious to provide the expected timeframe postinjection where physiological side effects were most likely observed. The second group was injected under isoflurane gas anesthesia and kept at a light anesthetic plane for the expected timeframe. Anesthesia was individually administered with a nosecone and mice were recovered postanesthetic in an oxygen chamber before transfer to a warming cabinet. Hydrodynamic tail vein injections of sterile saline were given weekly for five wk to both groups. The anesthetized group did not display the same range and severity of physiological side effects as were observed in their unanesthetized counterparts in 3 of the 4 strains evaluated. Isoflurane gas anesthesia dramatically lessens the physiological side effects of hydrodynamic tail vein injections in the Balb/c, C57BL/6, and SCID Beige strains. However, although an initial decrease in physiological effects was seen in the CD-1 strain, repeated hydrodynamic tail vein injections under isoflurane gas anesthesia were not tolerated.

P28 Animal Use Training Sessions and the 3Rs

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We examine the incorporation of the 3Rs into animal use training sessions (AUTS). Our program reduces the number of rodents needed for the training program through humane reuse of donated animals. Recently we have begun teaching a new bleeding technique (submental bleeds) that greatly adds to refinement. Finally, we have begun to expand our tissue donor program, which is used by many researchers enabling them to replace live animals with tissue generated from the training classes. Under the reduction section, a spreadsheet and cage cards used for tracking individual rodent procedures are illustrated, as well as a chart of the number of procedures each training animal is allowed before euthanasia. The tracking system allows maximum use of each donated mouse. In the refinement section, a description of teaching orbital bleeds (the primary method of obtaining larger quantities of blood at our institution) is contrasted to the newer submental bleed technique. The number of animals required for teaching orbital bleeds versus submental bleeds is examined. Preliminary results indicate an average of 15-20 mice is used for each successful orbital bleed taught. To date, only 1 animal has been euthanized during the teaching of submental bleeds and none for postbleed complications. The replacement section covers the growing tissue donor program. Testimonials from researchers illustrate how they are able to conduct research using donated tissue or carcasses obtained at the end of rat, mouse, or surgery labs without having to use their own animals. Some researchers are able to avoid having a colony of their own altogether. Implementation of these 3 programs allows maximization of the colony and minimization of painful procedures, while also directly benefits the researchers by replacing the number of live animals needed with in vitro tissue.

P29 An Improved Method of Implanting a Programmable Continuous Infusion Pump in Mice

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A study was conducted in which mice were to be continuously exposed to a drug for 2 wk. The constraints of the study required the surgical implantation of novel, relatively large programmable continuous infusion pumps in young C57BL/6J (44-d-old) mice. Sterile technique was followed in both the filling and surgical implantation of the pumps. The initial surgical procedure, performed under isoflurane anesthesia, began by making a longitudinal incision parallel to the lumbar vertebrae between the pelvic crest and last rib on the right flank. A subcutaneous tunnel was bluntly dissected in a cranial direction and the pump placed within as ventrally as possible. The antenna and delivery line were tacked in separate locations using nonabsorbable suture; the skin incision was closed with 7mm clips and skin glue. During the postoperative and experimental infusion period, the incidence of wound dehiscence was 56% and ulcerative skin lesions were 63%, thereby increasing the morbidity of the mouse. Both issues were considered to be the result of the large pump size, the relatively small size of the mouse, locations of the antenna and delivery line, and position of the pump that put significant pressure on the surgical incision. The procedure was refined with the goal of decreasing postoperative morbidity and creating a more viable research study. The surgical incision was changed to a transverse incision near the scapulae, caudal to the neck; presurgically suturing the antenna and delivery line together; and shortening the delivery line. After 2 wk the pumps were explanted and the mice recovered. Dehiscence and ulcerative skin lesions were decreased to 0%, therefore meeting our goal.

P30 Replacement of Ear Biopsies for Genotyping by Noninvasive Oral Swabs

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Ear biopsies for genotyping result in physical discomfort and pain experienced by the animal (*Oryctolagus cuniculus*). Here we describe the evaluation of oral swabs as an alternate method to obtain genetic material for genotyping of transgenic rabbits via polymerase chain reaction (PCR). The advantage of oral swabs is that no incision of any body tissue is necessary, but rather the constantly renewing upper layer of the mucosa lining of the whole oral cavity is used as a source of DNA material, analogous to human paternity or forensic testing. After several rounds of test establishment, analyzing relevant test criteria including material, equipment, handling, reproducibility, and reliability, we conclude that oral swab-based genotyping is, if not superior, of equal quality as conventional biopsy-based genotyping. Furthermore, we demonstrate the reduction of the potential risk of contaminating DNA "spill overs" during the skin biopsy preparation. The animal welfare principle of refinement has been successfully applied to reduce discomfort and pain of the research animal and to improve confidence of genotyping results.

P31 A 3Rs Impact in Rodent Health Surveillance

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Until recently rodent sentinel programs were limited by the available testing modalities which often required euthanasia of animals to obtain adequate samples for testing. With the advent of polymerase chain reaction (PCR) and dried blood spot (DBS) technology, it became possible to obtain samples for comprehensive testing without necessitating euthanasia of animals. This nonterminal method of testing 4 samples – a dried blood spot, an oral swab, a fur or cage swab, and a fecal sample – became known as the 4-sample method. In our AAALAC-accredited institution we evaluated this 4-sample method (4SM) of testing to determine the advantages and disadvantages compared to our previous method of whole body (WB) testing. Parameters that were analyzed during the evaluation process included a review of the pathogens on our bioexclusion list compared to the range of pathogens covered by the 4SM, the time needed to collect samples, the turn-around time for receiving results, cost, ease of use, and the quality of the test results. We collected DBS samples, fur swabs, oral swabs, and fecal samples on 16 mice and 3 rats and sent them to 2 different vendors for 4SM testing. We then shipped those same animals to a vendor for WB testing. The results demonstrated that all the pathogens on our bioexclusion list which could be tested for with the WB testing could also be tested for with the 4SM. In addition, the 4SM was lower in cost, had a faster turn-around time for receiving results, had equivalent or better accuracy of results, allowed for the ability to test colony animals without euthanasia, and provided the opportunity for a reduction in animal use since the same 2 sentinel animals could be resampled for confirmatory testing. Due to its numerous advantages, we ultimately chose to convert to the 4SM which also led to a 50% reduction of sentinel animal usage. In addition, once the testing results return as negative, all sentinel animals are available to be transferred for training use on our IACUC-approved rodent training protocol. This has also reduced the need to order animals for training creating a further reduction in overall animal usage in our facility.

P32 Establishing an African Green Monkey Dermal Fibroblasts System to Test Hypothesis on the Biology of Aging

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Aging-associated diseases are afflicting an expanding portion of the population as longevity increases. To improve health span it is imperative to develop therapeutic and preventative interventions to limit the impact of age-associated debilitations. This will be critically enabled by the development of preclinical test systems to evaluate the impact of age on biological processes. Dermal fibroblasts promise to provide a high-throughput in vitro system to evaluate mechanisms of aging as they consistently undergo damage accumulation and maladaptation. To establish such in vitro assays and the ability to interrogate the molecular basis of aging in a cell type highly homologous to humans, without terminal in vivo studies and associated animal utilization, we derived primary fibroblast lines from St. Kitts green monkeys (*Chlorocebus sabaeus*). Intrascapular biopsies were collected from infant (~5 mo), young adult (~10 y) and geriatric monkeys (~30 y), then cultured in vitro to generate primary dermal fibroblasts. Fibroblasts were expanded and cryopreserved at early passages to develop a sufficient cell bank for subsequent analysis. Quantification of cell growth behavior was performed in the initial phenotypic analysis of age-associated differences. Outgrowth of keratinocytes and fibroblasts were observed within 2-3 d after plating. The migration of cells and growth rate (time to confluency) were observed to be most rapid in the infant (2 d) and slowest in the geriatric (1.5-2 wk) cultures. Future studies will focus on structural and functional characterization of fibroblasts utilizing various cell metabolic assays to expand characterization of phenotypic differences and establish a platform for testing hypothesis about the molecular basis of aging.

P33 An Automated System for Positive Reinforcement Training of Group-Housed Macaques at Breeding and Research Facilities

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Behavioral training through positive reinforcement techniques is a well-recognized refinement to laboratory animal welfare. Behavioral neuroscience research requires subjects to be trained to perform repetitions of specific behaviors for food/fluid reward. Some animals fail to perform at a sufficient level, limiting the amount of data that can be collected and increasing the number of animals required for each study. We have implemented automated positive reinforcement training systems (comprising of a button press task with variable levels of difficulty using LED cues and a fluid reward) at the breeding facility and research facility to compare performance across these different settings and to prescreen animals for selection and refine training protocols. Animals learned 1- and 4-choice button tasks within weeks of home enclosure training, with some interindividual differences. High-performance levels (~200-300 trials per 60-min session at ~80% correct) were obtained without food or

fluid restriction. Moreover, training quickly transferred to a laboratory version of the task. Animals that acquired the task at the breeding facility subsequently performed better both in early home enclosure sessions upon arrival at the research facility and also in laboratory sessions. Automated systems at the breeding facility may be used to prescreen animals for suitability for behavioral neuroscience research. In combination with conventional training, both the breeding and research facility systems facilitate acquisition and transference of learning. Automated systems have the potential to refine training protocols and minimize requirements for food/fluid control.

P34 Air-Activated Hand Warmers as an Alternative to Conventional Warming Methods for Intravenous Tail Injections in Mice and Rats

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Constant improvement of technical procedures is a key component in all great animal research programs. As a small team with increasing husbandry and study support demand, we are constantly looking for ways to refine procedures to minimize animal distress and optimize time. One of the major areas for improvement identified by our team was intravenous tail injections in mice and rats. Common concerns associated with this procedure are inconsistent heating methods that can lead to potential animal welfare issues such as dehydration, overheating, and undue stress. In order to reduce these issues and improve the procedure, vivarium staff members proposed air-activated hand warmers in replacement of heat lamps and warming boxes to dilate the tail vein in both mice and rats prior to injection. An in-house comparison study using 60 C57BL/6 mice and 30 Sprague Dawley rats conducted between these 3 heating methods showed distinct advantages for the use of air-activated hand warmers over the more commonly used methods. Unlike with heat lamps and warming boxes, the hand warmer process does not require postprocedural veterinary care as the warming process is only momentary, lasting roughly 15-30 s depending on species. Hand warmers maintained a constant temperature through our longest procedural time of roughly 2 h with no replacement required. To date, there have been no adverse effects observed associated with the use of hand warmers for tail vein dilation. Once animals are placed into a restraint device, a hand warmer is immediately applied and the animal is dosed. This reduces the overall amount of handling and transferring between cages experienced by the animal. In summary, air-activated hand warmers are a viable alternative to conventional heat lamps and warming boxes when used for vein dilation in mice and rats for intravenous tail injections.

P35 Effects of Music Enrichment on Individually Housed New Zealand White Rabbits

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Beneficial effects of music exposure have been demonstrated in many species, including humans, dogs, cats, livestock, rodents, and fish. As yet, no evidence has been presented supporting the use of music enrichment for rabbits. We hypothesized that outcomes would be similar to other captive prey species, demonstrating lower cortisol and stress leukogram levels. Using an existing colony of 5 geriatric male New Zealand White rabbits, samples were collected during routine health checks for baseline (before music) fecal cortisol and leukogram levels. We then provided soothing music via a commercial rabbit-targeted CD Monday through Friday during the work day. After 6 mo, leukogram and cortisol levels were again collected at routine wellness checks. Music enrichment was stopped, and final levels were checked 6 mo later. Each rabbit in the study served as his own control before and after music enrichment. Fecal cortisol decreased significantly from baseline, when music was provided ($P = 0.002$). When music enrichment stopped for 6 mo, fecal cortisol levels rose significantly, compared to levels during which music was provided ($P = 0.0466$). This study was a good first step into providing evidence that music enrichment benefits rabbits. Unfortunately, we found no effect on stress leukogram, perhaps owing to the rabbits' advanced ages. All rabbits maintained cortisol levels over the normal range in all phases of this study, suggesting that they were in a chronic state of stress not completely ameliorated by music. Future work should expand upon the physiologic and behavioral effects of music enrichment. Resting versus active postures have been shown in dogs to be affected by music enrichment and could be observed in rabbits. Heart rate and blood pressure could also be measured as a part of future studies. To best cope with the demands of a laboratory setting, a comprehensive enrichment strategy would be more appropriate than any single intervention. Still, music enrichment has minimal cost, easy application, and lack of demonstrable negative effects that endorse it as a good place to start.

P36 Benefits of Play and Fraternization in a Rat Training Colony

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We were concerned that our training colony rats, due to repeated exposure to nonproficient handlers, associated humans with discomfort. This could lead to more fractious rats, as well as increased distress for both parties. We predicted that socializing training rats would result in lower stress levels and increased fraternization (a rat voluntarily approaching a human's hand). First, baseline blood was collected from the existing cohort of experienced and aged Sprague Dawley rats as a nonsocialized control. We then divided the rats into 2 groups. One group was hand fed a treat in cage 3 times a week and one group was played with by the head researcher 3 times a week. During round 2, we replaced the colony, predicting that younger rats would be more moldable to positive human interactions. Again, we collected baseline blood. We divided these rats into 3 groups: hand treat only, played with by the head researcher, and a group played with by 3 individuals. The third group was added to examine if the rats only became comfortable with the head researcher and positive interactions would not translate to other handlers. In both rounds, socialized conditions were provided for 4 wk, after which blood (via medial saphenous vein) and approach latency times were collected. Final stress leukograms (NE:LY) from those played with in round 1 showed slightly decreased levels, although not statistically significant. Approach latency also showed quicker times for those rats which were played with, but not with statistical significance. For the younger rats in round 2, the final CBC showed statistical significance in the group played with by head researcher for NE:LY ($P = 0.0136$), supporting that socialization leads to lower stress levels. Approach latency times for both play groups were statistically significant when compared to the treat only group ($P = 0.0042$). Compared to rats that were only hand fed a treat and not taken out for play, socialized rats were much quicker to approach a stranger's hand. While not showing decreased NE:LY levels, the group played with by 3 different people were quickest to investigate. We believe that the 3 handlers had different comfort levels with the rats, and these rats seemed more willing to gamble on fun times with a stranger.

P37 The Effects of Mouse Tunnels on Intake and Output of Mice Housed in Metabolism Cages

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Metabolism cages are typically nonenriched, grid-bottom cages with no nesting materials present for single animals, which can all be environmental stressors to rodents. We investigated the effects of the placement of a mouse tunnel within the metabolism cage on body weight, the amount of urine and feces collected, and food consumed by mice to determine if this device could be used as a refinement for this caging without impacting research outcomes. We also assessed the amount of time spent within the mouse tunnel. Adult female C57BL/6 mice were housed in either a typical metabolism cage or a metabolism cage with a mouse tunnel and all were provided with food and water. After a 4-d acclimation period, body weight, urine and fecal output, and food intake were measured daily for 4 d. Those cages with tunnels were recorded for 3 h daily with assessments occurring every 10 min to evaluate the use of the mouse tunnels. Mice were observed to spend approximately 60% of their time within the mouse tunnels during the light period. There were no significant differences in food consumed, urine and fecal output, or food intake between mice housed in metabolic cages with or without a mouse tunnel. The inclusion of a solid surface in a grid-bottom cage is a refinement for this type of housing commonly used for metabolic and pharmacokinetic studies without affecting the body weight, amount of urine and feces collected, or the amount of food consumed by mice.

P38 Use of Vaginal Impedance Measurements to Stage Estrous in Rats Given Luteinizing Hormone Releasing Hormone

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Estrous monitoring is one way to potentially assess whether rats are in the correct stage of estrous prior to mating with either intact or vasectomized males in order to ensure successful timed pregnancies or induce pseudopregnancy in ET recipients, respectively. Vaginal cytology is the oldest method of staging estrous in female rats; however, it requires technical skill beyond that of the average laboratory or animal care technician and is subject to interpretation based on the person performing the cytological exam. Vaginal impedance offers a quicker and less technical alternative to cytology. A slim, metal rod inserted into the vagina is used to measure the fluctuations in the inherent electrical resistance of the inner lining of the vaginal wall during different stages of estrous. Vaginal impedance measurements have been used successfully in normally cycling and breeding female rats. Our hypothesis is that vaginal impedance measurements can also be used to stage estrous in female rats hormonally primed with luteinizing hormone releasing hormone (LHRH). Twelve adult female Sprague Dawley rats were injected with LHRH intraperitoneally. Three days later, vaginal impedance was measured and vaginal cytology was performed. The next day, females were placed with proven Sprague Dawley males for breeding. Early the next morning, females were separated from males and checked for vaginal plugs. All females were euthanized at day 10 postmating to assess breeding success. In summary, this study simulates the steps taken to produce pseudopregnant females or ensure timed pregnancy and evaluates the effectiveness of using vaginal impedance measurements to stage estrous in female rats given LHRH to synchronize estrus. Results revealed that vaginal impedance measurements are equally as successful at identifying proestrus in the rat as vaginal cytology; however, impedance measurements were quicker and less technically challenging. Successful estrous staging using vaginal impedance has the potential to reduce animal numbers by increasing efficiencies and reducing costs due to downstream failures related to the use of animals that are not in the correct estrous stage when mated.

P39 Assessment of Barne's Maze Protocols Indicates that Fewer Test Sessions and a Single Aversive Stimulus Works Well

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The Barne's Maze test (BM) is a cognitive test for rodents that addresses spatial learning and memory and was developed by Carol Barnes as a physically less demanding alternative to Morris Water Maze test. In the BM, subjects are exposed to a brightly lit (500-700 Lux) open circular space elevated above ground with the possibility to find an escape hole among a series of symmetrically spaced holes decorating the space periphery. Loud noise and/or wind are often added as aversive stimuli. More recently Attar and colleagues developed a shortened protocol which they claimed was more sensitive to detect early cognitive disturbances. Here we evaluated a shortened version of BM developed by Attar et.al. and furthermore the impact of adding aversive stimuli as loud noise (90 dB) and wind, respectively. Three to 20-mo-old C57Bl/6J female and male mice were tested (n=59). For comparison, female Swiss RjOrl mice were included (n=16). The aged C57Bl/6J mice were tested for startle response prior to BM and due to compromised hearing, these mice were not tested with loud noise as added aversive stimulus. The results were similar with the original BM and the shortened version of BM. Performance in the BM was not different between age groups, gender, or strain tested with the exception of aged C657Bl/J6, which showed an impaired reversal learning. We could not detect any marked difference in learning when adding loud noise or wind but considering body positioning and moving velocity, loud noise seems to be more stressful. The shortened version of BM produce results consistent with the longer and more elaborate original protocol. No clear benefit could be detected when adding wind or the more stressful loud noise as aversive stimuli. Both sets of observations suggest that the refinements of cutting down the extent of test sessions and avoiding additional stressor are useful for the strains tested here.

P40 Enterprisewide Animal Governance

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Animal work is conducted in many locations worldwide with varying laws and differing cultural views with respect to such work. Our corporate policy defines principles for the care, welfare, and treatment of animals, independent of location. Our scientific and ethical principles, consistent with company values and a program of enterprisewide governance ensure we apply high standards of animal welfare to the development of novel and valued treatments in pursuit of our mission to enable people to do more, feel better, and live longer. Within our governance framework, 2 elements, namely, enterprise oversight and independent business monitoring will be the key topics of focus. Enterprise oversight is accomplished through our global Animal Welfare and Quality Council (AWQC) which serves as the governance board for assessing and mitigating the risks associated with animal work, ensuring an effective overarching risk management process. The AWQC endorses cross-business written standards and provides enterprise oversight and coordination. The AWQC contribute to the development of strategies and plans initiated by the Office of Animal Welfare Ethics and Strategy (OAWES) and are advisory the global risk owner for animals and chief of animal welfare ethics and strategy. Independent business monitoring within OAWES is a formal program of quality assurance tied to the care, welfare, and treatment of animals risk area. Enterprisewide process assessments are conducted by looking across multisite animal care and use programs. Monitoring against policy, procedures, laws, regulations, and guidance in relation to animal work provides management with up-to-date information on the current status of internal control and supports the identification of risks and business issues. Being respectful of local differences and by adopting our pillars of professional judgement, performance standards, and harmonized approaches have been conducive to the success of the monitoring program.

P41 Improving Intramuscular Injections in Small Non-Rodent Animal Models: A Demonstration of Technique in Ferrets and Felines

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IM injections are used for delivery of sedatives and test materials. Consistent IM injection technique provides improved data quality and decreases possibility of tissue damage. Felines and ferrets have limited muscle mass and are used in injection animal studies, making them ideal candidates for development of improved IM injection methods. Three separate IM injection protocols compared needle length and angle of approach in hind limb and epaxial muscles in a euthanized feline and in the hind limb in euthanized ferrets. Tissue dye, in a maximal standard dose volume, was used to mark the injection site. Injection sites were dissected and tissue staining compared to determine correct approach, needle length, and site for optimal future dosing. In the feline, injections with longer (5/8 in.) needles in the hindlimb or epaxial muscles resulted in delivery of injecta at the deep margin or even outside the muscle, in the deep fascia. In the hindlimb, use of a 5/8 in. needle or dosing with a caudal to cranial approach resulted in delivery of material around the sciatic nerve when injecting the biceps femoris, a standard IM injection site. A lateral approach and a shorter (3/8 in.) needle were successful in keeping the injected material within the target muscle in both feline and ferrets. Use of a short (≤3/8 in.) needle is sufficient for delivery of injected material into the biceps femoris, quadriceps, or epaxial musculature of small non-rodent model species. Injections in the hind limbs should utilize a lateral approach to avoid delivery outside the target muscle or damage to the sciatic nerve. Proper care and attention to details of injection approach, anatomy, and reduction of needle length will decrease potential trauma and produce more consistent dosing.

P42 Impact of Laboratory Animal Science Training on Scientists' Attitudes and Practice

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The implementation of principles and guidelines that govern the different areas of research in an educational institution is one of the key factors for international recognition of its research integrity and value. The privilege

of conducting research using animal subjects requires adherence to international regulations and standards governing the humane care and use of laboratory animals. Our IACUC found that it was critical to have an animal care and use training program to raise their researchers' understanding and knowledge. Recently, the IACUC designed a training program in the principles of laboratory animal science and the ethical issues involved in animal use. This study aims to measure the impact of such training on scientists' attitudes and practice. During 4 successive training courses, the participants (n=100, 72% females and 28% males) were surveyed in a self-administered questionnaire during the course. The participants were surveyed with the same questionnaire twice; the first one before the introductory of the course and the second one after the completion of the course. Questions were focused on ethical consideration for care and use of animals in research, ethical committees, international guidelines for humane care of animals, and 3Rs concept and its interpretation. The results revealed that the scientists' knowledge and awareness increased effectively after the introduction of the training courses. The scientists gained knowledge about the 3Rs. They recognized the importance of standardization of animal handling on their scientific results, and finally they can differentiate between different ethical committees and their roles. It is clear that establishment of training program in the principles of laboratory animal science and the ethical issues is a valuable means of educating and raising awareness about animal welfare. The legal requirement for training those responsible for designing and performing animal experiments may thus contribute to improving and harmonizing animal research practice. Training leads to standardization of animal care and use practices that is vital for reproducibility of results fundamental to quality scientific research.

P43 Comparison of Gavage Needles in A/J and CD-1 Mice

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Oral gavage is a common laboratory animal procedure for administering liquid compounds directly into the stomach. Gavage needles come in multiple sizes and lengths and should be equal to the distance from the mouth to just beyond the last rib. During a recent study, complications occurred during oral gavage dosing that were new to our technical team. The same feeding needles that have routinely been used, a flexible stainless steel 20 gauge, were causing esophageal bruising and perforations despite being appropriate size for the weight/age of the mice on study. The strain of mouse used in this study (A/J mice) had not previously been used in dosing studies in our facility. A colleague notified us of similar difficulties with this strain of mice. We conducted a comparison study of 4 different types of gavage needles using 2 strains of mice (A/J and CD-1). To rule out operator error, mice were divided into groups and each assigned to 1 operator for the duration of dosing. Mice were dosed via oral gavage twice daily, 8 h apart, with PBS for 5 consecutive days. Body weights were recorded daily for 7 consecutive days. No obvious differences were observed between operator groups. The plastic shaft 22 gauge gavage needles resulted in the lowest degree of body weight loss across both mouse strains with very similar results from the plastic shaft 20 gauge. There was no mortality in either of these groups. The flexible stainless steel 20 gauge had an overall 14% mortality and although weight loss is observed during active dosing days, weight gain resumes when dosing is completed. The nondisposable stainless steel 20 gauge needle was removed from week 2 of training after 83% mortality during the first week. In general, the CD-1 mice tolerated oral dosing better than the A/J mice. This indicates that researchers should be aware of strain-to-strain variation in tolerance to the oral gavage procedure and choose gavage needles accordingly.

P44 A Clinical Pathology Comparison of Lubricated And Unlubricated Saphenous Blood Sampling in Rats

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Application of sterile ophthalmic ointment or lubricant to the skin is a common practice to optimize saphenous blood volume collection in rodents. However, blood contact with the lubricant during collection results in contamination of the blood sample. To determine if lubricant contamination affects downstream clinical pathology results, blood samples obtained using lubricant versus nonlubricated saphenous venipuncture technique from 12 age-matched, female Sprague Dawley rats were obtained, and complete blood counts (CBC) and serum biochemistry analyses were compared. The right saphenous vein was used for unlubricated sampling and the left saphenous vein was used for lubricated blood sampling. The unlubricated collection sites yielded significantly lower collection volumes, increased clotting times, and required increased restraint time compared to lubricated collection technique. Lubricated collection sites yielded a significantly increased sample volume. Lubricated collection sites decreased restraint time, and a minimum required sample volume was easily obtainable. Upon visual inspection, samples from unlubricated collection sites were observed to be 1 to 2 grades of hemolysis greater than their lubricated counterparts. The clinical pathology results depicted no significant change in CBC or serum chemistry values, suggesting that saphenous blood collection from lubricated skin does not artifactually significantly alter blood values. However, using lubricant in blood collection does yield increased blood volumes, decreases hemolysis in serum samples, and decreases required restraint times to achieve acceptable blood collection volumes. When training individuals to sample blood for analysis, consistency among sampling and lubricant use remains an integral factor to limit variability.

P45 Three-Dimensional Model for Teaching Body Condition Scoring in Mice

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As members of the laboratory animal science community, we are constantly looking for alternatives to animal usage whenever possible. Practical, noninvasive methods for assessing the health status in mice are particularly useful and common methods include clinical and behavioral observations, monitoring weight gain/loss, and body condition scoring. Body condition scoring is an effective way to monitor the wellbeing of mice and is frequently used by veterinary staff, researchers, and technicians at many institutions. For best results, training is required for consistency between personnel performing the technique; however, finding mice that exhibit the full spectrum of the universal body condition scoring system (BC1: emaciated through BCS5: obese) can be challenging. Mice that exhibit body condition scores of 1 (emaciated) and 2 (underconditioned) are hard to find at most institutions due to their poor condition and the ethical and humane concerns that arise for keeping them for training purposes. Therefore, we have created a three-dimensional (3D) mouse model from puppets, and other easily acquired materials, that exhibit the 5 stages of body condition scoring. These puppet mice can be reused, are easy to make and transport, and alleviate the need to use live animals for body condition training. These mice have been introduced into our institutional training program and have received high praise. We hope to share our idea with others in the laboratory animal science field. Our invention helps to decrease mouse usage while still maintaining a high level training program for body condition scoring.

P46 Cecal Ligation and Puncture Model in Rats for Infectivity Testing of Medical Devices

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The 2002 version of the Food and Drug Administration's (FDA) *Guidance for Resorbable Adhesion Barrier Devices for Use in Abdominal and/or Pelvic Surgery* lists several special considerations for testing, including evaluating whether an implanted medical device may increase rates and/or severity of sepsis. The potential for a device to enhance sepsis is to be evaluated by challenge with gut organisms in the presence and absence of the test device and comparing rates of mortality and abscess formation between groups (for example, infectivity testing). Since the FDA guidance does not specify a preferred model for infectivity testing, a desirable and reproducible model needed to be developed. The cecal ligation and puncture model (CLP) was identified via a literature review as an acceptable animal model for evaluating infectivity potentiation. While CLP models generally have lower mortality rates than other models, specific CLP procedures and mortality rates varied between publications. To design a model with high reproducibility for these endpoints, we tested the effect of multiple variables on CLP mortality and abscess formation rates. The variables identified in pilot studies as most important in producing low mortality and consistent abscess for-

mation were the number of puncture holes created, needle gauge used for puncture and fasting vs. no fasting. These variables were incorporated into the final model design, which was shown to be highly reproducible for low mortality and consistent abscess formation. The finalized CLP model was tested in a definitive study using a resorbable adhesion barrier device and the model was found to meet the requirement for infectivity testing of resorbable adhesion barriers.

P47 Highlighting 3Rs Progress: Creating an Alternatives Knowledge Notebook, Dashboard, and 3Rs Champions to Advance Our 3Rs Culture

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With growing public opposition to animal research, it is more important than ever for the biomedical research community to share our progress with adopting alternatives, known as the 3Rs. We adopt refinements, reductions, and replacements, but often fail to give ourselves credit. We must find ways to emphasize the ethical nature of animal research and our ongoing progress in adopting the 3Rs. Our company has a fulltime 3Rs scientist/coordinator as well as an alternatives committee. Despite these dedicated efforts to promote the 3Rs, we had no way to catalog our 3Rs advancements. The public is largely uninformed about biomedical research but once educated, a majority quickly supports animal research if animal welfare is upheld. Alternatives are a foundational element of a strong culture of animal welfare. Having a way to highlight these alternatives helps us to share meaningful examples of 3Rs impact. We worked with our colleague from the data solutions group to develop a knowledge notebook (KN) to collect and categorize our alternatives efforts. A KN is a highly configurable and collaborative workspace that can capture and manage a variety of important information. In this case, alternatives that have been implemented and the efforts we are making to promote the 3Rs such as publications and consortia involvement. In addition to the KN, our colleague developed a dashboard that would pull the metadata and information from the notebook, creating graphs and charts, helping to paint the picture of our 3Rs progress in a more meaningful way. To facilitate input into the KN, we asked managers to appoint 3Rs champions from each therapeutic research area, serving as experts about their 3Rs efforts. We are learning about new alternatives as we gather information from our widely diverse research teams. This information will help us to better recognize and reward individuals or teams that have adopted the 3Rs. It will also help us to highlight the historical information and the progress we are making in replacing animal models with less sentient species or nonanimal methods, refining our methods to minimize pain or distress, and reducing our overall reliance on animals.

P48 Development, Refinement, and Optimization of the Rat Tail Vein Infusion Model

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Researchers needed the capability to deliver compounds intravenously in tumor-bearing rats, but surgically cannulating the rats posed several risks. A pilot study was performed with 6-8-wk-old female nude rats (n=6) to assess the feasibility of our tail vein infusion model, using 2 different catheters. The rats were placed into a restrainer and catheters were placed into a lateral tail vein. Once placement was confirmed the infusion line was connected to the catheter, a stainless steel tether was placed over the infusion line and secured to the tail. The rats were placed into their home cage and the tether was connected to the swivel and infusion line above the cage. During the infusion, the rats had free mobility and access to food, water, and enrichment. We determined which catheter was best suited for up to 8- or 24-h infusions based on how well the catheters were tolerated for the duration of the 24-h pilot study. The catheter identified for up to an 8-h infusion reduced animal restraint time from 7 to 1 min for catheter placement. Animal welfare benefits of this model versus surgically cannulated models include its minimal invasiveness (no surgery or anesthesia required); the animals are harness free during infusion; multiple dosing sessions are possible, resulting in fewer rats being used; and there is an immediate return to social housing postinfusion. To date, we have used this model in approximately 500 male and female Han Wistar and nude rats. The rat tail vein infusion model has the potential to be applied to any body of work that has the necessity for infusion administration but cannot be bound to the restrictive time constraints imposed by surgically prepared animals, such as catheter patency and maintenance. This model has added value to our oncology portfolio in areas of both efficacy and safety, helping to assess margins in therapeutically relevant dosing regimens.

P49 Physiological Response to Ear Punch Is Equivalent to Routine Husbandry

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Ear punching has proven to be a useful tool to laboratory investigators, both as a means of obtaining tissue samples for PCR genotyping, as well as in the identification of socially housed rodents. While the practice of ear punching is commonplace in laboratory settings, minimal research has been done to evaluate the potential stress and/or discomfort to subjects receiving an ear punch. As part of a parent study exploring the influence of environmental enrichment on various neuroendocrine markers, 6 adult female Swiss-Webster mice, equipped with ETA-F10 radiotelemetry devices, had their ears punched to allow for ease of subject identification. In addition to being handled for ear punching, mice in the study were periodically handled to obtain their weight and to collect fecal pellets for corticosterone analysis. In order to allow for the direct comparison of heart rates, telemeters were left running; for both the events involving ear punching and the handling events that did not. Independent samples testing of the radiotelemetry data show no statistical difference in average heart rate between handling when ear punching and for routine husbandry (P= 0.771). Further, by comparing the average heart rates on days the mice were not handled to average heart rates on the day mice received an ear punch, we were unable to identify evidence to suggest that ear punching results in a statistically significant increase in average daily heart rate (P = 0.786). While the raw data show an increase in heart rate at the time mice are handled, there was no evidence to suggest a longer recovery period to baseline heart rates following ear punching, as opposed to being handled for routine husbandry procedures (P = 0.897). Collectively, these data suggest that ear punching does not represent a significant welfare concern to laboratory mice.

P50 Target Training Pigs within an Isolation Unit: A Pilot Study

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In our high containment facility we aim to control and prevent exotic viral diseases of livestock. For porcine diseases we use female, large, white pigs between 6-8 wk old. Weighing them for the studies involves ushering them in and out of a weigh crate. This is usually stressful for both staff and pigs and could take a long time. The aim was to introduce target training using positive reinforcement as a way of refining this procedure to reduce stress and shorten the time taken. The pigs were required to touch a ball (the target) with the end of their nose to receive a food reward (positive reinforcement). After a few consecutive touches (~5-10) the pigs clearly understood the task and the target could then be moved around for the pigs to follow. This meant we could put the target into the weigh crate and the pigs would walk in to touch it and receive the reward. Adding the method of target training as a refinement to this procedure means the pigs now enter the weigh crate on their own terms which reduces their stress levels and improves welfare. Staff who were not involved in the training commented on how much easier it was and felt like it took less time overall. We also hope to be able to use this training with regulated procedures and also with other species in the future.

P51 Employing the 3Rs in Surgical Skills Training

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A neurosurgery lab was established to facilitate hands-on training of neurosurgery residents and fellows. The laboratory employs mixed-modality training modules ranging from high-fidelity, nonbiological simulators to live animal models. Here, we demonstrate how our use of nonbiological

simulators optimizes the need for live animal models, and thus inherently follows the principles of the 3Rs across a variety of neurosurgical training scenarios. The cadaveric duck wing model has replaced the requirement for live rats during the initial stages of training. Brachial vessels are perfused with dye enabling students to visually skeletonize and perform anastomoses using a surgical microscope. Once the duck wing model has been mastered, the student advances to using the anesthetized rat for femoral vascular anastomosis. A spine laminectomy-durotomy model has been developed for teaching a common microsurgical procedure. Polyvinyl alcohol, and in addition to bovine abattoir pericardium, has been demonstrated to effectively mimic the tactile properties of the dura during durotomy and closure. This model eliminates the requirement for live pigs. Brain tumor and stereotactic models are being developed as training simulators. Rats scheduled for euthanasia are acquired from other investigators. Pigs used for endovascular aneurysm coiling, stent, and embolization training are often used for other acute resident training such as craniotomy, durotomy, and brain retractor placement which decreases the number of pigs by half. Animals are housed for 72 h with environmental enrichment to avoid stress. The day of surgery a general anesthetic is given with continuous hemodynamics measurements until endpoint. Overall, our laboratory effectively employs the 3Rs principles across a diverse range of surgical training modules while in parallel fostering the development of technical skills outside of the operating room theatre.

P52 Reducing Stress Associated with Hand-Catching Owl Monkeys (Aotus nancymaae)

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Reducing stress in nonhuman primates through positive reinforcement training improves animal welfare and benefits researchers and husbandry staff. Desensitization prior to the start of an experiment is 1 way to reduce stress associated with experimental protocols. An experiment requiring hand catching 3 male/female pairs (n=6) of owl monkeys daily for blood draws and injections was scheduled. Approximately 2 to 6 wk were allotted for positive reinforcement training before the start of the study. Leather gloves were worn for hand catching the monkeys, so a plan involving familiarizing the monkeys with the gloves was drafted and implemented. The placement of the gloves in proximity to the monkeys progressed from being within view outside the cage to touching the monkeys inside the cage. The monkeys were rewarded with highly valued treats as they tolerated each step of the plan. Group A (n=2) had 14 d of positive reinforcement training, which consisted of 14 training sessions. This group showed signs of anxiety and fear during handling as true desensitization was not established. Group B (n=4) had 42 d, or 40 sessions, for desensitization. This group progressed through most of the steps in the plan before they were subject to daily hand catching for procedures. These monkeys were used to the presence of the catch gloves and thus showed little or no signs of stress when being caught and restrained. The time spent out of the cage for blood draws and/or injections was also reduced because the monkeys in Group B were less resistant to handling. This allowed the researchers to spend less time in the housing room, thus reducing stress further. In order to reduce as much stress as possible, it is recommended that a desensitization plan is completed prior to the start of future studies.

P53 Successful Behavioral Monitoring of Nonhuman Primate Colony Using a Multiaction Approach

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Behavioral monitoring for abnormal behaviors has always been, and will always be, a vital part of primate behavioral management. However, with increasing scrutiny from sponsors and regulatory agencies, documentation of these activities while maintaining inter-observer reliability, efficiency, and easy-to-understand records is critical. In order to improve the monitoring our colony of nonhuman primates, multiple strategies were initiated to construct a more defined behavioral monitoring program. The approaches included refining abnormal behavior definitions and observation techniques, retraining of vivarium staff, placing notification tags on cages of animals on behavioral treatment, and implementation of an electronic data management system. Refining and retraining provided more consistent behavioral abnormality reporting for animals but did not account for animals without any noted abnormal behaviors. Implementation of the tagging system reduced duplicate submissions of behavioral assessment requests, thus saving time for both the submitting and receiving parties. Using an electronic monitoring program which interfaces with our electronic animal records, staff responsible for behavioral monitoring are able to quickly assess all colony animals for basic behavioral parameters (alopecia, fecal smearing, and pacing). This data is then automatically updated to the animal's electronic health record. The combination of these strategies has lead to a significantly stronger behavioral monitoring and more robust behavioral program.

P54 A Veterinary Technology Student Outreach Program to Increase Awareness of Careers in Laboratory Animal Science

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Veterinary technicians are uniquely equipped for careers in laboratory animal science (LAS). However, many veterinary technology degree programs may incorporate only minimal exposure to LAS in their didactic programs. To increase awareness of the many career opportunities in LAS for veterinary technicians, we developed an outreach program in collaboration with a veterinary technology program at our state university. This outreach program consists of an introductory lecture to second-year veterinary technology students focused on veterinary technology career opportunities at our institution. Additionally, on an annual basis, we host third-year veterinary technology students for an onsite campus visit. During this full day visit, the students learn more about biomedical research and the differences between companion animal practice and LAS. The students also participate in a hands-on workshop in mouse handling and biomethods from various members of the veterinary staff. Another component of our outreach program includes providing clinical externship opportunities to those veterinary technology students who wish to spend more time at our institution gaining hands-on experience working with mice. Although the main goal of our outreach efforts is to increase awareness regarding fulfilling careers in LAS for veterinary technicians, this program has also been very valuable to our institution and has helped us successfully recruit talented veterinary technicians to our institution. Based on the success of our current outreach program, we see this as a first step to expanding our program to all veterinary technology programs in Maine and other New England states in the future.

P55 Ensuring Ethical Animal Use and Care Globally: What Happens When There Is Not an Established Oversight Body?

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Animal research, testing, and teaching is an integral part of developing safe and effective medications and is mandatory to fulfill regulatory requirements. In order to meet ethical and regulatory obligations, our facility maintains a corporate policy on animal care and use. This policy demonstrates our absolute commitment to the ethical and humane treatment of animals and alternatives to animal-based biomedical research. In most countries, the role of an ethical oversight body or an IACUC is to ensure animal welfare compliance. Our research, testing, and teaching is global in nature and encompasses many species that may fall outside of the scope of an ethical oversight body. For this reason, it is necessary to ensure that research, testing, and teaching is conducted ethically. This is independent of where the research, testing, or teaching takes place or what animal model is used. Additionally, we seek to minimize business risk by ensuring ethical review of protocols takes place prior to activities involving animals. Where an ethical oversight body or IACUC is not available, we have established regional ethical review boards (ERB) located at multinational sites. The ERB is responsible for reviewing study protocols that are not under the governance of another ethical oversight body. This ensures animals are used in a responsible and ethical manner. The ERB reviews protocols for statistical rigor in the experimental design and analysis and ensures animal procedures are conducted according to the Global Animal Care and Ethics Council Guidelines. The ERB role ensures that in vivo activities have ethical review prior to commencement of planned work, in accordance with

the corporate policy and in line with humane care and use publication requirements by many peer-reviewed journals.

P56 Laboratory Animal Science Training South America: An Experience from the Academic Arena

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Latin America lags behind in adopting many modern practices concerning care and use of animals in research, academic, and teaching practices. The region's economy generally does not help to improve this situation and contributes to the problem. There is poor funding, leading to lack of specialized equipment, lack of adequate facilities, animals of uncertain genetic background and health status, and insufficient staff qualified as LAS trainers. The available educational tools are intended for online training and most of the resources are in English. Moreover, there is a significant deficit of hands-on style training opportunities for undergraduate and graduate students of scientific disciplines, as well as for technicians in laboratory animal science. Wesought to update and renew the animal care and use practices at our university by starting a program to train fellows and technicians with any previous level of expertise in routine operations at the facility, knowledge of biological data of different species, animal handling, substance administration, sampling, welfare, and euthanasia. The goal of the program was to offer training according to updated literature and techniques to refine and support ongoing research, solving many problems due to poor, outdated, or no training at all. This program was implemented in 2015. The program consists of 2 theoretical modules, a visit to the animal facility, a hands-on session, and a final integrative evaluation quiz. Thus far, 170 people have taken and approved the course, being certified to go into and to work at the facility. In addition, the IACUC asks for the completion of this program in order to approve the qualification of researchers. We believe that is it possible to implement a specific training offered in LAS in the academic arena. It took time and dedication but it represents a benefit that will be reflected in the daily work with animals, the animal welfare, and the research results.

P57 Colitis Index Scoring on Il10^{tm1Cgn} Mice Model of Inflammatory Bowel Disease

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Inflammatory bowel disease (IBD) includes a set of pathologies that result from a deregulated immune response. A murine model, which has been extensively used to study IBD etiology, is B6.129P2-*Il10*^{tm1Cgn}/J. These mice spontaneously develop chronic inflammatory bowel disease (IBD) with an incidence of 100% by 3 mo of age. Clinical signs of inflammation are diarrhea, perianal ulceration, intestinal bleeding, and rectal prolapse. Intestinal histopathology shares some features with human Crohn's disease. In these mice, the entire intestinal tract can be affected; lesions are segmental and variable but duodenum, proximal jejunum, cecum, and proximal colon are the most severely affected tissues. In order to assess welfare in ongoing protocols, a disease activity index (DAI), body weight, and hydration status are currently used. However, spontaneous development of colitis has not been related to a decrease on DAI, although rectal prolapse results in humane endpoint. According to the data collected during a year from our mice colony, rectal prolapse occurs on females around 18 wk and males at 20 wk, but occasionally females between 6 to 8 wk showed a reversible rectal prolapse. For this reason, colitis score for this model was performed with 24 mice, using a scale from 0 to 3, in order to assure the early detection of colitis and the adequate decision of euthanasia. We established the colitis score that include the following parameters: rectal alterations (normal, edematous, or mild rectitis; intermittent rectal prolapse; permanent rectal prolapse), stool consistency (moist, soft, diarrhea/absence of feces); and bleeding in stool (negative/positive) and were defined as normal (0), mild (1), moderate (2), and severe (3). We found that 100% developed mild and moderate colitis along its life, but not necessarily result on permanent rectal prolapse. The developed system allowed us to better identify humane endpoint and the severity of colitis. Since animals were usually euthanized when the colitis was grade 2 (intermittent prolapse) and we could observe that this state is reversible, we optimized the use of the animals and avoided the unnecessary withdrawal of the experimental groups.

P58 The Langendorff Isolated Perfused Heart Assay: Improving Preclinical Cardiovascular Testing during Lead Optimization

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The International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use (ICH) S7A and B guidelines have significantly reduced drug attrition due to QT prolongation. However, cardiotoxicity is still a leading cause for drug attrition and market removal. Gaps still remain in cardiovascular testing, particularly when assessing functional endpoints that include left ventricular mechanics. The Langendorff Isolated Perfused Heart Assay is an ex vivo technique that offers enhancements to cardiovascular safety assessment by providing measurements of chronotropy, inotropy, lusitropy, electrophysiology, and coronary perfusion flow rate, independent of systemic influences such as sympathetic, parasympathetic, and nonheart-related metabolism. In this study, 4 antineoplastic agents with known cardiovascular liabilities were assessed. Guinea pig hearts were perfused with escalating concentrations of sunitinib (0.05, 0.5, and 1.5 µM), lapatinib (0.27, 2.7, and 8.1 µM), erlotinib (0.43, 4.3, and 13.0 µM), and doxorubicin (0.5, 1.0, and 10.0 µM) in Modified Krebs-Henseleit solution. Cardiovascular endpoints assessed included left ventricular pressure (±dP/dt), end diastolic developed pressure, electrocardiograms (RR, PR, QRS, QT, QTc), heart rate, and perfusion pressure. Evaluating clinical results, the results from this study validate the translatability of the cardiovascular endpoints assessed. By using these techniques early in drug development cardiovascular liabilities can be de-risked, reducing drug attrition and removal from the market, subsequently reducing the cost of drug development and the number of animals needed.

P59 Breeding a Better Training Rat

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In a university setting, much time is spent teaching students and researchers the proper way to handle laboratory rodents. The first step for many is overcoming fear and feeling comfortable with the animals, especially the rats. This is most easily achieved with friendly animals. Despite the numerous commercial strains of rats available, there always seemed to be a limiting factor such as size, temperament, prolificacy, or hardiness. NIH nude rats (RNU) and Sprague Dawley rats were readily available in the facility. RNU heterozygous are not immunodeficient and are extremely docile. However, genotyping was not an option at the time, so there was a potential for immunocompromised rats to be born. Sprague Dawleys are friendly and produce sizeable litters. Unfortunately, they grow too large too quickly and are often intimidating to new users. Since none of our available rats proved to be an ideal fit, the presenter created the Rutgers rat. Heterozygous RNU males Crl:NIH-Foxn1rnu were crossed with CD IGS (Sprague Dawley) females Crl:CD(SD). After 5 generations of breeding, it was perfected. The "Rutgers Rats" have been instrumental in training for research procedures, but also in promoting compassion for the laboratory rat. Instructors now teach the 3Rs in theory, but also promote it by example. Rats were historically purchased from a vendor, but now we breed our own. These rats further go on to educate other students and researchers in various techniques and procedures, minimizing the total number of rodents used. Although the Rutgers rats are not an officially recognized strain, they have played a huge role in the education here. They may never be involved in curing a disease or solving a medical mystery, but have been instrumental in educating both students and researchers in proper rodent handling and techniques.

P60 A New Approach to Advancing the 3Rs: The North American 3Rs Collaborative (NA3RsC)

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Several groups across North America include advancement of the 3Rs in their objectives. Recognizing a lasting need to foster improved communication and collaboration around the science, reporting, and implementation of the 3Rs, several members of the research community founded the North American 3Rs Collaborative (NA3RsC). The mission of the NA3RsC is to advance the education and science of the 3Rs. Members include academia, industry, and government. In our first year, the NA3RsC elected a board of directors, approved its bylaws, and gained 501(c) 3 status. The NA3RsC is sponsoring presentations at WC10, AALAS, and is co-sponsoring the 3Rs Sharing Conference. Our online collaborative space, the Virtual Education Community (VEC), is one of our most value-added tools. The VEC includes a theater to host live symposia, a community pavilion for 3Rs groups, an area for 3Rs-related vendors, a resource hub, and a platform for 3Rs-related discussions. The NA3RsC looks forward to working collaboratively for broad reaching impact in order to set a new precedent for the 3Rs culture in North America.

P61 Ethanol as a Refinement to CO_2 for Euthanasia of Chickens (Gallus gallus domesticus)

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According to the AVMA *Guidelines for Euthanasia of Animals*, injectable pentobarbital and inhalant CO_2 are acceptable and accepted with conditions methods of euthanasia for avian species. However, barbiturates are controlled substances and challenging to use in the field and laboratory setting. Additionally, there is limited literature on the use of CO_2 in avian species and flow rates are extrapolated from mammalian studies. Most importantly, CO_2 has been reported to induce anesthesia and euthanasia at inconsistent time intervals and is cited by users to be visibly distressful to avian species when used as a euthanasia agent. A recent study was conducted in mice investigating IP ethanol as an alternative to CO_2 euthanasia. Results suggested IP ethanol overdose is a suitable euthanasia agent in mice. Ethanol is easily accessible, noncontrolled, and its pharmacological properties suggest that it could induce a nonreversible humane death. Thus, we sought to determine if intracoelomic (IPc) ethanol overdose could be used as an alternative euthanasia agent in chickens. To evaluate this, we compared IPc ethanol to IPc pentobarbital. Chickens were fitted with ECG, noninvasive blood pressure, and videotaped throughout the experiment. Birds were randomized into 3 groups: 20mL of 100% ethanol, 20mL saline, or 0.5mL of pentobarbital IPc. Chickens receiving either pentobarbital or ethanol both exhibited a smooth transition into anesthesia with 5/7 birds in the ethanol group declared euthanized at approximately 10 m postinjection, and 5/7 pentobarbital birds declared euthanized at approximately 7 m. Loss of consciousness was assessed by intubation time and capnography was used to confirm respiratory arrest prior to loss of cardiovascular activity. In birds euthanized by ethanol, loss of respiration occurred prior to cardiac arrest and no overt signs of distress were observed. Postmortem examination revealed successful IPc placement of agent in all but 1 chicken, demonstrating that this euthanasia method is user-friendly. We conclude that IPc ethanol overdose induces euthanasia, similar to the gold standard IPc pentobarbital, exemplifying that IPc ethanol could be an alternative, refined method of euthanasia in chickens.

P62 Alternative Swimming Pools for Nonhuman Primates with Cranial Chambers

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Swimming pools used in the play cages provide advantages for rhesus macaques' enrichment. The idea of using a swimming pool is based on the natural behavior exhibited by primate species. Allowing socially housed animals to experience this behavior encourages natural play and serves as an additional form of exercise. Submerging treats and food such as seeds and nuts into the water has had a positive impact in rhesus macaques that have no cranial chamber. Primates that do have a cranial chamber are limited in participating in such activities, as these apparatuses cannot get wet. This limitation led us to fill the pools with alternative swimming materials so these animals can still forage for food and participate in this enriching experience. Rather than filling the pool with water, materials such as shredded paper, alfalfa, and cardboard rolls were used. These alternative means to water allow the primates to solicit natural foraging behaviors, cognitive thinking, and stimulate different tactile senses all while providing a versatile enrichment technique without compromising the integrity of the cranial chamber. The behavior was studied of 9 male adult rhesus macaques, some of whom have cranial chambers, as they displayed species-specific behavior in swimming pools containing a variety of materials.

P63 Effectively Training New Laboratory Animal Personnel

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With the majority of new animal users rotating through laboratories, one major challenge is ensuring that the new laboratory members are appropriately trained on regulatory, local, and university policies and procedures. Even with all the required training both online and hands-on training, we were still facing issues with laboratory members not truly understanding the requirements. This includes members new to working with animals, and members with experiences from other institutions within the U.S. and internationally. So we were faced with a challenge of how to engage these new members in an interactive way and to ensure they leave with a working understanding of what is required in order to have the privilege of working with laboratory animals. With over 300 principal investigator laboratories, the number of new laboratory personnel added to an animal study protocol (ASP) ranged from 10-30 new members a month. Some arrive with previous experience from other institutions, but the majority are new to working with laboratory animals. Even though they are required to take certain online courses and a hands-on session, there still seemed to be a gap in understanding the requirements and techniques. To fill that gap, the IACUC started hosting a monthly brown bag session during the lunch hour, inviting all the new members added to a protocol from the previous month. The session is a combination of a brief lecture and an interactive Q&A session, which helps engage the audience throughout the session. The questions are based on the topics discussed, along with any items noticed during the semiannual inspections, PAM visits, and minor deficiencies. This program was implemented 5 mo ago and so far has been a success, with a decline in minor noted deficiencies and understanding of the requirements. It has even encouraged long-time members to request an invite as a refresher.

P64 Refinement and Reduction Strategies for Neural Stem Cells Research

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Neural stem cells (NSC) are an invaluable tool for the development of therapies for neurodegenerative disorders. The time consuming and laborious continuous workflow consisting of isolation of fetuses, tissue dissection, cell isolation, and cell experiment/application is known to demand a considerable number of animals due to the low yield. Additionally, it is difficult to guarantee that every single tissue dissection comprises the same cell pool. Refining the process to isolate the target cells to increase the yield can greatly reduce the number of animals required, as well as improve the reproducibility of results. This work proposes a method that combines the isolation of NSCs from a specific region with a cryopreservation method of isolated cells for later use, hence minimizing the number of animals and decreasing the time consuming continuous workflow. The striatum of Sprague Dawley rats at embryonic day 16 (E16) were isolated and dissociated into single cells. Dissociated cells were cultured for 3 d to form cell aggregates known as neurospheres. Obtained neurospheres were then dissociated and cryopreserved as single cells. Cryopreserved cells can then be thawed and cultured (passage

2) for only 3 to 4 d to obtain neurospheres which can be further dissociated to single cells for all types of studies and applications These cells are comprised of neural stem cells which can be differentiated to all major types of brain cells: neuron, astrocytes, and oligodendrocytes. The method described in this work consisted of isolating cells from the rat brain striatum and culturing cells for 3 to 4 d to allow the selective expansion of NSCs, granting higher homogeneity of cell pool and reproducibility of results. An appropriate cryopreservation method can greatly decrease the time-consuming continuous workflow, since cryopreserved cells can be stored for extended periods, and then thawed when needed to form neurospheres within only 3 to 4 d of culture, avoiding the use of extra animals.

P65 Patency of Jugular Vein Catheters in CD1 Mice: Evaluation of 3 Catheter Maintenance Schedules in Standard External Catheter and Transcutaneous Buttons

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Pharmacokinetic (PK) studies in mice are conducted using chronically implanted jugular vein catheters (JVCs) that allow central venous access in conscious animals for repeated blood sampling and dosing. However, maintaining continuous patency sets practical limits on its uses. We conducted a study investigating 3 different catheter maintenance schedules for mice with JVCs with standard externalization (STNRD) compared to a transcutaneous button (BUTTON). Seventy-two adult male 27-30 g CD-1 mice (Crl:CD-1 IGSBR) were randomly allocated into 6 groups (n=10 each) based on catheter flushing frequency, for example, once every 3 (q. 3), 5 (q. 5) or 7 (q. 7) d, starting 3-4 d after surgery. For groups 1-3 the catheter was sealed with a metal plug and the extravascular portion was extended subcutaneously, exiting at the interscapular region. In groups 4-6 the catheter was connected to a transcutaneous button located in the same interscapular region. A locking solution of heparinized (500 IU/ml) 50% dextrose was administered and used for the duration of the study. Animals were shipped to another site for patency checks to mimic a standard customer order. The catheter was considered patent if blood was successfully withdrawn. Animals were clinically healthy throughout the study. Catheter patency rates were 100% in all animals on the first assessment. At approximately 2 wk postsurgery (10-14 d), 100% of q. 3, and q.7 catheters with the BUTTON remained patent, and 90% of the q.5 were patent. The STNRD dropped to 20%, 30%, and 50% patency for q.3, q.5, and q.7 respectively. At the conclusion of the study day 45-46, 10% (1 animal) of the STNRD remained patent for blood collection from each group; however, 80%, 40%, and 70% remained patent for the BUTTON. In summary, 90% of catheters using the BUTTON remained patent 2 wk postoperatively, irrespective of the catheter maintenance schedule. The use of transcutaneous buttons kept the catheters patent for up to 5 times longer than the standard externalization.

P66 Comparison of Ammonia Levels in the Microenvironment of Singly and Group-Housed Mice: Validation of 28-Day Cage-Change Intervals to Enhance Animal Welfare and Operational Efficiency

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Ammonia production occurs as a normal biological consequence of animals excreting waste following metabolism of their food and water. We measured ammonia in singly housed and group-housed (n = 5/cage) mouse (C57BL6/J) cages over 28 d and 19 d, respectively, to determine whether the cage-change interval could be extended from 14 d to 28 d. Less frequent cage caging has the benefits of less stress to the animals, less personnel time to change cages, and cost savings from using less equipment and supplies. Animals were divided into 4 groups of 6 cages each (singly housed/continuous cage ventilation, singly housed/periodic static cage condition, group housed/continuous cage ventilation, group housed/periodic static cage condition). Cage ammonia concentration was measured daily using an ammonia sensor for up to 28 d. We used 50 ppm as the maximum acceptable ammonia level based on literature reports. Ammonia concentration was <50 ppm in all continuously ventilated cages and in single-housed periodic static cages for the study duration. Ammonia concentration was >50 ppm in 3/6 group housed periodic static cages after 14 d. Based on our results, we conclude that the cage-change interval for singly housed mice may be extended to 28 d. Although group-housed mice also did not exceed 50 ppm for continuously ventilated cages, we maintained a 14-d cage-change interval due to cage soil and moisture. For group-housed mice under periodic static conditions (which represents typical conditions experienced for experimental manipulations), acceptable cage change interval should be identified based on consideration of variables such as bedding type, animal strain, gender, housing density, ventilation flow rate, and amount of time spent off of the ventilated rack.

P67 Creation of a Clinical Diagnostics Team in a Transgenic Rodent Breeding Facility

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A clinical diagnostics team was established at our institution in order to help gather information on clinical cases in a large scale transgenic rodent breeding facility. Valuable information can be collected from performing necropsies on any found clinical cases. However, managing the large number of cases generated in a production facility can be difficult with limited veterinary staff. By establishing a team of animal care technicians and research associates to perform this function, under the direction of the veterinary staff, larger numbers of necropsies can be addressed on a daily basis and technical staff can be further developed through the training and skill development involved. When appropriate, necropsies include collection of samples for histology, microbiology, parasitology, and photo imaging to further define the pathology of these cases. Records of the observations and sample analysis are entered into a searchable clinical case database which veterinary staff and investigators use to reference individual cases and analyze trends. This data helps to further characterize transgenic strains and monitor pathogenic status of the colonies in the facility. By setting up a standardized process and criteria for submission, and creating a rotating schedule of staff to process necropsies, a daily flow of cases can be handled efficiently to maximize the information gathered.

P68 Health Monitoring of Accelerated Aging SAMP8 Mice: Use of Nest Quality and Grip Strength for Defining and Refining Humane Endpoints

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Health oversight by animal care and animal health technicians was provided for a colony of senescence-accelerated; SAMP8/TaHsd (SAM) mice. Mice were needed to perform behavioral assays for Alzheimer's disease (AD) research studies with approximately 100 mice received every month for 1 y. Male SAMP8 mice were singly housed due to aggression, and retired breeders (either sex) were singly housed from vendor. In addition to age-related issues, many of the mice developed more chronic and subtle clinical signs (increased respiratory effort, mild weight loss, mild lethargy, unkempt hair coat) that did not clearly reach criteria for humane endpoints. Nest quality and grip strength provided additional measures to improve humane and study endpoints for these mice. During the AD study assessments, measurement of grip strength played a role in overall study performance evaluations. Body weights and cognitive scores were also evaluated. Overall, SAMP8 mice had lower body weights compared to SAMR1 mice (normal aging control). Shortened lifespan was also seen in SAMP8 mice compared to SAMR1 mice. Mice were observed weekly during cage change out by animal care technicians and any health concerns reported to animal health technicians (AHT). During health assessments, a correlation was noted between poor grip strength and poor nest quality, leading to increased health observations (from weekly to 1-2 times daily plus treatments or supportive care as needed) and timely euthanasia. Out of 1,100 mice, about 20% developed chronic health conditions and the nest quality/grip strength observations provided faster identification of mice with deteriorating health conditions, allowing for study-specific data collection prior to euthanasia of the mice. Monitoring of nest quality and grip strength provided insights into the health status of aging SAMP8 mice. Mice displaying poor nest quality and grip strength were placed on daily health observations which provided for more timely euthanasia. Behav-

ioral assessments and other study data were then collected from these mice prior to euthanasia, thus positively impacting study timelines. Monitoring nest quality and grip strength improved our health monitoring efforts and helped to better define and refine humane endpoints for these aging mice.

P69 Incorporation of Fluorescein into the Training of Retroorbital Sinua Injection Technique in Mice to Provide Visual Confirmation of Successful Agent Administration

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Intravascular administration of agents via retroorbital sinus infection in mice allows for rapid drug distribution and onset of effect, but the procedure is technically challenging. Adequate training and ongoing practice are required to develop proficiency with this methodology. One of the challenges of training to perform retroorbital sinus injections is that it is often difficult to verify whether or not an injection attempt is successful. Since the retroorbital sinus is unable to be directly visualized, it is difficult to determine whether the agent administered during training. It can be difficult to tell if saline has been injected intravascularly or whether it has instead been retained in the orbit. Successful retroorbital sinus infection can, however, be confirmed with the use of fluorescein. Fluorescein is an organic compound that intensely fluoresces when illuminated under ultraviolet light. Fluorescein sodium ophthalmic strips are readily available and were used to create a sterile solution of pharmaceutical-grade fluorescein to aid in the training of retroorbital sinus injection techniques in mice. Upon successful injection of fluorescein solution intravascularly, fluorescence under ultraviolet light was appreciated in the pinnae and paw pads of injected mice. In contrast, lack of fluorescence in the extremities or intense fluorescence around the globe indicated an unsuccessful attempt. While fluorescein is administered to people intravenously for angiography, caution must be taken when administering fluorescein to mice because little documentation is available regarding the safety of intravenous administration in this species. Fluorescein was successfully incorporated into retroorbital sinus injection techniques training without significant impacts to animal welfare, as mice were anesthetized for the procedure, then euthanized after success of administration had been determined. On the other hand, incorporation of fluorescein in the training of intravascular injection techniques that involve conscious mice should only be considered after the safety of this agent has been more thoroughly evaluated.

P70 Pair-Housing Female Rabbits in a Laboratory Setting

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For rabbits in research settings, there has been a push to identify an optimal social paradigm that minimizes stress, optimizes the time spent in direct contact, and enhances opportunities for species-specific behavior. Our initiative for pair-housing rabbits was to determine whether a sibling relationship improves the success rate for maintaining socially housed rabbits by performing a comparative study with pairs of young female littermates and non-littermates. All rabbits were housed in conventional cage units and assigned to 1 of 4 groups comprised of various arrangements of littermate pairs and non-littermate pairs. Throughout the course of the study, behavioral observations were conducted by trained staff on a routine basis. Results showed a relative increase in social incompatibility with increasing age across all groups except the group of preestablished littermate pairs; however, the size of this group was smaller due to attrition. Over the course of 5 mo, there was a 75% success rate in the group of littermates that were paired at the facility, and a 25-50% success rate in the 2 groups of non-littermate pairs. While the rate of success for maintaining pair housed rabbits may be aided by acquiring preestablished pairs of littermates from the vendor, we concluded that the most significant factor to maintaining compatible pairs of female rabbits is age rather than familial relationship. We further concluded that success declines when attempts are made to socially house older rabbits, as our inability to re-pair decreased as rabbits lost their pairs after 16 wk of age.

P71 Comparing the Efficiency of Large-Volume Blood Collection from Cynomolgus Macaques (Macaca fascicularis) Using a Procedure Cage versus Sedation

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Blood samples greater than 20mLs are required to perform a variety of laboratory tests, such as assays using peripheral blood mononuclear cells (PBMC). Typically, large-volume nonhuman primate blood collections require sedation due to the increased restraint time. Sedation requires monitoring the recovery of animals, increasing the overall duration of the procedure. Sedation also increases health risks due to associated drug side effects. In an effort to increase efficiency of collection for samples 20mLs or greater, 2 methods were evaluated: awake using a procedure cage and sedated. Blood parameters, supplies, number of personal required, and length of time were evaluated for each method. In the first method, conscious animals were restrained using a commercially available enclosure space, which functions by using a squeeze-back mechanism to restrain the animal. This method requires 2 people, 1 to restrain and 1 to collect blood. In the second method, the animal is weighed, sedated, bled, and monitored for recovery. This method requires 2 people, 1 to assist the collector and monitor the animal, and 1 to collect blood. In both methods a femoral vein was accessed by extending a hind limb to visualize and palpate the femoral vein. A 21-g, butterfly blood collection set attached to a needle holder was inserted in the vein. Blood was collected by attaching 8mL capacity collection tubes to the needle to collect volumes of 20mL and 35mL. Results show that time was decreased for collections of 20mL and 35mL blood samples using the procedure cage for restraint when compared to restraint by sedation. Both methods produced similar quality samples based on assessment of blood parameters and required the same supplies for the collection; however, sedation increased cost due to the use of controlled substances and increased technician time for recovery monitoring.

P72 The Evolution of a 3Rs-Based Training Method: Provision of More Effective and Humane Hands-On Training through the Use of Inanimate Tools

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Page 4 of the *Guide for the Care and Use of Laboratory Animals* states"the 3Rs have become an internationally accepted approach for researchers to apply when deciding to use animals in research and in designing humane animal research studies." We believe that it is equally important to apply the 3Rs concept to the humane use of animals during hands-on training. We created a training team mission statement that articulated our desire to implement the practical application of the 3Rs, while providing the best training possible for our trainees. We developed a curriculum that primarily uses hand-crafted training aids, specifically designed to tackle the most challenging concepts and steps for our trainees to grasp and for our trainers to convey. We referred to these challenges as the key learning issues (KLIs). We identified procedure-specific KLIs for each of the most common rodent techniques that we routinely teach, and we used these KLIs to guide us in creating inanimate training tools that are designed to target the specific KLIs for each procedure. Our approach to hands-on teaching has evolved into a trademarked method known as "Translational Training Tools" also known as tthe 3Ts serving the 3Rs. The 3Ts training method aims to promote and share ideas for affordable and effective means to implement the 3Rs alternatives into hands-on training programs. We outline the creation of the 3Ts method and implementation of the 3Rs for delivering effective and humane hands-on training.

P73 Efficacy of an Enrichment Device for Barbering in Female C57BL/6J Strain Mice

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C57BL/6J mice have been seen to barber during longterm breeding. One

possible cause of barbering is fighting among mice. Therefore we used an enrichment device for male C57BL/6J mice because such a device provided a place for inferior mice to escape and hide from superior mice. We previously found that the enrichment device we used could reduce barbering in male C57BL/6J mice. However, it was not clear that this device would work for female C57BL/6J mice. As a result, we examined the efficacy of using the same device for the barbering in female C57BL/6J mice. Thirty-two female C57BL/6J mice were divided into 2 groups. One group was housed with the enrichment device (E group) and the other group was housed without a device (C group). Each cage housed 4 mice. We observed mice from 4 to 37 wk of age. Body weight anbd food and water consumption were measured once a week. We checked barbering of mice once a week. At 37 wk of age, the mice were euthanized by collecting blood under isoflurane anesthesia. Blood cell counting and biochemical examinations were performed. The area of barbering was measured. After necropsy, the main organs were weighed. Statistical analysis was conducted using an unpaired t-test. A *P* value less than 0.05 was considered statistically significant. There were no significant differences in body weights (except at 37 wk) and organ weights between the 2 groups. There were no significant differences in food and water consumption (except at 33 wk) between the 2 groups. There were no significant differences in the blood examination results. However, the total number of barbering mice in E group (15/16) was significantly higher than that of C group (7/16). The mean value of the barbering area in the E group (5.09 cm^2) was significantly larger than that in the C group (0.53 cm^2) at age of 37 wk. In summary, the enrichment device we used could not reduce barbering in female C57BL/6J mice. In addition, the device facilitated barbering in the E group. This result was totally different from that of male mice. The cause or mechanism of barbering might be different between male and female C57BL/6J mice. Therefore, further investigation into the relationship between enrichment devices, sex, and barbering in mice is required.

P74 Heart Base Teratoma in the Western African Clawed Frog (Xenopus tropicalis)

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Two adult wild type and 1 adult transgenic Western African clawed frogs (*Xenopus tropicalis*) presented dead in tank or died shortly after treatment over a 2-mo period. The affected frogs were used for spawning and were previously treated for an infestation by an external protozoan ectoparasite (*Epistylis* spp). Treatment for the *Epistylis* consisted of manual removal of protozoan accumulations from the keratinized epithelium of the hind claws along with topical application of high-salt solution, methylene blue and pronase solution, high-salt solution a second time, and malachite green formalin or potassium permanganate. A single, dark red, round, soft tissue mass, ranging in size from 0.75-1.0cm in diameter with a mineral component and associated with the heart base and cranial border of the lungs, was identified in all 3 frogs on gross necropsy. Well differentiated tissues from multiple origins were identified on histology of 2 of the masses, including skeletal muscle, hyaline cartilage, thyroid gland with well-formed follicles, transitional epithelium with squamous metaplasia, and nervous tissue. Histologic findings were consistent with a teratoma. These cases may represent spontaneous teratoma development within this colony, however, this may also be secondary to exposure to malachite green formalin or methylene blue, which are both known teratogens. The teratomas are unlikely to be related to the genetic modification as 2 of the frogs were wild type. Spontaneous mediastinal teratomas have been previously described in African clawed frogs (*Xenopus laevis*), and experimentally induced teratomas have been described in Marsabit clawed frogs (*Xenopus borealis*). To the authors' knowledge, this is the first description of teratoma development in the Western African clawed frog.

P75 Off-Target Upper Respiratory Effects in a Xenograft Model of Metastatic Prostatic Adenocarcinoma

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Off-target effects of test article administration can be a significant cause of morbidity and mortality in preclinical studies. A subset of male athymic nude mice [CAnN.Cg-Foxn1nu/Crl] receiving an Endothelin-A (ETA) receptor antagonist (test article), experienced significant morbidity on study. Intact or castrated male mice were administered either test article or Polysorbate-80 vehicle via oral gavage, once daily. A majority of castrated animals receiving test article experienced significant weight loss and gastrointestinal ileus. In contrast, intact mice treated with test article or vehicle were not clinically affected. Several affected mice presented with an audible respiratory click, suggestive of intermittent upper respiratory obstruction, so the nasal cavity was examined histologically from all mice. Histopathology showed a test article-related effect in all drug-treated mice, characterized by marked rhinitis, olfactory and respiratory hyalinosis, marked olfactory epithelial atrophy and degeneration, and olfactory nerve atrophy. Lesions were also observed, but to a much milder degree, in vehicle control animals, suggesting the complication of gavage-related reflux effect of the vehicle, independent of test-article effects. Preliminary expression studies using immunohistochemistry showed evidence of a group 2 innate lymphoid cell (ILC2) mediated inflammatory response, characterized by upregulation of YM1/YM2 protein in hyalinosis lesions and significant eosinophilic inflammation, driven by release of IL33 from damaged olfactory epithelium. Endothelin-1 (ET-1) serves a neuroprotective role in the olfactory epithelium, and it is hypothesized that direct drug exposure or receptor-mediated effects in the nasal epithelium due to ETA receptor antagonist exposure may lead to the test article-induced injury seen in the treated animals. The lesions resulting from direct drug exposure in the nasal cavity, likely due to gavage reflux, are thought to have led to recurrent obstructive respiration and aerophagia, resulting in clinically evident bloat. This study provides a good example of why nasal lesions should be considered in any study utilizing oral gavage, where there is unexplained morbidity or mortality associated with gastrointestinal bloat.

P76 Atypical Multifocal Pododermatitis Lesions in a Laboratory Beagle

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An intact 2.5-y-old purpose-bred male beagle presented with focal swelling on the right forepaw digit P4, and left hindpaw digits P3 and P4. The areas were alopecic, erythemic, with small multifocal pustules. No pain was appreciated during ambulation or palpation. Five weeks prior to presentation, the dog had been placed on a canine prostate cancer study. Briefly, cyclosporine A (20mg/kg) was given daily for 10 d followed by an ultrasound guided intraprostatic injection of canine prostate carcinoma cells. Daily dosing of cyclosporine continued throughout the remainder of the study. Given the history, initial differential diagnoses included dermatophytosis, pododermatitis, and study-related metastatic disease. A trichogram and DTM fungal culture were negative. An impression smear indicated mild neutrophilic inflammation with degenerate neutrophils, however, no bacteria were identified. Twice daily dilute chlorhexidine (2%) warm water soaks and 72 h of oral meloxicam were provided, however swelling was unresolved and lesions progressed to hyperkeratosis. The dog was placed on cephalexin (22mg/kg) twice daily for the remainder of the study resulting in only mild improvement. At week 8 postinjection, a grossly enlarged prostate gland (+20cm) and palpable abdominal nodes were noted on physical exam in addition to weight loss (12%). An MRI confirmed a mass was present within the prostate gland and surrounding tissue. Euthanasia was performed at week 10 postinjection and histopathology of the foot lesions showed mild lymphoplasmacytic dermatitis and orthokeratotic hyperkeratosis consistent with resolving deep pyoderma. Pyoderma can have infectious, inflammatory, or neoplastic etiologies. Histopathology did not support systemic inflammation or immune suppression, and neoplastic cells were not identified in the affected areas. These results strongly suggest a bacterial pododermatitis which often requires weeks to months of antibiotic therapy for complete resolution.

P77 Molecular Hydrogen Improves the Oxidative Stress-Induced Low Motility of Mouse Sperm

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Oxidative stress caused by the imbalance between reactive oxygen species (ROS) and biological antioxidant system leads to damaged sperm

and subsequent male infertility. Recently, it was reported that molecular hydrogen (H_2) acts as a therapeutic antioxidant by selectively reducing cytotoxic oxygen radicals. To investigate the effects of H_2 on damaged sperm, we prepared oxidative stress-induced low motility sperm. Suspension of fresh B6D2F1/Crlj mouse sperm, which motility rate was 82.4%, was treated with 0.3 mM hydrogen peroxide for 30 min, resulting in low motility rate (14.6%). We further incubated the suspension for 20 min with or without H_2, and found that H_2 significantly increased the motility rate (63.9%) accompanied by improvement of ATP accumulation in sperm. To investigate fertilizability of H_2 treated sperm, we used them for in vitro fertilization (IVF) and found that H_2 markedly improved the fertilization rate (59.2%). Transfer of the 2-cell stage embryos to pseudopregnant ICR mouse showed normal ontogeny (94.6%). Because of the rapid diffusion and high membrane permeability, H_2 can reach and react with intrasperm ROS, including hydroxyl radical, and ameliorate low sperm motility. Our results strongly suggest that H_2 is a new promising tool for male infertility treatment.

P78 Decreased Appetite in a New Zealand White Rabbit Inoculated with Treponema pallidum

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A 3-mo-old intact male, 3.2kg, New Zealand White rabbit was examined for decreased appetite 2 wk postintratesticular injection with *Treponema pallidum*. On physical examination, the rabbit was bright, alert and responsive, normothermic, euhydrated, and had a normal heart and respiratory rate. There were no signs of orchitis, dental disease, or abdominal discomfort. A 2 cm linear mass was palpated in the cranial abdomen. Due to continued partial anorexia and weight loss, additional diagnostics were performed to rule out gastrointestinal obstruction, infection, or systemic causes. Radiographs showed decreased serosal detail in the abdomen with increased opacity in the caudal lung fields and possible cardiomegaly. Clinical pathology revealed regenerative anemia, few spherocytes, markedly elevated blood urea nitrogen, creatinine, phosphate, and mild-moderate elevations in gamma-glutamyl transferase and bilirubin. Due to a poor prognosis, euthanasia was elected. A urinalysis performed at necropsy demonstrated isosthenuria, proteinuria, glucosuria, and hematuria. The kidneys were bilaterally pale tan with multifocal pinpoint dark foci within the cortex. Additional necropsy findings included hydrothorax, ascitic modified transudate, hepatomegaly, and minimal flaccid cardiomegaly. The lungs failed to collapse and were doughy with multifocal foci of varying size. Histopathologically, there was systemic thrombotic microangiopathy (TMA) in the kidney, lung, heart, and brain. The vascular lesions were often accompanied by secondary changes such as edema, hemorrhage, necrosis, and degeneration. Affected vessels were characterized by acute to organizing intravascular thrombi with prominent endothelia and classic onion skinning' of the renal arterioles. Glomerular lesions ranged from acute to chronic glomerulonephropathy. In the proximal small intestine, there was segmental severe acute necrotizing enteritis with villar blunting and fusion, severe crypt apoptosis, mucosal and submucosal edema, congestion, and mild hemorrhage. A re-review of the initial blood smears demonstrated few schistocytes. The clinicopathologic and histologic findings are most consistent with hemolytic uremic syndrome although a definitive cause for TMA in this rabbit cannot be determined.

P79 Cecal Inversion in a Naive Beagle

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A 1-y-old male beagle dog (*Canis familiaris*) housed in an indoor kennel facility presented with unformed stool with mucus and hematochezia on arrival from the vendor. Physical examination revealed no clinical abnormalities. The dog was bright, alert with a responsive demeanor, had pink mucus membranes, a CRT <2 s, and heart rate and respiratory rate within normal limits. During the following 3 mo, the animal continued to present with hematochezia that was at times intermittent. Diagnostic evaluations included a rectal exam, bloodwork, fecal floats, serial rectal cultures for *campylobacter*, *calmonella*, *shigella*, and *E. coli* 0157, survey and contrast radiographs, urine dipstick, and urinalysis. With the exception of a mild increase in BUN and thickened intestinal walls on imaging, all results were within normal limits. The diet was changed to hypoallergenic feed for 2 mo. Concurrently, treatment included courses of metronidazole, bismuth subsalicylate, and tylosin. During this time, the animal exhibited no other clinical signs and had a consistent weight gain of approximately 12%. After 3 mo of inconclusive diagnostics and no change in clinical signs, euthanasia was performed and the animal was necropsied. At necropsy, a complete cecal inversion was discovered, and no other abnormalities were noted. Histopathologic evaluation confirmed the cecal inversion and revealed a band of mature fibrous connective tissue within the cecum, possibly indicative of previous trauma. Cecal inversion is rare in the dog and has also been described rarely in other species, such as the red wolf, cat, and horse. Some treatments have been described, including reduction or typhlectomy. To our knowledge, this is the first report of a cecal inversion in a naive laboratory beagle.

P80 Standard Observations Result in Pup Survival Findings

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Macroautophagy is an essential maintenance and protective catabolic process involving the digestion of cellular components and damaged organelles within lysosomes. Macroautophagy occurs constitutively at a low level, but is accelerated by cellular stressors, such as starvation, lack of growth factors, and DNA damage. Unc-51-Like Kinase 1 (ULK1)-dependent autophagy is a protein that is essential for some macroautophagy processes. ULK1-deficient mice on a mixed (C57B6/129Sv) background exhibit numerous abnormalities, including elevated mean corpuscular volume (MCV), anemia with delayed mitochondrial clearance form red cell precursors, and reticulocyte counts, due to a defect in autophagy-mediated clearance of mitochondria during red blood cell maturation. As animal research technicians, we were maintaining the mouse ULK1 gene knockout colony for related experiments. Heterozygous ($ULK1^{+/-}$) mice were interbred and once the dam gave birth, the pup's weight, phenotype, litter size, gender, and toe sample were collected for identification and genotyping. All genotypes were born in the expected Mendelian ratios. However, homozygous null pups exhibited increased death rate prior to weaning. Specifically, survival rates of pups at 3 wk were: [WT] $ULK1^{+/+}$ 91% (n=36), [HET] $ULK1^{+/-}$ 89% (nonsignificant vs WT: n=28), and [KO] $ULK1^{-/-}$ 64% ($P = 0.018$ vs WT: n=14). We observed similar reduced survival rates of $ULK1^{-/-}$ mice that were studied on the C57BL/6 background. Despite numerous studies of $ULK1^{-/-}$ mice over more than 10 y, this is the first time that reduced survival of homozygous null animals has been noted and our observation skills as research technicians contributed to this finding. Reasons for postnatal lethality of $ULK1^{-/-}$ mice require further study and may provide new insights into the in vivo functions of the ULK protein.

P81 Hyperglycemia and Predictors of Declining Health in Aged Sand Rats (Psammomys obesus)

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The use of sand rats (*Psammomys obesus*), a member of the gerbil family, for age-related spontaneous lumbar disk degeneration requires long-term housing of animals prone to the development of diabetes. Aged sand rats were studied to determine if the diabetic-prone state develops even in animals fed a low-energy diet and to identify predictors of age-related decline. After an overnight fast blood, glucose (obtained from a tail vein using a monitor and strips programmed to dog code), body weight, and body condition scores were measured every 2-wk in sedated sand rats over 24 mo of age. Preliminary data demonstrated hyperglycemia (>100 mg/dL) in 68% of aged animals. Blood glucose was not found to correlate with body weight or body condition. Animals that were flagged clinically due to body weight loss and/or poor body condition did not differ from nonclinical animals in maximum or average blood glucose concentrations nor baseline body weight ($P > 0.05$). Clinical cases only differed from nonclinical cases in percent change in body weight from baseline ($P = 0.006$), which was part of the criteria for identifying clinical cases. It is interesting to note that during the study period a younger, 22-mo-old sand rat had to be euthanized for acute weight loss and had a blood glucose of 341 mg/dL prior to euthanasia. These results indicate that sand

rats can develop hyperglycemia even when fed a low-energy diet. Weight loss seems to be the best indicator of declining health in aged sand rats, although a longer-term study in a wider age range of animals may be necessary to adequately determine the impact of glycemic control on long-term health.

P82 Idiopathic Dermatitis in a Colony of Siberian Dwarf Hamsters (Phodopus sungorus)

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A prolonged history of dermatitis was observed in a colony of Siberian dwarf hamsters (*Phodopus sungorus*) maintained at our institution. The prevalence of hamsters presenting with clinical signs within the colony was estimated to be 9%. Clinical signs included mild to severe alopecia, erythema, and scaling of the skin, primarily of the axillae and forelimbs. Occasionally, dermal lesions progressed to involve the ventrum and inguinal regions without additional clinical abnormalities. Historically, diagnostic measures such as bacterial and fungal cultures and tape tests failed to identify an underlying etiology outside of normal flora. The lesions were generally unresponsive to husbandry changes, such as alternative bedding types, and medical management with a variety of topical and systemic treatments. *Demodex* spp. mites were rarely observed on tape tests. A treatment trial was initiated based on the observation of *Demodex* mites and the lack of response to other management and treatment options. Affected hamsters (n=33) were treated twice, 3 wk apart with 1 of the following: topical selamectin (15 mg/kg), oral ivermectin (0.2 mg/kg), or SC ivermectin (0.2 mg/kg). None of the treatments resulted in clinical improvement, and all affected individuals were subsequently removed from the colony. All treatment groups remained PCR positive for *Demodex* based on pooled samples at the time of euthanasia, approximately 5 wk after the second treatment. Histologic evaluation of the skin from 4 severely affected hamsters (3 treated, 1 untreated) failed to identify a definitive cause of the dermatitis, although there was significant mixed bacterial involvement beyond what is expected for healthy animals. The cause of the observed clinical signs is suspected to be multifactorial.

P83 Spontaneous Cold Agglutinin Disease in a Male Rhesus Macaque (Macaca mulatta)

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Cold agglutinin disease (CAD) is a rare clinical presentation in animals where complement fixing autoantibodies bind to red blood cells (RBC) at a temperature below the core body temperature and subsequently induce intravascular hemolysis when reexposed to the body temperature. Here we describe a spontaneous case of CAD induced hemolytic anemia in a study-naïve, 12-y-old male rhesus macaque. The animal was seronegative for MaHV-1 and SRV. The animal was presented with self-limiting sporadic episodes of hemoglobinuria. A sample of the peripheral blood indicated regenerative anemia in the presence of erythrocyte agglutination, spherocytes, and eccentrocytes. There was also moderate leukopenia. A followup bone marrow biopsy confirmed regenerative anemia and ruled out lymphoproliferative disease. Blood smears made at 4°C and 40° C revealed marked agglutination at the lower temperature. Cross incubation of serum from the index animal with RBCs from a clinically healthy male macaque revealed agglutination. A diagnosis of CAD was made. We believe this case to be the first reported incidence of spontaneous CAD in a rhesus macaque.

P84 Outbreak and Infection Control of Balantidium coli, Isospora suis, and Enterotoxigenic E.coli in Minipigs

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Hygiene is very important to a laboratory animal facility for standardization of animal experiments. So, microorganisms should be blocked and removed as soon as possible. Unfortunately, infection of 3 microorganisms occurred in our laboratory animal facility, including *Balantidium coli, Isospora suis,* and *Enterotoxigenic E.coli.* Our animal laboratory raised 61 minipigs in 8 rooms. Our animal facility could not be cleaned for about a year because of the facility problem. After the problem had been fixed, reddish and phlegmatic diarrhea and anorexia from 12 minipigs (male: 6 of 12 minipigs; female: 6 of 12 minipigs) occurred during cleaning. In order to solve this problem, we prepared 4 plans. First, we immediately isolated minipigs with diarrhea from normal minipigs. Second, we treated with antibiotics (toltrazuril 200mg/kg PO to treat *Isospora suis,* oxytetracycline 10mg/kg IM BID to treat *Balantidium coli,* and amoxicillin 10mg/kg IM BID to treat *Enterotoxigenic E.coli).* These were chosen due to the results of microscopic examination, identification of bacteria, and antibiotic resistance test. These tests were performed on the feces of 3 minipigs with diarrhea. Third, we reduced the supply rate of feeds to ease the burden on the intestine tract. Fourth, we enhanced appetite by appetite catalyst injections and mixing sugar with feed. Clinical symptoms are completely asymptomatic a week after treatment and they remain healthy after a year. Microorganisms seem to have trespassed on the facility about a year ago. ETEC can live for 16 mo in a dried environment and they can live for a longer time in a wet environment, including feces. An exact cause is still unknown but minipigs could be infected by fecal-oral route when we cleaned feces with high-water pressure. Changes in the environment of gastrointestinal tract induced from ETEC infection increased explosively the number of *Balantidium coli* and *Isospora suis* which exacted in small numbers. This event and therapeutic process can be used to guide other minipig animal laboratory facilities

P85 Management of Postanesthetic Hyperthermia in Duroc Pigs (Sus scrofa domestica)

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The Duroc pig is an increasingly popular animal model for wound healing studies in the biomedical research setting. Our facility routinely receives Durocs for IACUC-approved research studies. Induction and maintenance of anesthesia for these animals are often necessary to achieve the research aims. Hyperthermia, ranging from 104.3°F to greater than 107.0°F, was detected in 6 adult female Duroc pigs recovering from general anesthesia on multiple dates. Our staff monitors vital signs including temperature, pulse, and respiration at 5-min intervals while intubated and 15-min intervals postintubation. Clinical signs following the detection of hyperthermia ranged from muscle rigidity, open mouth breathing, mucous membrane redness, ataxia, and a generalized warm to touch feeling. Supportive and treatment measures included the administration of intravenous fluids, acepromazine, and topical 70% isopropyl alcohol. Ice chips and frozen enrichment items were provided as additional supportive care measures. Unlike with malignant hyperthermia, we were able to successfully return each patient's temperature to within normal limits without antidote and had no signs of organ failure or death due to hyperthermia.

P86 Search for Definitive Senescence Biomarkers in Mice: What Changes Will Occur in Naturally Aged Mice?

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Our facility has kept many naturally aged mice and rats used for gerontology and geriatric research. If a scientist conducts research using these aged animals, it is important to know what changes will occur in them with aging, especially at individual levels. However, a clear criterion for defining aged mice has not been established. In the present study, we evaluate various characteristics as senescence biomarkers of naturally aged mice kept in our facility. Four-wk-old male (n=90) and female (n=30) mice (C57BL/6N) were obtained from Japan SLC every 3 mo and were kept over their lifetimes (male, n=1419; female, n=473, in total). Physiological assesments (measurement of body weight, food/water consumption and survival rates), behavioral tests (rotarod tests and grip strength tests were conducted every 3 mo), and morphological analyses (autopsy, MRI, and histological examination) were performed. Additionally, some male mice were checked with blood tests. Each analysis was

conducted using a different number of mice (n=2-129/ age groups). Body weight of male mice peaks at 18-19 mo-old (approximately 45.0g) and decreases at around at 25 mo, while female mice peak at 18-20 mo (approximately 35.0g) with no significant changes up to 27 mo. Food/water consumption increase rapidly with over 25-mo-old mice. Survival rates start to decline in both sexes at 18 mo, and show a relatively high score in elderly mice (>24 mo) than those in other research institutes. Rotarod performance peaks at 3 mo in males and at 6 mo in females and then continues to decline. Their grip strength does not significantly change with aging in both sexes. Enlarged seminal vesicles in male mice (78.9%) or splenic tumors (28.8%) were often found in dead animals at autopsy. Mice with abnormalities in various organs are mostly over 20 mo old. Additionally, blood tests show the composition of each type of white blood cells (WBC) tend to change with aging. Various age-related changes (such as food/water intake, motor performance, the composition of WBC, or pathologies) found in aged B6N mice can be senescence indicators at individual levels. We will analyze these parameters in detail and continue to search for novel biomarkers. Our analyses were conducted using different numbers of mice (n=2-129/ age groups). Only a small number of elderly mice (>24 mo) were used.

P87 Comparison of Direct and Indirect Methods of Arterial Blood Pressure Measurement in Healthy Male Rhesus Macaques (Macaca mulatta)

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Blood pressure is a critical parameter for evaluating the health of an animal, assessing effects of drugs and procedures, monitoring physiological status during anesthesia, and making clinical decisions. The placement of an arterial catheter for direct measurement of blood pressure is the most accurate method for measuring blood pressure; however, this is invasive, technically challenging, and often impractical during brief sedation. The objective of this study was to determine which method of indirect blood pressure monitoring was most accurate when compared to direct arterial catheterization. The indirect methods evaluated were ultrasonic Doppler flow detection (Doppler) and oscillometry. Additionally, we sought to determine the relative accuracy of each indirect method (as compared to direct arterial measurement) at a given body location and to assess whether the accuracy of each indirect method was dependent on body location. Fourteen healthy, male rhesus macaques (*Macaca mulatta*) ranging 3 to 16 kgs and 1-14 years of age were anesthetized with ketamine (20 mg/kg IM) and maintained within a stable surgical plane under general inhalant anesthesia using isoflurane. Blood pressure measurements were taken via direct arterial catheterization of the saphenous artery and compared to ultrasonic Doppler flow detection and oscillometric measurements at 3 body locations (forearm, distal leg, and tail-base). Results from this study indicate that oscillometry on the forearm is the best method and location for accurately and consistently determining blood pressure in healthy male rhesus macaques.

P88 Myocarditis in a Common Marmoset (Callithrix jacchus) after Adeno-Associated Virus Vector Injection

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A 6-y-old male common marmoset (*Callithrix jacchus*) being used in a terminal adeno-associated virus variant (AAV-PHP.B) vector injection displayed moderate, multifocal myocarditis on histopathological examination. The monkey had IV injection of an AAV-PHP.B vector and an intracortical AAV-PHP.B vector injection at 6 wk and 4 wk respectively prior to termination. The monkey also underwent a unilateral intravitreal tetrodotoxin injection 24 h prior to euthanasia as part of a retinal inactivation study. The animal had a history of clinical signs consistent with chronic wasting disease, but had successfully maintained body weight with budesonide treatment. Histopathological examination revealed moderate multifocal, chronic lymphoplasmacytic, and histiocytic myocarditis. The cardiac lesions were associated with myocardial degeneration, necrosis, and fibrosis. The skeletal muscle was unaffected, with no evidence of parasites or viral inclusions. Differential diagnoses for myocarditis in a marmoset include spontaneous cardiomyopathy and chronic myocarditis; infection with bacterial (for example, *Clostridium piliforme*), protozoal (for example, *Trypanosoma cruzi*), or viral (for example, Encephalomyocarditis virus) agents; dietary deficiencies (for example, vitamin E/selenium deficiency); and experimental manipulations. Heart tissue was negative on Gram, Giemsa, and Warthin-Starry stains, suggesting the cardiac lesions were of noninfectious origin. Immunohistochemical staining using an antibody against virally induced green fluorescent protein revealed strong expression in cardiac muscle and low expression in the brain. All other organs tested were negative. The AAV-PHP.B vector used in this case was designed for use in mice, where off-target expression has been observed in the liver, heart, and muscle. Use of this vector in primates has not been previously reported. Although AAV vectors are invaluable tools in nonhuman primate neuroscience research aimed at mapping neural circuits, our diagnostic results suggest that specialized viral vectors developed in other species may have the potential to exhibit altered tissue expression profiles in a marmoset, which may result in unexpected pathology.

P89 Facial Abscesses in Mice: Response to Treatment and Development of a Clinical Scoring System

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Three mice from separate cages were reported with facial masses and poor body condition scores in a colony of cytoglobin knockout (global) mice on a C57BL/6 background. Mice had been maintained in individually ventilated cages for 8 mo without prior clinical issues. Within 2 wk, 5 additional mice were identified with facial masses and were euthanized. A detailed evaluation of the colony revealed 18 of 62 (29%) mice with facial masses of varying severity. Animals were experimentally naïve, and no sex or age prevalence was identified. On necropsy, masses were identified as multifocal botryomycosis with intralesional cocci bacteria. Samples cultured from 5 mice revealed mixed infections of *Staphylococcal* species *(S. aureus, S. sciuri, S. epidermidis).* Following a sensitivity assay, treatment was initiated with oral enrofloxacin in reverse osmosis (RO) water provided via water bottle for 7d. Abscesses were refractory to treatment and lesions progressed rapidly. Treatment was switched to trimethoprim/sulfamethoxazole in RO water for an additional 2 wk. At the conclusion of the second treatment, 52 animals remained in the colony, and 17 had facial abscesses. *Staphylococcal* organisms are opportunistic pathogens and are common surface bacteria of healthy animals. Case reports have linked similar mandibular abscesses to barbering, suggesting excessive grooming leads to hair shafts becoming impacted in gingival tissues leading to infection. In this colony, barbering was noted in 30% of animals with abscesses and 25% of animals without abscesses. Cytoglobin is a cytoplasmic protein with protective effects against oxidative stress. Cytoglobin knockout mice have a high occurrence of multiorgan lymphoma, suggesting this colony may have a compromised immune status. A scoring system was developed as a practical method for determining removal. Using this approach we were able to reduce and maintain colony prevalence of abscesses at 3%.

P90 Report of an Adverse Phenotype: The Case of the Chirping Mice

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We report an unexpected phenotype that was observed in a closed-mouse breeding colony used to study calcium regulation. The breeding scheme involved multiple crossings of genetically engineered mice on 2 different background strains. The mice presented with a history of wheezing or chirping. Upon physical observation, the mice were observed with noise upon inspiration (inspiratory stridor) and increased respiration. The mice appeared otherwise normal. Three mice were submitted for necropsy. Upon gross examination, a collapse of the middle 1/3 of the trachea was observed in each mouse. Tissues were fixed in 10% formalin, processed for routine hematoxylin and eosin staining, and examined histologically. Histological examination revealed a narrowing of the tracheal lumen with malformation of the tracheal rings. After review of the breeding history with the investigators, it was determined that the chirping mouse phenotype was not resultant of a common breeding pair. Each mouse

with the chirping phenotype was homozygous for a null allele of the Cacna1h gene (Cacna1h -/-) and either homozygous or heterozygous for a flox allele for a second calcium channel. A literature review revealed that mice lacking a functional Cacan1h gene have been described with tracheal stenosis. This report emphasizes the importance of monitoring for unanticipated phenotypes when crossing genetically-engineered mice.

P91 Simian Varicella Virus Infection in Transplantation Research NHP Center

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Simian varicella is a natural erythematous disease of old world monkeys (*Cercopithecoidea cercopithecine*), involving patas (*Erythrocebus patas*), African green or vervet (*Chlorocebus aethiops*), and various species of macaque (*Macaca* spp.) monkeys. These outbreaks are sometimes associated with high morbidity and mortality and the loss of valuable research animals. Simian varicella virus (SVV, Cercopithecine herpesvirus 9), a primate herpesvirus, is the etiologic agent of the disease. Twelve Chinese rhesus monkeys (*Macaca mulatta*) at our nonhuman primates research center developed simian varicella, characterized by fever, vesicular skin rash, and acute hepatitis. Four of the 12 infected monkeys died within 48 h of the appearance of the rash with high AST and ALT elevation. Nine of 12 infected monkeys were undergoing transplantation research (corneal, pancreatic islet, liver transplantation) and 7 monkeys were treated with immunosuppressants. Serologic detection (ELSIA) and antigen detection (PCR) were conducted to confirm SVV. All of 12 infected monkeys were positive to ELISA and PCR. To control SVV outbreaks, an antiviral agent was used to reduce morbidity and mortality and minimize economic and research losses. After antiviral treatment (15mg/kg, PO, SID, or 5mg/kg, IV, SID), 8 survived and monkeys were recovered from SVV. To prevent SVV outbreaks, we decided to exclude SVV seropositive monkey before importing and during quarantine and recommend prophylactic treatment of antiviral agent for transplantation research included immunosuppression.

P92 Easy and Safe Endotracheal Intubation: Using the Endoscope and Inhalational Anesthesia for Mice

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Anesthesia strongly infuences the vital signs of laboratory animals, and it can also greatly affect the experimental data. Therefore, appropriate methods are critical for safe and reliable animal experimentation. In experiments using small laboratory animals such as rodents, injectable anesthesia or inhalational anesthesia using a mask is common for general anesthesia. These methods depend on spontaneous breathing for own breathing and/or are difficult to control the depth of anesthesia. Therefore, these are both simple and easy, but inferior in safety. On the other hand, in clinical practice for humans and pets, when performing general anesthesia, inhalation anesthesia after the endotracheal intubation is mostly performed using a ventilator. Therefore, in order to overcome these problems, we tried endotracheal intubation using an endoscope, and inhalational anesthesia was maintained using a ventilator. To each 8 male and female mice, M/M/B composed of 0.3 mg/kg b.w. of medetomidine, 4.0 mg/kg b.w. of midazoram, and 5.0 mg/kg b.w. of butorphanol was administered intraperitoneally as a pretreatment agent, and an endotracheal intubation using an endoscope was performed. Inhalation anesthesia was maintained with 1.5-2.0% of isoflurane and a ventilator. During anesthesia, some vital signs including SpO_2 (percutaneous arterial oxygen saturation %), heart rate (beat/min) and breath rate (breath/min), pulse distention (μm), and breath distention (μm) were measured and recorded using a pulse oximeter. Tracheal intubation was safe and easy. During inhalation anesthesia, the state of the murine vital signs and the respiratory system was relatively good. It was suggested that by using the endoscope, endotracheal intubation becomes safe and easy for the mice. It was also confirmed by checking the vital signs and indexes of the respiratory system. There were no major problems for the mice during inhalation anesthesia.

P93 Vitamin E/Selenium Deficiency in Juvenile and Young Adult Farm Pigs

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A series of unexpected intraoperative complications and deaths occurred while developing a surgical model in farm-raised Yorkshire pigs. The goal was to develop a porcine model of pediatric kyphosis and involved a lateral thoracotomy with pulmonary manipulation to access the ventral spine of 10kg piglets. Animals were placed postoperatively in a recovery area where they were monitored and remained for up to 48 h. All piglets appeared clinically normal prior to surgery. Postoperatively, some animals did not recover well or at all. Changes noted included posterior paresis, head pressing, and death. Necropsies were performed on all pigs that were euthanized or died. Consistent necropsy findings included excess fluid in the thorax and pericardial space along with hemorrhages in the myocardium. Due to the nature of the procedure, postmortem changes in the first pig were initially suspected to be intervention related. After similar findings in a second animal, frozen, banked preoperative serum samples were submitted for analysis of vitamin E and selenium levels. Vitamin E and selenium were both below age-specific lab reference ranges. Subsequent samples for all pigs in-house were submitted and found to be below normal levels for vitamin E and selenium in pigs of several ages. In retrospect, it was determined that vitamin E and/or selenium deficiency contributed to numerous unexplained morbidities which had not been previously identified in animals in this surgical lab. These changes affected several active projects and included intraoperative tachycardia and respiratory acidosis, drug-resistant surgical site infections, arthropathies including septic arthritis, and multifocal suppurative dermatitis. Clinical signs of vitamin E/selenium deficiency are most commonly seen in fast growing weaner piglets, and we suspect that the added stress of a major surgery exacerbated the condition of these animals. Resolution of morbidity and mortality occurred upon purchase of pigs from an alternate vendor providing feed analyses that met industry standards.

P94 A Comfortable Orthopedic Device that Provides Strength, Support, and Long-Term Stability of the Lower Leg in Small Ruminants

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Mature sheep with normal skeletal structure were used as a pain model. After a single common perineal nerve damage, exaggerated flexion and collapse of the left rear hock developed. Frequent dicubital ulcers would develop to the lower hock while trying to maintain a normal stance. External bandages would not provide mechanical support needed. We challenged conventional casting material for a unique external device to support the lower rear hock. A polycarbonate resin was molded to fit the lower 1/2 rear leg. The mold was divided into 2 pieces joined together with 25 mm texture rubber duc allowing expansion or decreasing the diameter around the lower leg. The boot stabilized the joints and is flexible, strong and avoided moisture breakdown. In the preceding 11 mo, one sheep reestablished normal stance within 2 mo after placement of the boot and continues 95% weight bearing without the boot. The other sheep continues to use the boot to maintain normal stance and weight of the rear leg. The polycarbonate resin boot can be cleaned and adjusted to fit the lower rear leg. Both sheep receive pain medication.

P95 Sterile Hemorrhagic Cystitis in a Sheep Following Repeated Cyclophosphamide Administration

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A 2-y-old, 60 kg female sheep received 3 high doses of cyclophosphamide (CPA) (37 mg/kg or 1453 mg/m^2) at day 0, day 13 and day 20 to render it immune deficient in the context of research on DNA-transfer. Three to four after the first injection, the animal developed stranguria without further clinical abnormalities. There were no signs of dehydration, no fever, and no loss of appetite. Urinalysis at day 8 showed no white blood cells and culture was negative. The urine, however, contained red blood cells (RBC) (86/µl) and was positive for Hb. protein. Creatinine were respectively 448.2 and 88 mg/dl with a ratio of 5.09. Our diagnosis was sterile, hemorrhagic cystitis (SHC), a side-effect from CPA caused by the metabolite acrolein, already described in humans, dogs, and cats, but not in sheep. A second dose of CPA, scheduled 1 wk after the first one, was postponed for 1 wk. To overcome this side effect the following 2 administrations of CPA, 1 mg/kg of furosemide was given daily until 1 wk after the last administration. From then on, the animal showed no more signs of cystitis and urinated normally during the rest of the study. Daily clinical exams revealed no abnormalities. Urinalysis was repeated 1 d after the third administration of CPA and revealed a decrease in RBCs (33/µl), no Hb, a decrease in proteinuria (144.2 mg/dl), and normalization of protein/creatinine (0.85). The animal was euthanized 20 d after the third CPA administration. Urinalysis showed no RBCs and a further decrease in proteinuria (42.5 mg/dl) and protein/creatinine ratio (0.26). Postmortem examination of the urinary tract showed several pinpoint mucosal nodules in the bladder. Histology of the urinary tract showed multifocal urothelial ulceration, mild subepithelial splitting, and moderate diffuse superficial mucosal fibrosis in the bladder. Kidneys and ureters showed no abnormalities except for a mild subepithelial splitting in one of the ureters. We found that CPA administration in a sheep led to SHC. Although treatment with diuretics successfully overcame the clinical symptoms during follow-up, damage to the bladder was obvious on histology. Whether starting diuretics from the first CPA administration on would prevent this damage still needs to be studied.

P96 Cell Collection Using Bronchoalveolar Lavage in Nonhuman Primates

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Optimization of a terminal procedure to collect bronchoalveolar lavage fluid (BALF) from the lungs of nonhuman primates (NHP) was developed to recover either radiolabelled tracers or resident airway and infiltrating inflammatory cells. Prior to the procedure, NHPs were administered a radiolabelled compound (IV) or lipopolysaccharide (LPS) via an intratracheal instillation. Animals were anesthetized with ketamine (15mg/kg, IM) and xylazine (2mg/kg,IM) and buprenorphine (0.05mg/kg, IM). The animal was placed in a supine position and a midline incision was performed and the underlying tissue was teased apart with blunt dissection to expose the trachea. Once exposed, a cut was made between the cartilage rings and a modified 20-24 french feeding tube (approximately 2 in. in length) was inserted and secured with a suture. Saline (20-40 mL) was injected into the airways, and the chest was palpated for 30-45 s. Following the chest palpitation, the animal was euthanized, and the BALF was extracted. BALF was then processed and analyzed for radioactive material or cells. This optimized procedure is effective for the collection of BALF from NHP to quantitate radioactive agents, cells, and potentially cytokines and other biomarkers.

P97 Doxorubicin-Induced Cutaneous Toxicity in a Beagle Canine

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An adult female beagle (*Canis lupis familiaris*) used in a model of doxorubicin-induced cardiomyopathy presented with epithelial desquamation on the left shoulder and ventrum after receiving the eighth weekly (20mg/m^2) intravenous dose of the free form of doxorubicin. No evidence of ectoparasites was found on trichogram or skin scrape. The lesions were empirically treated with topical disinfectants and antibiotic ointment. An oral cephalosporin antibiotic was initiated to limit secondary infections. Despite treatment, desquamation developed on the right shoulder, and the lesions increased in size and became ulcerated. Samples were collected for skin biopsies, trichogram, skin scrape, and bacterial and fungal cultures 5 wk following initial clinical presentation. Bacterial culture revealed *Staphylocccus aureus*, but trichogram, skin scrape, and fungal culture were negative for microorganisms. Skin biopsies revealed epidermal and apocrine gland hyperplasia, apocrine gland dilation, abnormal maturation of epithelial keratinocytes, and perivascular lymphocytic infiltration. These histopathologic findings resemble those described in humans and canines following chronic administration of doxorubicin containing pegylated liposome. However, clinical presentation in this case followed chronic administration of the free form of doxorubicin. In dogs, the pattern of cutaneous toxicity has been described to be localized to the paw pads, limbs, axillary, and urogenital regions. In this case, lesions were localized to the ventrum and trunk, but did not involve the paw pads, axillary, or urogenital regions. This is the first known report of this unique distribution of lesions following chronic administration of free doxorubicin.

P98 Unexpected Procedural Complications during Intraosseous Infusions

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Intraosseous (IO) cannulation has long been used in veterinary practice as a means to achieve vascular access in neonates or in species in which venous access is difficult. The advantage of IO access is that bone marrow provides a network of noncollapsible venous sinusoids. However, bone marrow is confined by a rigid compartment and subject to shear forces during IO infusions. Recent studies suggest that fat intravasation is common with IO infusions. We report a rare complication and unexpected ultrasound findings associated with IO infusions attributed to fat embolization. In this study, femoral venous ultrasound imaging was performed in 70 experiments on 35 female swine (*Sus scrofa*, 51± 4 Kg) with IO cannulas placed in the proximal and distal tibiae. Animals were maintained under general anesthesia with ECG, arterial (AoP) and pulmonary artery (PAP) pressures, and ventilatory status continuously monitored. Infusions varied from bolus injections of saline to progressive increases of infusion rates using a programed infusion pump. A postmortem volumetric method was developed to assess bone marrow volume loss (n=6 tibiae). Acute cardiopulmonary decompensation occurred in one animal at an IO infusion rate of 2 mL per second. In this animal, PAP increased rapidly followed by a fall in AoP, the appearance of ECG irregularities, a slow rise in central venous pressure, and a fall in arterial O_2 saturations and ET CO_2. At necropsy, lung histology revealed numerous large fat emboli consisting of bone marrow cellular elements and adipose cells within medium-sized arteries. Also observed in animals following high IO infusion rates, was the sequestration of echo-bright densities consistent with fat embolism on the nondependent endovascular margin of the femoral vein and 3 femoral vein thrombi formed at sequestration sites. Finally, post mortem assessment of medullary bone marrow loss in this study ranged from 7.7-38%. In conclusion, although acute hemodynamic complications associated with fat embolization from IO infusions are rare; providers must be cognizant of the potential for pulmonary embolism. Additionally, the latent effects of sequestration of fat emboli and formation of thrombus in the venous drainage from IO infusion sites is poorly understood.

P99 Diamond Burr Superficial Keratectomy of a Chronic Superficial Corneal Ulcer in a Rabbit

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A 7-y-old male 4.5 kg New Zealand White Rabbit housed individually in a standard rabbit bank in an AAALAC-accredited facility presented with mild ocular discharge and blepharospam of the right eye. Physical exam revealed corneal opacity and edema. On exam, no foreign object was found and a superficial central corneal ulcer was diagnosed with positive

fluorescein stain uptake. The cause of the ulcer was not determined, but it was speculated that the rabbit may have inadvertently injured the cornea with a piece of hay from its feeding bowl. Treatment was standard for the first 6 wk with topical ophthalmic antibiotics and topical atropine solution. The eye was r-stained at approximate 2-wk intervals and removal of loose corneal epithelial tissue was removed twice by debridement with sterile cotton tip applicators and topical ophthalmic anesthesia. After 6 wk the ulcer was determined to be nonhealing and the rabbit was treated with a diamond burr superficial keratectomy using a diamond burr with a 3 mm round tip with medium grit on a handheld powered drill. This type of burring technique is thought to remove more abnormal basement membrane than simple debridement, and thus enables new epithelial cells with stronger adhesion complexes to grow. Although rabbits in general have a 30% thinner cornea as compared to humans and dogs, there was no special consideration or adjustment made to the burring technique. Approximately 8 wk after the diamond burr treatment, and with the only continuing treatment being topical ophthalmic antibiotic, the rabbit cornea was negative for stain uptake. Although there was some residual corneal scarring, the cornea was considered to be healed. Diamond burr debridement was determined to be a safe, feasible, and successful treatment in a New Zealand White rabbit for a superficial, refractory corneal ulceration.

P100 Babies Helping Babies: Meeting the Critical Care Needs of Preterm, Nonhuman Primate Infants Treated with Continuous Positive Airway Pressure

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In order to support a nonhuman primate (NHP) animal model for moderate-to-late preterm (MLP) human neonates, our research group, along with veterinary and animal care staff, created a special care nursery (SCN) for preterm neonatal macaques. The goal of this study was to determine whether continuous positive airway pressure (CPAP), administered 12 hours per day for 10 d postbirth, improves the function of the MLP lung by promoting growth and suppressing airway reactivity. NHP neonates were delivered by cesarean section at approximately 140 d gestation to model the timeframe of human MLP neonates. Neonates received either CPAP or sham CPAP under light sedation and were evaluated at day 11 postbirth to determine if CPAP treatment resulted in improved lung growth and developement. The SCN had to address all the medical needs and potential emergencies that could arise with MLP neonates receiving extended CPAP. Our SCN is innovative in its ability to mirror the management of human MLP infants in the neonatal intensive care unit (NICU). The neonatal subjects required specialized housing and intensive care 24 h a day, by personnel with highly specialized skills including training in both basic and advanced life support. We developed a critical care managment plan that includes acute respiratory support, thermoregulation, and continuous physiological monitoring during CPAP administration. Immediate access to emergency drugs, intubation supplies, suction, infant ventilators, and IV access via peripheral inserted central catheter (PICC) lines were also necessary. We found that the most prevalent complications were transient apnea and extended low SpO_2. To address these issues efficiently, the respiratory support controls were repositioned so they were reachable from the isoletts to make administration a one-step process. If CPAP is shown to be effective in MLP nonhuman primates, it could be a simple, safe and nonpharmacologic way to improve lung function in MLP infants and prevent the high incidence of respiratory illnesses, hospitalizations, and use of asthma medications that may continue on through adulthood.

P101 Spontaneous Pulmonary Adenocarcinoma and a Subcutaneous Cavernous Hemangioma Arising in a Squirrel Monkey (Saimiri sciureus)

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A 12-y-old, wild-caught, male squirrel monkey (*Saimiri sciureus*) was transferred from another research institution. It had a history of a chronic indwelling catheter placement for an unknown duration for use in an addiction study. The animal arrived and was assigned to a neurobehavioral protocol but was experimentally naïve at the time of clinical presentation. The monkey presented with a gradual decrease in activity, though was eating and drinking normally. On day 12 after initial presentation, the animal would not leave the bottom of the cage. Due to concerns of declining health the animal was used in a terminal experiment. Gross evaluation revealed thickening of the skin over the dorsal tail with multiple raised, alopecic nodules (0.7-1.0 cm in diameter). The right accessory and right middle lung lobes were firm, enlarged, and white to tan to red, sections of which sank when placed in formalin. Microscopic evaluation of the tail nodules showed cavernous, blood filled spaces lined by bland endothelium (subcutaneous hemangiomas), partially occluded by organized thrombi. Histologic evaluation of the lung mass showed neoplastic cells with round to oval nuclei and 1-2 large nucleoli along with moderate to abundant, amphophilic cytoplasm. Tumor cells were simple cuboidal to tall columnar cells growing in a lepidic (linearly spreading along the alveolar walls) to papillary growth pattern. Mild anisocytosis and anisokaryosis along with a mitotic rate of 3 per 10 high-powered fields were noted. Alveolar spaces contained a lightly basophilic Alcian Blue positive secretory material. Small, intracytoplasmic secretory granules within the tumor cells were PAS positive. Histologically, the tumor is consistent with a well-differentiated papillary adenocarcinoma of bronchioloalveolar origin. It is unusual to see dual independent tumors arising in a nonhuman primate, and even though rare in squirrel monkeys, pulmonary tumors must be considered as a differential diagnosis.

P102 Staphylococcus xylosus Cystitis and Struvite Urolithiasis in Nude Mice Treated with Sustained-Release Estrogen Pellets

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Six-wk-old, female, nude mice (*Foxn1*^{nu}) were injected with 1 million MCF7 human breast cancer cells in the fourth mammary fat pad and received a 21-d sustained-release estrogen pellet (0.25 mg) implanted subcutaneously in the dorsum of the neck. All mice were maintained in sterile housing with sterile water and autoclaved rodent chow. Approximately 6 wk after surgery and implantation 4 of the 30 mice showed clinical signs of depression and dehydration. The animals most severely affected were euthanized and presented for necropsy. The urinary bladders of 2 of the animals were distended with variable sized white, opaque uroliths. Urinalysis revealed coccal bacteria, erythrocytes, neutrophils, and struvite crystals. Urine cultures from multiple animals grew heavy, pure growths of *Staphylococcus xylosus*. The organism was sensitive to all antibiotics tested with the exception of erythromycin (Intermediate). Analysis of the uroliths revealed 100% struvite composition. Remaining animals in the study were evaluated clinically for dehydration, the ability to urinate, and were manually palpated to detect stones. One additional mouse was identified as having a firm, nonpainful bladder (urolithiasis suspected). Based upon the sensitivity of the urine cultures, the remaining mice were placed on enrofloxacin in the drinking water (0.5mg/ml). All remaining mice completed the study without further morbidity or mortality. Previously published reports show estrogen supplementation association with urinary bladder pathology including infection and urolithiasis. Here we report a case of urolithiasis and cystitis associated with *Staphylococcus xylosus* which has not been previously reported in nude mice receiving estrogen supplementation. In this case we were able to visualize stones within the bladder through the skin of the nude mice. Bladder palpation and expression was helpful in identifying affected animals.

P103 Pseudoparasites Noted in Electric Eel (Electrophorus electricus) Aquaria

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Electric eels (*Electophorus electricus*) at our institution are studied for predation behaviors and are maintained in 80-120 gallon aquaria with gravel substrate. The environment is enriched with rocks, plastic branches, and plants. Thermostatic heaters maintain the water between 24°–28° C. Eels are fed live prey (fish and earthworms) 2–3 times a week. Approximately 1 week following addition of feeder fish, a large number of white, thread-like organisms were noted floating in the tank, adhered to the eel, and on the artificial plants/branches. These organisms exhibited purposeful

movement and ranged from 5–8 mm long. Since these organisms were present on the skin of the eel, there was concern of a parasitic infection. Specimens were collected from the tanks for examination. Microscopic examination revealed segmented worms with long hairs (chaetae) present on each segment. Initial identification of these segmented worms placed them as Oligochaetes within the family Naididae. Oligochaetes are free-living organisms feeding off of organic material and are often used as an environmental indicator of water quality. The organisms have not been shown to be parasitic or detrimental to the eels. However, their presence is an indication to be mindful of feeding (schedules and amounts of food), as well as providing adequate filtration and cleaning (especially gravel). Subsequent cleaning of the filters and additional vacuuming of the water in this case resulted in markedly decreased population of the oligochaetes. Although oligochaetes are nonparasitic organisms that do not present a direct health concern, they are reflective of the water quality within the tank system.

P104 Design, Construction, and Implementation of a Novel Apparatus for Urine Collection to Be Used for an Extended Period of Time

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Urine samples may be required from animals in pharmacological studies to determine excretion of test articles, their metabolites, and for urinalysis. Urine samples are also used to isolate pathogens in challenge models for biologicals (for example, Leptospira vaccines. Catheterization of the bladder is common in companion animals, however; anatomical characteristics and handling logistics make this procedure difficult and dangerous in cattle. To assess the environmental impact of a test article, urine from adult cattle required collection over a 24-h period. The urine was analyzed for amounts of test article and its metabolites. Our aim was to create a device to be used for collection of an uncontaminated urine sample from male bovine over a 24-hperiod. The animal was acclimated to and contained in stanchions. A rubber harness was placed on a 1-yold, 300 kg steer for 24 h, once weekly, for 4 consecutive wk. The harness consisted of an abdominal section with rubbermade laces tied to the dorsal area. A funnel was placed around the preputial area that was connected via tubing to the urine collecting tank. Feces were also collected through a receptacle placed underneath the plastisol-coated flooring. Total volume of urination and total amount of feces was measured and weekly sampling was allowed to test for drug kinetics. Urine collected was sufficient for purposes of study (averaged 2 gal. per animal/24-h collection period). No animals suffered any injuries or complications. The animal was safely restrained during collection. The design of stanchion and device contributed to handler safety. Minimum investment in construction of collection device was required and urine leaks were minimal to none. Repeatability of collections with no contamination is possible and requires minimal human resources (only necessary at installation and removal of apparatus). A number of improvements were or will be made for future studies, including funnel placement, shut off valve on hose, bigger capacity of collection tank, hose flexibility, and larger stanchions.

P105 Subcutaneous Melengestrol Acetate Implant as a Treatment for Endometriosis in Rhesus Macaques (Macaca mulatta)

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A 19-y-old, 10 kg female rhesus macaque used in cognitive neuroscience research sedated for routine suture removal was bright and alert, and without clinical signs of illness. On physical examination, a large, firm, palpable mass was present in the caudal abdomen. Abdominal radiographs revealed a large soft tissue-opaque mass in the caudal abdomen, associated with the uterus. The diagnosis of endometriosis was confirmed by visualizing a 12cm x 16cm x 10cm, hypoechoic, fluid-filled cyst on ultrasound examination, and aspiration of 140mL of chocolate-colored fluid. Endometriosis in rhesus macaques is commonly treated by once monthly intramuscular methylprednisolone acetate injections. However, as this particular animal was no longer needed by the investigator for neuroscience research, she was eligible to be transferred to a sanctuary for retired research primates. The sanctuary was unable to accept an animal requiring monthly treatment for a chronic disease, so an alternative, long-lasting treatment option was explored. In humans, endometriosis can be successfully treated with the use of progesterone analog contraceptives, including subcutaneous progestin implants. Subcutaneous implants impregnated with 20% MGA have been successfully used in primates and other species in zoos for contraceptive purposes. In this case, the female macaque was treated with an approximately 3.5 in.long, 0.38 in.diameter MGA implant that was placed subcutaneously between the scapulae. Serum progesterone and estradiol levels were monitored by LC/MS/MS, which is specific for endogenous hormones, at 2 time points pre (48 mo: progesterone 2260 and estradiol 11.6 pg/mL and 1 mo: progesterone 9810 and estradiol 36.6 pg/mL) and posttreatment (2 mo: progesterone 3.24 and estradiol 18.5 pg/mL and 4 mo: progesterone 3.0 and estradiol 16.1 pg/mL). These results suggest that the luteal phase of the menstrual cycle is being maintained by the implant. The endometrial cyst was monitored for regression using serial radiology and ultrasonography, which revealed involution of the cyst over time. This report demonstrates the utility of a subcutaneous MGA implant for the treatment of endometriosis in a laboratory rhesus macaque.

P106 The Mitey Chicken: Ornithonyssus sylviarum Outbreak in a Research Facility

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The chicken, *Gallus gallus domesticus*, is a commonly used avian species in biomedical research. Our university houses chickens of varying ages for biomedical, teaching, and agricultural purposes. In February 2017, a ~50% reduction in daily egg production in 3 floor pens of 1-year-old chickens was observed, and average daily egg production dropped from ~40 eggs/pen/d to ~20 eggs/pen/d. Upon closer examination, multifocal black, dry, round-to-coalescing lesions on waddles and combs, along with soiled, blacked vents were observed. Three affected birds were necropsied and tested for avian virus isolation, avian infectious bronchitis virus, and avian paramyxovirus-1. All results were negative. Necropsy examination revealed a significant mite infestation and the mites were positively identified as *Ornithonyssus sylviarum*. A treatment plan was developed as follows: battery birds were treated with topical Imidaclo-prid (4mg/bird) and moxidectin (1mg/bird) once, topical spray with permectrin II (10% permethrin) (20mL/gal) of both the caging and the birds once a month for 3 mo. Floor pen birds were treated with ivermectin (0.08% solution) (7.5mL/gal) in the drinking water for 24 h, every 10 d for 3 treatments and topical spray with permectrin II (10% permethrin) (20mL/gal) of the environment and the birds once a month for 3 mo. Following initiation of treatment, egg production increased and returned to normal in less than 1 mo. Additionally, the lesions on the waddles and combs healed and vents returned to clean white feathers within 1 mo. In conclusion, a combined topical treatment of imidacloprid and moxidectin and permethrin, or oral ivermectin and topical permethrin, in addition to environmental treatment with permethrin, effectively reduced *Ornithonyssus sylviarum* infestation, eliminated clinical signs, and resulted in an increase in daily egg production.

P107 Depression and Decreased Ambulation in a Common Marmoset (Callithrix jacchus)

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An 11-y-old female nulliparous, experimentally naive common marmoset presented with a 4-y history of previous intermittent depression and decreased ambulation upon arrival from another institution. The medical history included obese body condition, hepatomegaly, and mild intermittent jaundice. After 2 y in stable condition, the animal developed difficulty ambulating and tachypnea. Diagnostic assessments included physical examination, abdominal ultrasound, serum chemistry, complete blood count, and survey radiography. Initial clinical differential diagnoses included various etiologies of chronic renal disease, as well as metabolic bone disease, secondary nutritional hyperparathyroidism, and marmoset wasting syndrome. Physical exam revealed generalized muscle wasting as gauged by thickness of epaxial musculature, a gas-filled gastrointestinal tract, and small heterogeneous kidneys. Abdominal ultrasound confirmed physical exam findings. Bloodwork revealed anemia, hypoalbuminemia, hypocalcemia, hyperglobulinemia, and an elevated blood urea nitrogen (BUN) and alkaline phosphatase (ALP). Radiographs

demonstrated spondylosis of the lumbosacral spine, which may have contributed to the patient's decreased physical activity. During the ensuing months, the animal was treated symptomatically with meloxicam, gabapentin, oral calcium carbonate supplementation, iron dextran IM, and oral lactulose. The stability of the animal's condition deteriorated when the patient presented with tachypnea, palpable constipation, anemia, leukopenia, hypoalbuminemia, and a significantly elevated ALP. At that point, the animal was euthanized due to deteriorating quality of life. Gross necropsy revealed pale, tan, and irregular firm kidneys and soft, pliable bones (long bones, skull, ribs and vertebral bodies). Histopathological diagnosis included bilateral chronic progressive nephropathy, fibrous osteodystrophy of bones (metabolic bone disease), and mild to moderate lymphoplasmacytic mucosal enteritis and typhlocolitis. These findings highlight the varied spectrum of clinical and pathological manifestations of marmoset wasting syndrome and associated challenges in diagnostic assessments and therapy.

P108 Use of Dexdomitor-Ketamine Cocktail Sedation for Improved Recovery in Rhesus Macaques (Macaca mulatta)

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The use of chemical restraint/anesthesia for short procedures is a common practice when working with nonhuman primates (NHPs). Our center frequently uses chemical restraint for blood collections and other short procedures such as swab collections and rectal biopsies. It was noted that some of our NHPs, routinely sedated via chemical restraint with ketamine alone, have subsequent lack of appetite and weight loss several days postrecovery. To address this concern, our center began administering a drug cocktail of dexmedetomidine and ketamine for sedation procedures, with atipamezole given as a reversal agent for the dexdomitor. Upon using the cocktail, we noted that animals under sedation with the dexdomitor/ketamine cocktail had increased heart and respiration rates as well as near-optimal body temperatures compared to those sedated with ketamine alone. Additionally, the NHPs not only recovered more quickly, but consumed more of their biscuit rations following recovery, suggesting less overall stress and improved drug clearance. Six juvenile NHPs, all within the same age and weight range, were routinely sedated for study-related blood collections. Half of the NHPs received ketamine alone, while the other half received a dexdomitor/ketamine cocktail. With the cocktail, the dosage of ketamine is reduced from 10 mg/kg to 3-5 mg/kg. During the procedure, which lasts approximately 5 min, the animals' temperature, pulse, and respiration (TPR) were monitored, in addition to obtaining the animals' weight and length of time until full recovery. The number of biscuits consumed following full recovery was recorded. We will demonstrate that a dexdomitor/ketamine cocktail sedation is a viable chemical restraint alternative for sedating rhesus macaques and may result in less stress for the animals, improved appetite postrecovery, and less ketamine used. This additional advantage reduces the need for purchased and stored ketamine as a controlled substance.

P109 Managing Ulcerative Dermatitis in Aging Alzheimer Disease Strains of Mice with a C57BL/6 Background

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Ulcerative dermatitis (UD) is a common, spontaneous condition in mice with a C57BL/6 background. Although initial lesions are milder, UD is often a progressive disease that can result in deep ulcerations or debilitating fibrotic wound contractures leading to euthanasia, thereby affecting study outcomes through the loss of research subjects. At our institution, UD is a common health condition in aging mice used for behavioral studies. The majority of mouse strains used for age-related studies were developed on a C57BL/6 background. While there is no definitive treatment for UD, in order to maintain health and welfare of study animals, we have employed several novel treatment modalities.Treatments employed included pedicure with or without low-level laser therapy or application of a moisture barrier depending on the severity of the lesion. Low-level laser therapy is reported to have analgesic, antimicrobial, and anti-inflammatory effects and has been shown to accelerate wound healing of non-UD origin in several species. However, low-level laser therapy requires purchase of the equipment and may be inappropriate for lesions on the face. Performing a pedicure reduces manual trauma to the skin while application of a moisture barrier (sterile lubricant or petroleum jelly) allows penetration of the moisture into the lesion. Regular observations were performed for each treatment option and lesions were scored based on size, location, and response to treatment. When lesions were small, the low-level laser therapy and/or pedicure procedure worked equally well. For larger lesions, the low-level laser therapy promoted areas of granulation tissue but did not reduce the overall size of the wound. Pedicure for more severe lesions along with moisture barrier provided better treatment response for larger wounds than the cold laser treatment. Low-level laser therapy offers similar advantage to pedicure for smaller lesions. Location and size of the UD lesions were the most predictive measures of treatment success.

P110 An Acute Cranial Swelling in a Golden/Labrador Retriever Mixed-Breed Puppy

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A 13-wk-old, 11.3 kg, intact, purpose-bred, pair-housed, female mixed-breed canine carrier of Duchenne muscular dystrophy was examined for the formation of an acute swelling on the skull located in the region of the sagittal crest coursing over the occipital bone. Past manipulations included routine preventative care, consisting of vaccinations according to the AHAA canine vaccination guidelines using modified live vaccines, deworming, and flea and heartworm prevention. Experimental history included 2 MRIs to obtain baseline cardiac structural data. During physical exam, no abnormalities were detected except for the acute cranial swelling. Mentation was within normal limits and no neurological deficits were displayed. The swelling was firm and approximately 4 cm in diameter and 2.5 cm in height. The primary differential list included subperiosteal hematoma secondary to trauma, hemostatic disorder, or collagen defect. Secondary differentials were seroma, subcutaneous abscess, sebaceous cyst, idiopathic calvarial hyperostosis, and multilobular osteoma or chondroma of the skull. Diagnostics performed involved a complete blood cell count, serum chemistry, coagulation panel, buccal mucosal bleeding time, and fine needle aspirate. Additionally, radiographs and ultrasound were conducted to characterize the swelling. Based on diagnostic results, differentials were ruled out including hemostatic disorders, cysts, abscess, and diseases related to dystrophy of bone. Radiographic and ultrasound findings in addition to FNA confirmed a diagnosis of subperiosteal hematoma secondary to trauma. The initial treatment of choice was cryotherapy QID for 10 minutes/session for 11 d which only yielded a reduction of 0.2 cm. Consequently, benign neglect and monitoring was chosen and the lesion regressed over the subsequent 5 mo without complete resolution. Although occasionally reported in general clinical practice, there are no reports documented in the literature of this type of lesion occurring in canines, making this an unusual presentation in the laboratory animal setting.

P111 Intravaginal Prostaglandin Administration as a Method of Fetal Abortion in the Rhesus Macaque (Macaca mulatta)

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An 8-y-old, female, pregnant, SPF rhesus macaque (*Macaca mulatta*) housed in an outdoor breeding colony presented with severe crush trauma inflicted by cage mates. While the fetus was viable on initial examination, fetal demise occurred due to complications of the dam's condition. Intrauterine fetal death (IUFD) is a potential complication in breeding colonies of any species. The causes of IUFD are varied and can include congenital malformation, hemorrhage, infection, or occult maternal disease (such as diabetes mellitus, autoimmune disease, or thyroid disease). Vaginal birth is the recommended mode of delivery for management of IUFD in most women, and many women with IUFD will spontaneously labor within 3 wk of diagnosis. However, prolonged management carries the risk of maternal complications, such as disseminated intravascular coagulopathy. The suspected cause of fetal death in the patient presented is extensive trauma or rhabdomyolysis of the dam. Initial treatment of the dam included fluid therapy, antibiotics, analgesics, and hematinics. Five days after presentation, IUFD was noted and the dam was monitored for abortion. On recheck exam 3 wk later, the fetus remained in utero, but the cervix was noted to be soft, allowing for an induced abortion. A 200 mcg

misoprostol tablet was administered within the endocervix. Misoprostol is a synthetic prostaglandin E_1 analog that is widely used for induction of abortion and labor in women. In the presented case, spontaneous abortion occurred within 7 h of misoprostol administration. Intravaginal prostaglandin administration is a rational alternative to more invasive methods to induce abortion for the condition of IUFD in the rhesus macaque.

P112 Chagas Disease in a Texas Rhesus Macaque (Macaca mulatta) Colony

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Chagas disease is a zoonotic vector-borne disease caused by infection with the protozoan parasite *Trypanosoma cruzi. T. cruzi* is found in Latin America and the southern United States, where it infects many species, including humans and nonhuman primates (NHP). It is a major cause of cardiomyopathy in endemic countries, and to a lesser degree disturbances of the gastrointestinal tract and nervous system. The disease has also been associated with reproductive complications, such as stillbirths, miscarriages, and cases of congenital infection. NHPs are susceptible to natural infection and can develop clinical symptoms consistent with human disease. Studies of individual NHP colonies in Texas and Louisiana have found as high as 23% seroprevalence depending on geographic location and age. Knowledge about infection status of these research animals is important with regard to colony health, the outcome of the scientific studies, and the safety of caregivers and laboratory workers. Due to evidence of Chagas transmission in Texas, this study hypothesized *T. cruzi* infection in a closed, outdoor-housed breeding colony of rhesus macaque in Bastrop, Texas. In addition, the seropositive females in this colony could have reproductive complications consistent with Chagas disease. The seroprevalence of *T. cruzi* infection was evaluated by 3 tests: a multiplexed fluorometric immunoassay, an in-house produced Tc24 antigen-enzyme linked immunosorbant assay (ELISA), and a commercially available Chagas stat pak test. Retrospectively, the breeding history of the females within the colony from 2011- 2015 was reviewed to evaluate the reproductive history, including no births, miscarriages, still births, and live births. An overall 9% seroprevalence of *T. cruzi* infection was found in the total population of adult, juvenile, and weanling NHPs. We conclude that there is a significant seroprevalence of *T. cruzi* infection in the colony. There appeared to be an increase in reproductive complications when comparing seropositive and seronegative females. Further studies will determine whether *T. cruzi* infection is significantly associated with reproductive complications.

P113 Practical Inhalant Anesthesia and Monitoring Techniques Used for Short-Term, Noninvasive Cranial MRI/µPET Imaging in Infant Macaques

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Medical advances and interest in neurodevelopmental and psychological disease, among various other central nervous system processes, has warranted an increased use of advanced imaging techniques of the neonatal macaque brain. Using such young nonhuman primate models can provide a learning experience even for those who already have extensive experience with other small nonhuman primates. Out of about 380 infant anesthetic procedures using the discussed methods, less than 3% of cases required additional veterinary assistance. These subjects, across the ages of 2 d up to 1 y, were scanned using inhalant anesthesia and pre-scan analgesics for possible laryngeal irritation. Limited space, poor lighting, and difficult visualization of the laryngeal anatomy are all common challenges faced in these types of protocols. Nursing infants can spasm or regurgitate during intubation, which may compromise the infant's well-being from a possible obstruction of the airway. Another consideration is selecting the appropriate nonrebreathing system with minimal respiratory resistance for neonatal lungs for the stereotactic devices provided. Anesthetic/analgesic monitoring in the magnified or radioactive environment will be discussed using nonMRI-compatible anesthesia machines, multi/single parameter monitors, and the MRI compatible probes required. All of these challenges will be discussed focusing on the difficulty of providing an adequate breathing system with intubation techniques for stereotactic µPET and MRI scans.

P114 Use of Point-of-Care Blood Analyzer in a Nonhuman Primate Facility

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We maintain a large population of Rhesus macaques (*Macaca mulatta*). The most common causes of morbidity are attributed to diarrhea and trauma. In managing the health of the colony, various diagnostic methods are used. One of the most important tools we use is a portable point-of-care blood analyzer, which allows us to rapidly acquire results for critical animals. Having access to immediate blood values allows the veterinarian to customize a specific treatment plan to each animal. The main purpose of using a point-of-care blood analyzer is the immediacy in identifying and correcting electrolyte and acid-base imbalances due to acute, ongoing fluid losses such as diarrhea. The particular blood analyzer our facility uses requires a small whole blood sample (less than 0.10ml) and uses individual self-contained cartridges. Depending on the type of cartridge used, we can evaluate electrolytes, blood gases, acid-base balance, and hematocrit and hemoglobin values in approximately 3 min. We are able to quickly assess an animal's needs and customize the use of crystalloids and colloids, along with specific additives, in order to correct significant imbalances. After a few hours post IV fluid therapy, the animals are reevaluated. The majority of these animals respond well to the initial treatment and corrective measures. The accuracy, speed, and utility of point-of-care style blood analyzer are essential when working with nonhuman primates. Not only do the animals benefit greatly from having their electrolyte and other imbalances corrected in a controlled and accelerated manner, but it also reduces the hospitalization time and the need for repeated IV fluids. As a result of the decreased hospitalization time, the risk of rejection toward these animals when reintroduced into their respective groups is decreased. The point-of-care analyzer is also highly cost effective; requires little maintenance, technical expertise, or expensive reagents; and cartridges have a long shelf life.

P115 Renal Dysplasia in a Purpose-Bred, Vendor-Acquired Dog

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A 10-mo-old intact female purpose-bred hound dog arrived at our institution from a vendor for use in a study involving bilateral adrenalectomy. No abnormalities were noted on physical examination. After 4 d of acclimatization, a laparotomy was performed, and bilateral nodular kidneys were observed. Due to the potentially confounding influence on the study, the dog was euthanized during the procedure. On necropsy, the left kidney was hypertrophied, while the right kidney was severely crenated. Both kidneys had an irregularly nodular surface with areas of grossly normal parenchyma divided by fibrous tissue. Histologically, evidence of renal malformation was present in the form of segments of densely fibrotic interstitium with a paucity of tubules and numerous tortuous arteriolar profiles, persistence of fetal-type glomeruli, presence of fetal glomerular structures in the renal medulla, and foci of primitive mesenchyme. Mild to moderate cystitis, pyelitis, ureteritis, and tubulointerstitial nephritis were also present. No evidence of renal insufficiency or uremia was present, and postmortem cystocentesis was consistent with mild lower urinary tract inflammation. While an infectious or inflammatory disease of the kidney could result in scarring and deformity, the young age of this animal and the microscopic features of renal maldevelopment suggest congenital renal dysplasia. This is a hereditary condition and was reported to the vendor.

P116 The Effect of Bronchoalveolar Lavage on Postprocedural Computed Tomographic Imaging in Rhesus Macaques (Macaca mulatta)

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Bronchoalveolar lavage (BAL) is a common clinical procedure to access and recover pulmonary parenchymal cells for research, diagnostics, and clinical purposes. A known volume of sterile saline is instilled into the lungs and is withdrawn. A portion of instilled saline, roughly 1/3, is typically unrecovered. In human medicine, the recommendation is to wait 24 h for the residual saline to dissipate before performing pulmonary imaging or additional pulmonary diagnostic procedures. However, no recommendation is available for nonhuman primates undergoing similar procedures. The purpose of our study was to determine 1) if residual PBS instilled into the lungs during BAL procedures is detectable on postBAL computed tomographic (CT) images, 2) if present, the extent of the post-BAL CT changes, and 3) how long the post-BAL imaging effects persist in macaque lungs. Baseline CT imaging was performed on 4 rhesus macaques (*Macaca mulatta*), followed by BAL, in which PBS was instilled in the right lung of study animals. The lungs were reimaged immediately after BAL and every 24 hr until no pulmonary changes were evident. The studies were performed twice on the same animals approximately 3 wk apart. During each BAL procedure, 40 ml of PBS were instilled into the right lung, and an average of 27 ml were withdrawn. Pulmonary changes, manifested by variable densities and opacities in the right lung, were subjectively evaluated and were quantified using percent change in lung hyperdensity (PCLH). Changes were detectable on CT imaging immediately after BAL in all animals, were detectable in 2 of 4 animals during both experiments at 24 h postBAL, and persisted for up to 3 d (72 hr) postBAL in at least 1 animal in each study. The studies demonstrate that invasive pulmonary procedures such as BAL may cause immediate CT-detectable pulmonary changes that can persist for up to 72 h after the procedure. These findings further suggest that postBAL pulmonary changes may be superimposed onto preexisting pulmonary changes, and could be misinterpreted as pathologic change.

P117 Contaminated Rodent Feed as a Source of Salmonella enterica Serotype Livingstone Infection in a Laboratory Mouse (Mus musculus)

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A group of 4 genetically engineered mice imported to our facility tested positive for *Salmonella* spp. by pooled fecal sample (PCR) during routine quarantine testing. The mice were shipped from the noncommercial source via ground transport in an undamaged, single, 3-compartment, plastic crate. On arrival mice were housed singly or in pairs in 3 cages. Following the PCR result, individual rectal and fecal cultures confirmed only 1 (singly housed) of 4 mice was positive for *Salmonella* spp. The isolate was confirmed as *Salmonella enterica* by while whole genome sequencing (WGS), single nucleotide polymorphism (SNP) analysis, and serotyping confirmed the isolate as a rare Livingstone serotype. The infected mouse shed *Salmonella* persistently; it was isolated 6 times over a 4-mo period. A naïve Swiss Webster mouse, cohoused for 1 mo with the positive mouse, remained *Salmonella* culture negative when tested weekly on 5 consecutive wk. The remaining 3 mice were *Salmonella* negative by culture in 5 tests over a 5-wk period. Two of them were subjected to a dexamethasone provocation test, and all 3 were ultimately euthanized and multiple organs cultured. The mice remained culture negative for *Salmonella*. The same bacterium, as determined by WGS, SNP analysis, and serotyping, was isolated from a commercial rodent feed sample at the facility of origin. Kirby-Bauer antibiotic testing also revealed that both isolates had identical antibiotic sensitivities and resistance patterns. *Salmonella* spp. infections are extremely rare in contemporary laboratory mice. Commercial rodent feed was the most likely source of contamination in this case. Other serotype Livingstone infections, have been traced to contaminated feed intended for other animal species.

P118 Ferret Enteric Coronavirus in a Pregnant Jill

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A 15-mo-old, pregnant jill presented with mild, green mucoid diarrhea 3 d after arrival from the vendor. At the time of arrival, she was estimated to be at 28 d of gestation. On physical exam, she was afebrile, in fair body condition (BCS 2+/5), and exhibited mild, intermittent tremoring. Fecal flotations were negative. She was treated with a 4-d course of Albon, subcutaneous fluids, and nutritional support. Diarrhea resolved after 8 d, and 2 d later, she gave birth to 7 kits, all of which were small in size and died within 48 h of birth. At 6 d postpartum, the jill presented lethargic and weak, with mild tremors and thickened small intestines noted on physical exam. She was euthanized and submitted for necropsy to investigate the cause of chronic, mild, intermittent diarrhea and acute mortality of all kindled kits. On gross necropsy, there was focal hyperemia of the ascending and transverse colon and enlarged, firm, mesenteric lymph nodes (approximately 2 cm in size). Histopathology results showed a moderate, multifocal to coalescing lymphocytic and plasmacytic enteritis with mild to moderate villar blunting and fusion and crypt hyperplasia. Serum chemistry was normal other than mild hypercholesterolemia (244 mg/dL). Feces tested positive for ferret enteric coronavirus (FRECV) by polymerase chain reaction (PCR). In this case, the clinical course of infection and gross and microscopic lesions of the intestine were consistent with epizootic catarrhal enteritis (ECE) caused by FRECV. While this disease is not generally fatal, infection of the dam during the time period from parturition to weaning has been associated with mortality of neonates in cases of epizootic catarrhal enteritis in mink, a similar disease to FRECV. If this infection is suspected based on clinical presentation, definitive diagnosis of FRECV can be achieved by PCR of fecal or intestinal samples.

P119 Considerations for the Veterinary Care and Management of an Older Ferret Colony

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Several of the older ferrets in our colony were exhibiting consistent weight loss over a period of 2-3 months with no marked clinical observations. The ferrets are group housed in rabbit/ferret caging at tan AAALAC-accredited facility. The poor body condition and significant weight loss (20-30%) in some of these ferrets necessitated euthanasia. Organs were evaluated grossly for any abnormalities. Liver was observed to be grossly abnormal with diffuse yellowish discoloration on all the liver lobes. The other organs, including intestines, stomach, spleen, kidneys, lung, heart, and the reproductive organs appeared grossly normal. On histopathology, eosinophilic portal triad vasculitis characterized by an inflammatory and some proliferative (hypertrophy of tunica media) vasculopathy of portal triad vessels with several arteries displaying fibrinoid necrosis of the tunica media was observed in the liver. In the intestinal tract, the lamina propria of mucosa had a moderate diffuse mucosal lymphoplasmacytic infiltrate with significant numbers of eosinophils. The sections were also stained with the Steiner silver stain and no organisms consistent with pathogenic *Lawsonia* or *Helicobacter* were observed. The intestinal section tested positive for ferret coronavirus upon molecular testing (reverse transcription-polymerase chain reaction). It was found that ferret corona virus, which is endemic in the laboratory ferret population, is also prevalent in our colony, but is not always associated with characteristic clinical signs, such as diarrhea, lethargy, or dehydration. One of the intervention approaches we adopted to manage weight loss in the colony was to provide the pelleted ferret diet mixed with water in a 1:2 ratio as it improved the palatability and subsequently the intake of food. This has been shown to be effective in previous literature and we also found that the body weights improved in these animals, though it was not significant compared to ferrets that did not have weight loss fed the regular diet. It also had the added advantage of providing an additional source of water.

P120 Treat-Driven Physical Therapy in Exercise Tunnels for Rhesus Macaque (Macaca mulatta)

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Rhesus macaques with musculoskeletal injuries may need increased incentives and space to regain muscle mass and range of motion of limbs than what is provided in standard macaque housing. Encouraging animal movement through a cage tunnel system could improve range of motion, muscle mass, and quality of life for animals with musculoskeletal injuries. An exercise tunnel connected a bottom-tier cage with a top-tier cage for continuous access to both top and bottom cages. Daily peanut physical therapy (PT) was prescribed for patients needing PT in the exercise tunnel. Peanut PT involved enticing patients to move up and down the exercise tunnel with treats such as peanuts or dry pasta. Treats were offered at upper-tier and lower-tier positions to encourage limb use in locomotion. Animal pairing was attempted whenever possible, and 2 patients could be treated with cooperation. Two patients were paired and housed with the exercise tunnel together in the hospital. Patient 1 was favoring his right leg, and after pain medication and 10 d of daily peanut PT, right leg use returned to normal. Patient 2 had a full triceps muscle transection that was repaired, and the animal was put in the exercise tunnel for 10 d of peanut PT to decrease muscle contraction, improve muscle mass, and improve range of motion of limbs. Both animals were later released successfully to social groups in large enclosures with high perches. Daily sedations for range of motion exercises were not necessary for rehabilitation. The exercise tunnel and peanut PT offered an environment to improve or maintain limb use following injury with a low risk of damage.

P121 Abdominal Dilation in an Electric Eel (Electrophorus electricus)

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A research naïve male electric eel (*Electrophorus electricus*) of unknown age was presented for dilation of the caudal abdomen. This dilation would contract as the eel lunged to eat, and then returned to its distended contour afterward. The eel had normal appetite and buoyancy. The eel had been in the facility for about 2 y and was housed with 2 other eels of similar age and unknown sex. The housing consisted of a 40-gal tank with an isolated recirculating system. The aquarium contained plastic plants and a substrate of small river stones. The water temperature was maintained between 24-28°C with a thermostatic heater, at pH 6.5. The diet consisted of earthworms and occasional live fish. Diagnostics were limited due to the electrical hazard presented by the species. Differential diagnoses included neoplasia, foreign body, obstruction, or ascites. Thirteen days after presentation, the dilation had enlarged and euthanasia was performed with tricaine methanesulfonate. Postmortem radiographs revealed multiple round mineral opacities in the caudal abdomen. On necropsy, a dilated stomach was found to contain 14 small river stones. Histologically, rare granulomas were found in the kidneys and gastric wall, which were unlikely of any clinical significance. To our knowledge, this is the third documented case of substrate ingestion occurring in an electric eel. This finding led to husbandry modifications for this species at our institution.

P122 Normal Physiological and Pathological Values for the Sinclair Miniature Swine

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Miniature swine overall are increasingly recognized as a nonrodent model in regulatory toxicology and dermal toxicology. The similarities of their cardiovascular, renal, and digestive systems to those of humans make them a suitable animal to model the human counterpart; they are also amenable to all routes of compound administration. Additional attractive traits that make them a good substitute to model humans are that they are omnivorous, easy to handle, prone to obesity, and will develop atherosclerosis and dyslipidemia when fed a high-fat diet. The Sinclair miniature swine (SMS) is the oldest strain of miniature swine developed for research; it is one of the smallest, as well. In an effort to generate a database of baseline information about the normal physiological status of the SMS, information was retrospectively collected from control animals in various toxicology studies, as well as from studies designed solely to collect baseline information. Animals selected were 3 to 5 mo of age and were required to be healthy and immunologically naïve. Animals used as negative or vehicle controls in systemic or dermal toxicology studies were included; control animals that were part of wound healing or surgical studies were excluded. Physiological data were collected from equal numbers of both male and female, and include weight and body measurements, hematology, serum chemistry, coagulation profile, urinalysis, ECG rhythm and segment intervals, and organ weights, including the brain and pituitary gland, thoracic organs, reproductive organs, and abdominal organs, excluding stomach, pancreas, and intestines. Body measurements such as height, width, circumference, and tail-head length were taken of 20 SMS at 3, 4, and 5 mo of age. Urinalysis samples were collected from 40 SMS via metabolic cages. Multiple-lead ECGs of 22 SMS were collected with the SMS in sternal recumbency in slings, and organ weights were collected from 12 SMS at necropsy. The resultant data from this retrospective study will benefit the SMS as one of the nonrodent species in research by providing baseline information with which to correctly interpret regulatory toxicity and other testing results.

P123 Normal Data on Selected Lineages of Miniature Swine

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We recently updated a comprehensive dataset on normal data (reference intervals or ranges) for our 4 lineages of miniature swine. Included are Yucatan, Hanford, Sinclair S-1, and Micro-Yucatan lineages. This effort collates and summarizes normal biological and physiological data collected over many years. Data categories include uses in biomedical research, body measurements (biometrics), growth, coat colors, clinical pathology (hematology, chemistry, coagulation, urinalysis), organ weights, background histopathology findings, blood glucose, ocular, diet/feeding, cardiovascular, ECG, dermal, reproduction data, normal rectal temperatures, and references. Over 200 tables of data are presented. These data are offered to veterinarians, biomedical investigators, preclinical clients, and university staff to facilitate research when using our miniature swine animal models. We outline the available data and present representative data tables.

P124 Development of a Nutritionally Complete, Shelf-Stable Diet for Egyptian Fruit Bats (Rousettus aegyptiacus)

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Our university houses captive Egyptian fruit bats *(Rousettus aegyptiacus)*, a frugivorous species that are fed a diet of fresh, highly perishable produce daily. However, during an earthquake, severe drought, or other types of natural disaster, the availability of fresh produce may become limited. Thus, we sought to develop a palatable, shelf-stable, and nutritionally complete diet for our laboratory bat colony. Research on the macronutrient and micronutrient requirements of the Egyptian fruit bats was conducted in order to select a suitable dry diet for the species. It was essential to implement a diet that contained a stabilized form of vitamin C, a nutrient that Egyptian fruit bats cannot synthesize in the body, while also minimizing the overall iron content to prevent hemochromatosis, a diet-related disease reported in captive bats. In addition to nutrient requirements, it was also important to factor in the natural eating behavior and taste preferences of the Egyptian fruit bat when developing a diet. A nutritionally appropriate dry diet was selected and combined with dilute juice and canned goods in small bowls, to provide shelf-stable nutrition for captive Egyptian fruit bats. The addition of juice and canned goods to the dry diet ensured the bats were provided sufficient vitamin C and carbohydrate content to mirror their current fruit diet. These items also served to increase the diet's overall appeal in terms of moisture and flavor. Use of the shelf-stable diet has resulted in decreased costs and labor associated with purchasing and preparing fresh produce daily, as well as decreased storage facility needs. By combining a dry diet with diluted juice and canned goods, we were able to successfully develop a palatable, shelf-stable, and nutritionally complete diet for captive Egyptian fruit bats.

P125 Breeding Optimization when Choosing Mating Formats of Genetically Engineered Conditional Models

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The complexities of breeding genetically engineered models (GEM) has increased with the abundance of techniques available to modify the genome. Pronuclear injections, homologous recombination, Zn finger TALEN's, and CRISPR/Cas9, among others, are all used to generate GEMs. Scientists looking to get the needed experimental cohort face a complicated task designing the mating format when various mutations are considered: homozygous versus heterozygous or potential deleterious phenotypes that may occur due to the effect of the mutated alleles that can be exacerbated when its introduction is through the maternal or paternal germline. Conditional models were generated to get around lethal phenotypes, namely, introducing the deletion at a certain age, or in a tissue-specific manner, etc. In mice, a widely used conditional model system is the CreESR1/lox inducible model in which the Cre recombinase gets activated by estrogen analogous compounds (for example, tamoxifen), excising the DNA at the lox target sequence. When intercrossing such inducible lines, the inducible CreESR1 may get undesirably activated. Other Cre lines are expressed under a tissue-specific promoter and therefore should only delete lox sequences present in the tissue where Cre is being expressed. In this study, data has been collected on over 40 different alleles with several of these conditional Cre deleters. When specifically using a Cre/ESR1, Cre deleter model, 63% of the alleles collected were not leaky while 37% of alleles were. For the leaky alleles, the overall percentage of penetrance varied from 0.05% to 80%. Data on other tissue-specific Cre deleters show an overall leaky penetrance ranging from 0.5% to 93%. One interesting conclusion from this study is the unpredictability of the affected allele by leakiness and the importance of a pilot study in order to assess the use of the conditional model.

P126 International Laboratory Animal Technician Week 2017: Celebrating the Unsung Heroes of Biomedical Research:

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Laboratory animal technicians are the voice for animals in research and play a critical role in making biomedical research a possibility. Despite their crucial role they are often not given the recognition deserving of their importance. International Laboratory Animal Technician Week gives us an opportunity to celebrate them as heroes of animal research and give the researchers an opportunity to recognize and thank them for the work that they do. This occasion allows us to boost morale and give the staff a sense of ownership over the research. At our university, the laboratory animal residents, with the help of the area supervisors, put together a celebration for a staff of approximately 70 technicians. The technician week events were spread over 2 wk to allow the staff on varying schedules to all be able to participate. The events were focused on food, fun, relaxation, and celebration. Participation was at an all-time high and we have established some morale building traditions for our team's future.

P127 Group-Housing Rabbits

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Single-housing of rabbits has been the standard method of caging system for rabbits in research. Group-housing rabbits can be difficult due to social hierarchies to assert dominance within the group. The purpose of this project is to demonstrate the functionality and success of group housing rabbits. This demonstration uses 5 female New Zealand White rabbits at 4 mo of age within a group housed caging system. Rabbits are known to establish dominance each time a new member is introduced to the groups and especially as rabbits age past adolescence, and so to help lessen these effects within our caging system we looked at changes that we could make to improve the animals' welfare and reduce stress levels. Two different group housing systems were used with no adverse events in either system. The rabbits were fed ad libitum in feeders with an automatic watering system as well that allowed all rabbits to have a source of feed, hay, and water source accessible at all times, which helped to decrease aggression when feeding or drinking. Wooden chew blocks and Himalayan salt blocks were also provided for additional enrichment, along with plastic hutches and hay for bedding for each rabbit to nest and hide from the group as needed when aggressors were present. Through the use of a group-housed caging system and daily handling, we were able to provide an enriching and safe environment that allowed for the rabbits to display behaviors such as play, foraging, and nesting. Upon the conclusion of the experiment, we were able to determine that if the rabbits were provided with ample enrichment, hiding areas, and free-choice feed and water sources, along with dedication to daily handling, that the rabbits could be group housed at 4 mo or older successfully, rather than singly housed. We found that our rabbits overall had reduced stress from the increased enrichment opportunities and natural stimulation, which resulted in the importance of these variables for a successful group housed system and overall betterment of the animals' wellbeing.

P128 Hopping toward the Future

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Our institution supports cancer research, and in that endeavor we maintain rabbits, a species known to be uniquely challenging in many facets, particularly enrichment. Over the decades, we have provided the standard enrichment recognized to meet the basic needs of rabbits. We have had an ordinary program, but it lacked dimension. The basic elements included a couple of toys, a water source, and food enrichment. Nevertheless, we determined that adequate enrichment was not necessarily satisfactory. As there was potential for significant improvement, we determined that exceeding minimum standards was achievable and practical in our population. Therefore, in our goal toward continuous improvement in our rabbit enrichment program, we approached this effort openly, to fully assess what was working well, and what required revision or augmentation. We wanted to develop enrichment that would maximize the natural behaviors expressed by the rabbits, reduce abnormal behaviors, increase normal patterns, and cope with the artificial environment and inevitable limitations of space imposed by a research facility. Additionally, we wanted to ensure that they would be ideal subjects to serve as research models. To that end, we solicited the creative contributions of our team and gathered the knowledge provided by other institutions to achieve transformation of our program through a deconstruction and reordering process. We created a new environment, replete with a variety of novel manipulable objects such as edibles, use of recyclables, a free-ranging yard, exercise, incorporation of a novel means of social enrichment, as well as human contact. Moreover, we have developed a system of enrichment rotation with overlapping forms of enrichment for our rabbits. Once complete, we achieved our original goal of a more enriched environment for our rabbits, which will inevitably lead to improved wellbeing and research outcomes.

P129 Pictorial Flipbooks as an Immediate Reference for the Safe and Precise Use of Clinical Equipment

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Training on the proper use of equipment in an animal research facility is essential to maintain the safety of personnel, animals, and to obtain accurate research data. Due to the various degrees of equipment use, different equipment models, and communication barriers, refresher training is often needed to ensure proficiency. As a way to reinforce face-to-face training and offer a constant and immediate refresher, easy-to-follow pictorial flipbooks were created. The flipbooks are a small 4 in. by 4 in. booklet that contains colorful pictures along with step-by-step instructions taken directly from standard operating procedures. They are hung on or near equipment such as anesthesia machines, euthanasia systems, and radiology machines. The pages of the flipbooks are laminated for ease of cleaning which enables them to be placed in animal housing and procedure rooms. They serve as an instant detailed reference and are tailored specifically for each piece of equipment. The veterinary team, operational staff, and research staff have all found them easy to follow and vital when

troubleshooting equipment. The flipbooks have nearly eliminated the need for retraining on the use clinical equipment which allows training efforts to be focused elsewhere.

P130 Rhesus Macaque (Macaca mulatta) to Owl Monkey (Aotus nancymaae): Fabricating Changes to Existing Caging to More Appropriately House a New Species

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Accommodating a new species in a laboratory setting requires proper housing as different species have different needs. While both are from the same order, new and old world primates have different natural histories. Appropriately housing them means not only taking these histories into account, but also how the staff will interact with the new caging system the animals require. Brand new, standalone, systems are expensive and require storage space when not in use. We have adapted a number of our cage fronts (originally designed for macaques) to more easily accommodate new world monkeys, specifically owl monkeys (*Aotus nancymaae*). The changes were made with not only animal needs in mind, but ease of use by personnel, storage requirements and also cost as compared to purchasing new world monkey specific caging systems.

P131 Maintaining a Large Colony of Beagle Dogs

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Maintaining a large colony of dogs comes with many challenges. Our colony of ~200 beagle dogs remain in the facility for 4-4.5 y. The challenge with this colony was the development of interdigital (ID) cysts which required a high degree of veterinary support to treat. After brainstorming how to improve the situation, staff adopted the use of compressed air (CA) to decrease the presence of water after the daily cage cleaning. The use of CA added an additional 45 s to 1 min to the cage-cleaning process. After piloting this over 3 mo, there was a significant decrease (~75%) in the incident of ID cyst occurring and it additionally resolved other ongoing issues. Each animal housing room was equipped with a CA supply and appropriate supplies to increase the efficiency for staff. The use of CA versus other options includes a safety benefit of no electrical hazard in the animal room when the cage cleaning is being conducted. This refinement in our animal care operations has significantly decreased our veterinary support to maintain the health of our chronic dog colony.

P132 Cellulose-Based Bedding as an Alternative to Corn-Cob for Breeding Colonies

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Bedding material used in rodent cages is constantly reevaluated in terms of cost efficacy, animal preference, and advances in animal welfare. Determining which bedding choice is ideal for a facility can be challenging due to lack of directly comparable metrics. This study was designed to determine if using cellulose-based bedding, instead of the facility standard of corn-cob, would decrease the frequency of off-cycle cage changes without negatively affecting reproduction (as assessed by litters born and deceased pups). Two breeding rooms containing 450 cages per room of mixed strains were placed on cellulose bedding for 4-6 wk, followed by a return to corn-cob bedding for 2-4 wk. The total number of cages spot-changed (cages that required changing during the non-changeout week), as well as total number of new litters and deceased pups were tallied daily in each room. Data was compiled over the course of the study and compared statistically to detect meaningful differences between bedding types. The total number of cages spot-changed on cellulose bedding showed a 3-fold decrease compared to corn-cob and did not affect breeding. Decreasing off-cycle cage changes reduces personnel time, supply cost, and animal-stress associated with re-establishing dominance and scent markers within the cage.

P133 Enrichment Items to Improve Current Treatment of Malocclusion in Laboratory Rodents

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Malocclusion is a common occurrence in laboratory rodents and is a result of improper alignment of teeth, namely upper and lower incisors, and cheek or molar teeth. Once diagnosed, routine technician intervention is required to manage the condition. We attempted to refine the treatment method to reduce technical intervention. Improper alignment of teeth prevents the normal wear of tooth surfaces, resulting in overgrowth and malocclusion. Tooth overgrowth can affect an animal's health by not allowing the animal to feed and drink properly, leading to malnutrition, dehydration, and oral or facial abscesses. Treatment requires restraining or sedating the animal and clipping incisors, as well as providing supplemental food enrichment. However, clipping of incisors only manages the misaligned teeth and does not address the problem which resides with the cheek or molar teeth. Incisor maintenance is required as often as weekly and requires attention throughout the duration of the animal's life. Tooth clipping can also result in complications, such as tooth loss, splintering of teeth, or injury to the animal's oral cavity. We will demonstrate that the refinement of our treatment for malocclusion with the addition of enrichment items such as wooden gnawing blocks or nylon bones can minimize the requirement for tooth clipping, with the aim of discontinuing this practice entirely. We will demonstrate the effectiveness of the enrichment by comparing the length of time between incisor clippings.

P134 Daily Water Intake of Common Marmosets (Callithrix jacchus) in Biomedical Research

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The typical daily water intake of common marmosets (*Callithrix jacchus*) in a research setting has not been well characterized. Because these New World primates are increasingly popular as animal models in neurophysiological and behavioral experiments, which can include the potential use of water regulation for training, veterinary and research staff need to understand how marmosets normally regulate their hydration status. A study was undertaken to measure the water consumption of older (5-12-year-olds, n=11) and younger (1-2-year-olds, n=10) adult marmosets every 3 h during the 12-h light cycle (0700 to 1900) for the months of January and July. The results show the average water intake per animal was 38.71 ml/kg/day with a wide range across animals (minimum: 14.57, maximum: 129.40 ml/kg/day). However, water intake by an individual marmoset was fairly consistent from day to day. Water intake did not vary across the 4, 3-h periods measured during the day, and minimal water was consumed overnight when the room lights were turned off. Also, there was no significant difference in daily water intake between the 2 mo ($P = 0.41$). A moderate but significant correlation ($R = 0.65$; $P < 0.01$) was found between age and water consumption, where older adults tended to drink more than the younger group. Singly housed marmosets drank more on a per kilogram basis than pair-housed marmosets ($P < 0.01$). Importantly, the considerable variation in water consumption among marmosets emphasizes the need for an individualization of fluid regulation protocols.

P135 Attempting Enrichment Harmonization across Multiple Sites and Embracing Our Differences

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We have a Global Enrichment Committee that incorporates active members from each of our 6 sites that works towards harmonization of enrichment practices. One of our hot topics is attempting to standardize rodent (specifically mouse) enrichment and social housing practices, which has proven to be quite challenging. There is a multitude of options regarding enrichment items such as the provision (or not) of group housing, food treats, nesting material and amount, gnawing devices and type, shelter/no shelter and type, caging (size/type), and bedding. The selection of each site also has to consider the needs of the strain(s) and sex(s) being used and the differing therapeutic areas and research goals. With regards to this and to the availability of specific enrichment items across the globe, the attempt at full standardization across all sites is somewhat limiting. We have found that, although we have our Global Enrichment Committee and are striving to make things the same at each site, it is understandable that we can't always harmonize practices, but instead harmonize when we can, and continue to do our best to communicate what each site is doing and stay on top of what is current best practice in the industry. The following breakdown includes a brief description of where we were and where we are now with changes we have been able to enforce across sites, current differences and the process(s) involved along the journey. Each site will have a section with pictures of current mouse enrichment preferences and reasoning/explanation as to choice(s). A conclusion and summary section(s) will incorporate a brief breakdown of the differences/similarities across sites, and what we have been able to incorporate across the board (specifically, nesting amounts and material).

P136 Assessing Hazard Risks for Instructional and Teaching Protocols at an Academic Institution

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Instructional and teaching protocols are used extensively within the academic setting. Standardizing training on occupational health and safety to new personnel such as students and visiting scientists is challenging due to the variety of their animal and hazard exposure. To evaluate training provided to this demographic, 48 instructional protocols were categorized based on type of animal involvement (for example., field study, curriculum courses, and core service). Protocols were analyzed and risk assessment was performed with Environmental Health and Safety to identify potential risks and hazards. Principal investigators described procedures for documenting training and provided additional course material such as emergency plans, course syllabi, or orientation lectures. Our review revealed that 35% (n=16) of protocols provided training information using a multimodal approach and 33% (n=15) provided training through in-person discussion alone. Training was documented for 61% (n=28) of the protocols, of which 43% (n=23) submitted this documentation to the animal care program. Laboratory and classroom supplemental materials detailed specific protocol hazards, though frequently contained outdated institutional policies or contact information. Overall, 70% (n=32) of the protocols had satisfactory coverage of animal and hazard exposure, while 30% (n=14) were considered unsatisfactory based on failure to address relevant hazards, including exposure to waste anesthetic gas, animal allergens, and zoonotic disease. To address the identified issues, the institutional web-based training system was updated to cover the overlooked hazards, and printed brochures were developed as a source of standardized information for students and new personnel. The ongoing assessment of hazard training provided to this demographic is essential to maintain a strong animal care and use program. Ensuring the safety and competence of those who work with animals is a responsibility shared by both the animal care and use and environmental health and safety programs, and requires continuous collaboration as new protocols are submitted and university policies continue to develop.

P137 Refining Exhaust Air Duct PCR Testing to Reduce Costs

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Exhaust plenum testing has proven to be a sensitive and specific means of screening for common murine pathogens. While this method is sensitive, its implementation may be cost prohibitive. Exhaust air duct (EAD) PCR of individual racks is much more expensive than using one soiled-bedding sentinel cage per rack. The ultimate goal of this study is to develop a cost-effective EAD testing alternative to soiled-bedding sentinels by using pooled rack samples. An additional aim is to assess the latency period for detection of common opportunistic pathogens by EAD, sentinel PCR, and serology. Success will fulfill the reduction and replacement arms of the 3Rs by replacing sentinel animals with a nonanimal alternative. Initial data showed that when 8 racks were sampled for a comprehensive list of organisms comparing 1 swab per rack (sampling all 10 plenums) versus 10 swabs per rack (gold standard, one swab per plenum, all swabs pooled as 1 sample), an average of 6 organisms were detected and there was no significant difference between the 2 sampling methods. Seventeen racks were then compared by using 1 swab per rack versus pooled samples from 3-4 racks (also 1 swab per rack). Although not all organisms were detected in every sample, there was no significant difference in the number of organisms detected between pooled and individual samples. Further, during this study *Aspiculuris* spp. pinworms were detected and both the single and the pooled samples detected the pinworms. However, when an experimental rack containing Myocoptes-infected mice was pooled with 9 samples from uninfected racks, detection was inconsistent. Preliminary data from a followup study comparing latency of detection for sentinel serology, sentinel PCR, and EAD swabs revealed no statistical difference between the number of organisms detected on sentinel PCR and EAD. However, sentinel serology detected no organisms. The number of organisms detected on individual racks (10 swabs per rack) versus groups of 4 racks pooled (5 swabs per rack) was also not statistically significant. These results suggest that pooled rack samples may be a cost-effective alternative to individual rack samples.

P138 Mobile European Primate and Canine Housing

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As a global drug development company, we provide drug development services to numerous clients. Preclinical drug development services include a wide variety of study designs. Length and type of study, approving bodies, laws/regulations, species of animals used in study, and target species may differ with each client and between each study. Maintaining flexibility allows us to provide high-quality data to our clients while streamlining the drug development process. Previously, we had a limited capacity to run preclinical nonhuman primate (NHP) studies in housing compliant with Appendix A of the *European Convention for the Protection of Vertebrate Animals used for Experimental and other Scientific Purposes (ETS123)*. Demand for this type of housing to be used began to exceed our capacity. We needed to find a solution to be able to run more studies with this housing, yet, also be able to maintain flexibility to convert vivarium space to other housing types, and/or other species, due to dynamic housing needs for our clients. To solve this issue, we worked with a vendor to have mobile housing units designed and built, which can be readily assembled and disassembled, and are in compliance with Appendix A of *ETS 123*. Currently we have the NHP version of this housing completed and in use, and we are now working on completion of similar units for dogs. Additionally we hope to obtain the ability to expand this capability to units that will house pigs.

P139 Food for Thought: Changing Feeding Practices Improves Efficiency

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A rodent facility must meet the needs of the animal population at the most fundamental levels of food, water, and housing. Because these tasks are performed daily and the animals are thriving, complacency can set in with current practices. Nevertheless, a more detailed examination of a process can often yield opportunity for improvement. Our team routinely analyzes our current practices and seeks out innovative and creative ways of improving efficiency while maintaining or enhancing animal welfare standards for our rodents. We determined that a technician practice of placing a large, heaping scoop of feed into the rodent hopper led to overfilling and had negative consequences. This practice resulted in feed wastage when feed was discarded and pellets were spread over the entire wirebar lid, potentially obstructing the normal flow of air within the ventilated cages. It also made replacement of the bonnets cumbersome for

research and health care personnel. To address this issue, we assessed the technicians' reasoning behind this practice, altered the culture of our husbandry team, replaced feed implements to facilitate the change, and successfully eliminated the practice. These changes resulted in savings for our facility. Additionally, we removed the potential for airflow obstruction within the cage, improved ergonomics, and improved the day-to-day working experience for our research and health care personnel.

P140 The Use of Positive Reinforcement Training to Minimize Handling Stress and Risk for Owl Monkeys (Aotus nancymaae)

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Owl monkeys (*Aotus nancymaae*) are nocturnal new world primates that are easily stressed by handling, restraint, and changes in routine and personnel. Our institution recently housed a colony (n=6) of male-female paired owl monkeys in 4-unit nonhuman primate cages with dividers removed. Cage changes were initially performed every 2 wk by hand-catching each individual. This process appeared stressful to the animals, evidenced by their vocalizations and attempts to escape capture. Furthermore, hand-catching required at least 2 technicians and risked injury to both personnel and animals despite taking appropriate precautions. To minimize handling stress and risk associated with cage changes, a positive reinforcement training program was established. The planned training paradigm involved progressively exposing the animals to a stainless steel transfer box, allowing them access to the box, closing them inside, and finally transferring them to a new cage using the box. While the response to training varied between cages, all monkeys entered and accepted hand-fed treats within the transfer box by the third week. Despite regular training for 8 wk, the desired behavior of voluntarily sitting in the box as it was closed was not achieved, and such attempts subsequently caused a brief reluctance of the monkeys to reenter the box. An alternate method of changing cages was instituted following the initial 8-wk training period, using cage dividers and the squeeze-back mechanism to encourage them into the transfer box. Using this method, it takes a single technician an average of 4.16 min to change 1 cage (range 1.25-6.41 min). Approximately 13 h were invested by 4 trainers over the course of 12 wk (15-20 min/trainer/week), after which training was discontinued due to the lack of further improvement. Based on the achieved behavioral outcomes, active training could have stopped after 3 wk, requiring a minimal time investment of only 10-15 min/day (2.5-3.75 h total). Although unable to attain completely voluntary use of the transfer boxes with this colony, the positive association with food rewards and the alternative method of cage changing successfully minimized stress, eliminated handling risk and reduced the personnel time requirement.

P141 Simple Solution to Rat Restraint for Intramuscular and Intraperitoneal Injections

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When performing repeated injections on rats, the rats will quite often learn to refuse entry into the most commonly used types of restraint devices. This can lead to an increased level of distress for both the animal and the handler. This concern prompted a search for a better methodology for conducting injections on rats when performed by only 1 person. Using a surgical towel, a simple and easy restraint can be made by folding it lengthwise once, allowing the rat's head to go into the fold, and wrapping the side pieces around the rat. The folded surgical towel and rat tail can be held by the user's nondominant hand, and IM and IP injections can easily be performed using the dominant hand. Lifting the rat gently by the tail exposes the abdominal area for an IP injection, or with a little manipulation, you can expose the hind limb for an IM injection. The use of this restraint technique has reduced the amount of time each injection takes, allowing the handler to feel confident, which in turn allows for an injection to be administered with less hesitation and less repeated needle sticks. This method of rat restraint has been extremely successful and accepted as a preferred method of restraint among technicians. Technicians with all skill levels have found this method to be the easiest and quickest way to perform these injections. This is also an economical solution to ordering expensive devices.

P142 Extending the Frequency of Cage Sanitation outside the Parameters of the Guide

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We evaluated cage sanitation using a partial bedding change model to determine if the current method of cage change practices for static and ventilated cages could be extended outside the parameters of the *Guide*. Currently, complete cage change occurs once and twice weekly for ventilated and static cages, respectively. Extending the frequency of complete cage change could help alleviate unnecessary stress on the mice, decrease the environmental impact of bedding waste, extend the duration of water pouch use, and decrease facility and personnel costs. Four control and test groups of 6 cages each were evaluated over a 16-wk period. The first groups (1-4) were breeders, housing 1 male/2 female C57BL/6 with no pup limit. The second groups (5-8) were nonbreeders, housing 7 male C57BL/6. Evaluation of animal health, litter size, nest structure, cage cleanliness, RODAC samples, and ammonia was recorded at each cage change. A complete cage bottom change (prefilled bottoms with wood chip bedding) occurred with control groups. Test group caging was prefilled with a known amount of wood chip bedding. For partial bedding change, a disposable paper tray was used to remove 2/3 of the bedding, and a fresh tray was used to replace the same amount with clean autoclaved bedding. Over the 16-wk period, ammonia levels remained well under 100ppm for all ventilated and static caging with the exception of one flooded nonbreeder (group 6) cage whose ammonia level was 152ppm and 3 pup-free breeder (group 3) cages whose ammonia levels were 117ppm, 110ppm, and 109ppm. All other parameters measured were comparable between control and test groups. In conclusion, it was determined that partial bedding cage changes could occur for all caging tested with no negative impact on animal health. Instituting partial bedding changes at the facility level seems feasible based on the results of this study.

P143 Finding the Balance with Rodent Enrichment: A Study of Pros and Cons in an Animal Facility

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The goal of a rodent environmental enrichment program is to provide a means for laboratory rodents to express natural behaviors without compromising the quality of the research by introducing variability. Parameters that must be taken into consideration when choosing an enrichment product include the use of the materials by the animals, visibility of the animals, cage environment effects, ease of use by the technician, and cost. This project evaluated 2 disposable types of enrichment relative to use by the mice, alteration of cage environmental parameters, and cost. Twelve static microisolator cages of group-housed female, CD1 mice (4-5 mice/cage) were given either a cotton square, 8 g of paper strips, or a half cotton square and 4 g of paper strips. Animals were checked daily and cage parameters (temperature, relative humidity, and ammonia levels) were measured on days 4-7 following cage change. For 3 consecutive days the animals were introduced to 1 of the 3 enrichment options (cotton square, 8 g of paper strips, or ½ cotton square and 4 g of paper strips). Behavioral response to enrichment was measured. The use of the enrichment was subjectively quantified by nest scoring. The results of the experiments were summarized and compared to cost. The cages with paper strips only had the highest ammonia values (84ppm) and cages with the cotton square only had the lowest ammonia values (62ppm) on day 6. There were no differences in temperature and humidity among the enrichment options. Although the paper strips were the least expensive at $4 per 100 cages, nest scores were highest (4.5/ 5) when the combination enrichment was used. Although there is increased labor associated with using only half cotton squares, this was counter-balanced with the cost savings. Factoring in all of these results, we concluded that the combination enrichment was superior to either the cotton square or paper strips alone.

P144 Individually Ventilated Caging versus Static Microisolator Cages: Analysis of Intracage Air Quality, Murine Lung and Fecal Bacterial Microbiota, and Lung Inflammatory Gene Expression

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The introduction of individually ventilated cage (IVC) systems allows for extended cage-change frequencies, reduced husbandry labor, and increased overall housing capacity compared to static microisolator caging. The reduced cage-change frequency also results in improved fecundity due to the reduced disturbance of newborn or neonatal litters. However, the increased interval between cage changes and the higher, intracage air exchange in IVCs has raised concerns about the potential for aerosolization of intracage particulate material and/or variations in the accumulation of ammonia (NH_3) and carbon dioxide ($C0_2$) which may result in subclinical physiological alterations. We compared the weekly intracage levels of NH_3 and CO_2, bacterial microbiota in the lung and feces, and the expression of inflammatory genes in the lungs of 6- to 7-wk-old female C57BL/6J (wild type) mice and immunodeficient mice [B6.129P2(SJL)-*Myd88*$^{tm1.1Defr}$/J] housed in either static microisolator cages changed every 7 d or IVC cages changed every 7 or 14 d for 10 wk. Intracage NH_3 levels were nondetectable for most cages at all time points with only 1 cage reaching 7 ppm in the first week. No cage exceeded 400 ppm CO_2 over the testing period, and no differences in NH_3 or CO_2 were observed between the 3 caging environments. No differences were observed in either the lung or fecal bacterial microbiota between any of the 3 caging types based on a false discovery rate (FDR) of 0.05. Minimal changes were observed in the expression of certain lung inflammatory genes in both the wild type and immunodeficient mice. However, none of these changes were significantly different. Based on our results, housing wild-type C57BL/6J or *Myd88*-deficient mice in IVCs changed every 7 or 14 d had no significant differences in the parameters measured compared to the same strains housed in static microisolator cages changed every 7 d.

P145 Improving Animal Welfare and Employee Engagement through Creative Treat Competitions

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We are committed to ensuring all animals have the highest level of care and welfare. It is essential that the personnel responsible for the care of the animals are engaged in efforts to improve welfare by encouraging opportunities for the animals to express species-typical behavior. To this end, our facility started the holiday treat competition among its global sites to promote friendly competition, while the animals receive the benefits. All food items created were required to be from the site-specific approved foodstuff lists, as many of the sites perform GLP toxicology research and the treats could not interfere with data collection. In a way, the restrictions bolstered creativity with the type of food items that could be used. Employees from all departments, whether directly working with the animals or not, were encouraged to participate in the creation of the treats and could submit pictures of their items for judging. The competition was a success with the majority of sites participating. Each holiday the competition grows and the treats are more creative. We have found this to be a positive way to promote improved welfare and increase employee engagement across the many sites.

P146 A New Biosecurity Paradigm: Design and Assessment

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A strong biosecurity program is key to protecting our research animals and preventing confounding factors from complicating our research results. An animal research facility tolerates very low risk of disease or infectious outbreaks and we commit many resources to accomplish this goal. The Epidemiologic triangle defines the relationship between the host, the environment and the agent involved. There is a causal relationship between exposure to disease and host resistance. Disease prevention strives to break the connection between the host and the environment or agent through bioexclusion, surveillance and containment. We are familiar with key components of a biosecurity program include vendor health assessments, quarantine, rodent sentinel programs, clean-dirty traffic patterns, a variety of personal protective equipment and caging types. This presentation will introduce a new approach to biosecurity program design that extends beyond these typical standards of infection or disease control. It represents a more comprehensive view of population health management. Case examples provide a structure to demonstrate a biosecurity audit system.

P147 An Accelerated Hydrogen Peroxide Disinfectant Produces Artefactual Auto-Luminescence in In Vivo Imaging System

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Accelerated hydrogen peroxide (aHP) surface disinfectants are sanitizing agents composed of hydrogen peroxide, surfactants, wetting agents, and chelators. Accelerated hydrogen peroxide has several benefits, including being odorless, having a quick contact time for a wide array of potential pathogens (1 min kill time, 5 min tuberculocidal), noncorrosive to equipment, ease of use, and being safe for personnel and animals, as the breakdown products are water and oxygen. As part of our evaluation transitioning to aHP, we assessed an aHP-soaked wipe for possible autofluorescence and auto-luminescence within an in vivo imaging system, which is a popular optical imaging modality that allows longitudinal evaluation of disease progression, cell trafficking, and gene expression. There was no auto-luminescence or auto-fluorescence displayed, and the aHP manufacturer confirmed that there is no chemical present in aHP that would auto-luminesce. However, after following established practices of spraying gloves with the aHP disinfectant prior to handling rodents, it was noted that mice were displaying auto-luminescence in anatomical areas that had no tumors present, making interpretation of images difficult. To rule out whether there was a chemical reaction between a material present on the gloves and the aHP, an experiment was performed to determine if the aHP alone was causing artefactual auto-luminescence. Mice without any fluorescent markers (for example, luciferase, green fluorescent protein) were placed within the in vivo imaging system and wetted with aHP. These mice showed auto-luminescence on the dorsum where aHP had been applied, which erroneously mimics tumor images. When mice were nude or have patches of fur shaved, there was no auto-luminescence present. Our theory is that a chemical component in the aHP reacts solely with the fur temporarily to produce the auto-luminescent artifact. It is important that gloves are allowed to fully dry when using aHP disinfectant to spray gloves in between handling rodents with an in vivo imaging system.

P148 Management of Mite Infestation of Etruscan Shrews (Suncus etruscus)

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The Etruscan shrew (*Suncus etruscus*), the smallest known mammal, has an adult body mass of 2 g. It has been used as a model to study physiology, and more recently, neuroscience. They have enormous metabolic demands compared to other mammalian species and rely heavily on tactile object recognition for live insect prey capture. Anecdotally, they present a unique challenge in that they are difficult to maintain in captive breeding colonies due to their dietary requirements of live insects and high sensitivity to vibrations and other ubiquitous qualities of laboratory animal housing. At our institution, they are housed in 10 gal glass aquaria with tunneled concrete blocks and PVC pipe for enrichment, all of which are autoclaved every 2 wk. The shrews are provided wood chips and autoclaved peat bedding, a live cricket diet, and water ad libitum. Dirty-bedding sentinel mice are used to detect primary rodent pathogens; however, little is known

about health status with respect to diseases common to *S. etruscus*. The shrews were noted to be infested with mites when an animal necessitated euthanasia for unrelated health reasons; a customary skin scraping revealed the presence of an unidentified mite species. Additionally, a different type of mite was discovered in the shrew bedding. Identification of these mites has been challenging, but sequencing results show that the environmental mites are members of the *Sancassania* genus, presumably introduced by the crickets. Further mite identification is ongoing; the sensitivity of this species to manipulation precludes further sample collection unless anesthetic procedures are warranted by the investigator, or clinical illness necessitates a diagnostic necropsy. The infestation has been treated with topical dilute selamectin on shrews and in their environment, a parasiticide selected based on precedent in other insectivorous species, with variable success.

P149 Laboratory Animal Allergy: Improving Occupational Safety though Allergen Exposure Monitoring

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Occupational allergen exposure is a common cause of occupational asthma and other allergic diseases, particularly in animal laboratories. Most laboratory animal species have multiple allergen sources (for example, hair, dander, urine, saliva, and serum), which are collectively called laboratory animal allergens (LAA). Rodent urinary allergens are predominantly hazardous as contaminants on inhaled airborne particulates; however, direct contact with the skin should also be avoided. Several studies have reported a high prevalence (20–40%) of sensitization in working populations. Exposure response studies provide evidence that exposure to laboratory animal allergens may pose a considerable risk for sensitization even at low exposure levels. Engineering controls and safety protocols can significantly reduce occupational allergens exposures. However, the implementation and performance evaluation of allergen avoidance measures require reliable methods to quantify actual exposures. In contrast to detection methods for small molecule contaminants, allergens are proteins produced by a variety of sources, including dust mites, rodents, cockroaches, pollen, furry pets, molds, and foods. Here, we describe available allergen-specific immunoassays used to evaluate animal allergen exposures, as well as airborne allergen sampling strategies using personal, task-oriented sampling, and exposure guidelines used in animal laboratory environments, to improve worker protection.

P150 Silicone Pad Is an Effective Floor Material to Reduce Pododermatitis of Guinea Pigs (Cavia porcellus) Housed in Perforated Cages

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We used stainless steel suspended cages with perforated floors and a mesh size of 8 mm to house our guinea pig (*Cavia porcellus*) colony. The prevalence of pododermatitis was approximately 9.6% (111/1160), in which the oversized animals were affected the most. Only 14.6% (6/41, number recovered/number treated, recovery rate) of the mild to moderate injured animals (swollen, redness, and/or ulcerative lesions without suppuration) responded to an intensive antibiotic treatment (subcutaneous enrofloxacin and topical beta-iodine and neomycin) for 7 wk, whereas 31.7% (13/41, number progressed/number treated, progression rate) worsened Fifty-seven percent of the animals (12/21) with severe suppurative lesions also did not respond to the treatment. Providing a silicon pad and aspen shaving on the cage floor along with the treatment regimen markedly increased the recovery rate (36.8%, 35/95) and decreased the progression rate (4.2%, 4/95) of the mild to moderate injured animals. In addition, the symptoms of 25.0% (4/16) of those severely injured animals were improved, and only 25% of them were exacerbated (4/16). Additional 10-wk observation revealed a 73.2% recovery rate (52/71) and a 0% progression rate (0/71) of the mild and moderate injured animals, and a 20.0% recovery rate (2/10) and a 50.0% progression rate (5/10) of severely injured animals housed on silicon pads with only the topical treatment. After routine application of the silicone pads, the incidence of pododermatitis was below 1% and mostly occurred in late pregnant females, which was reversible after parturition and medical treatment. Silicone pads are relatively cheap, easy-to-clean, autoclavable, and reusable, making it an excellent alternative to solid-bottom cage to control pododermatitis in guinea pigs.

P151 Detection and Characterization of Atypical Strains of Enteric Bacteria in a Purified Animal Diet

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For over 90 years, investigators have used purified diets to efficiently restrict potential diet-related variables in animal-based research or for the study of specific nutritional components. Despite their hygienically suggestive name, purified diets are not free of microbial contamination, and these organisms have the potential to cause foodborne disease or subclinical, physiological effects if used without prior sterilization. As part of our standard quality assurance program, we routinely screen all diets for microbial load and potentially pathogenic organisms. We have found that purified diets often harbor a variety of microorganisms, particularly enteric flora such as the nonpathogenic *Enterobacter cloacae*. By contrast, the presence of *Escherichia coli* in a diet, while rare, indicates an unacceptable contamination of the feed and justifies the rejection of a batch. We recently isolated several atypical strains of enteric bacteria, tentatively identified as *E. coli* from a high-fat, purified diet. However, these strains defy ready characterization of their identity by biochemical profiling, mass spectrometry, and molecular sequencing of the 16s rRNA (*rrnB)*and b-subunit of the RNA polymerase (*rpoB)* gene targets. Herein, we describe the unique properties of these strains and our results to date, in distinguishing them from closely related taxa. Knowledge of the microbial contamination in diets fed to research animals, especially if unsterilized, is important in the reproducibility of animal-based research studies, and the prevention of personnel exposure.

P152 Locomotor Effects of a Low-Frequency Fire Alarm on C57/BL/6 Male Mice

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Maintaining appropriate acoustic conditions for animal welfare and data collection is of paramount importance in biomedical research facilities. Negative impacts of disruptive sound are known and can include auditory damage, changes in immune function, and alterations in behavior. One type of disruptive sound occurring in these facilities is that of fire alarms. To attenuate this issue, many facilities have incorporated the use of low-frequency fire alarms that emit tones outside of the rodent audible range. The impact of these devices on experimental rodents have been assumed negligible. However, this has not yet been tested in the context of behavior. Thus, our objective was to investigate the impact of low-frequency fire alarm exposure on locomotor behavior using the open field, a test sensitive to acoustic stimuli disruption. To accomplish this, male mice were randomized to 3 alarm exposure groups (no-alarm, alarm-during, and alarm-before) and placed in individual, photobeam-activated locomotor chambers. The alarm-during group displayed significantly reduced horizontal locomotion and trended towards reduced vertical locomotion. Frequency analysis of the emitted alarm sound revealed 2 peaks (395 and 930 Hz) within the human, but not rodent, audible range. Although some harmonics of these peak tones were emitted that overlap the audible ranges of the 2 species, the decibel level of these tones was substantially lower than that of the peak tones, meaning that they were of low volume and likely not detected by the experimental subjects. Tones beyond the human audible range were not detected. These data suggest that a brief alarm tone can temporarily disrupt movement, a valuable insight should an alarm be deployed. Further, findings support close collaboration between researchers and institutional facility staff to ensure appropriate acoustic conditions for research animals are maintained whenever possible.

P153 Change, Wipe, Replace, Repeat: An Evaluation of Cleaning Water Valves

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The Guide for the Care and Use of Laboratory Animals (*The Guide*) states watering devices, such as drinking tubes and automated water delivery systems, should be checked frequently to ensure appropriate maintenance, cleanliness, and operation. To determine the cleaning method and sanitation frequency for detachable water valves on individually ventilated cage (IVC) racks, we used available disinfectants and cage washer systems with standard husbandry practices. Mice were housed in IVCs on corncob bedding according to the *Guide* standard for cage density, and were changed at 2-wk intervals. Water valves were wiped with a paper wiper soaked in 1 of 2 disinfectants or were removed and processed in a cage washer at the time of cage change. Water valves were tested before and after cleaning using a combination of ATP monitoring system, ATP luminometer, and sterile swabs for bacterial culture. Wiping with a disinfectant or processing in a cage washer provided "pass" (<17 RLU) ATP levels for 26 wk and had no impact on animal health or breeding. Volunteer animal care staff (n=15) trained on the disinfection process using the disinfectant were monitored at the time of cage change for quality assurance (QA) of water valve disinfection. The average time for cleaning of water valves during cage change was 3.2 ± 0.16s (n=116), and decreased ATP RLU from 106±22 to 7±1 (n=60). QA testing of water valves attached to the rack sanitized in a rack washer after 6 mo was negative for ATP and bacterial culture (n=10 water valves per rack, 176 racks tested). These data indicate that detachable water valves can be maintained on the rack for 6 months using disinfection with sterilant during cage change out, and water valves do not need to be detached from the rack for sanitation using a standard rack washer.

P154 Preparation and Use of Supplemental Diets as a Refinement for Weight Management and Clinical Treatment of Nonhuman Primates

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Managing individual dietary needs in a large primate colony generates high workload demands for husbandry and clinical staff. Clinical staff treats many conditions, such as body weight inconsistencies, gastrointestinal conditions, trauma, and dental care, all of which may require the use of dietary supplements. Husbandry staff is then tasked with creating and distributing these diets. It's paramount that dietary treatments are easy to administer, offer consumption compliance, and provide results. Identifying efficient and effective means to address varied food and medicine intake can be a painstaking challenge. To meet these unique needs, our organization has an onsite diet kitchen where dedicated staff develops clinical and research diets. We have a menu of various supplement options such as high fiber, high fat/calorie, soft mechanical, probiotic antacid, and low phytoestrogen variations. These offerings are produced as soft cubes, compromised of a dough or gelatin base. Having the infrastructure to manage supplementation through the creation of prepared diets has provided vast solutions such as decreased waste of both product and technician time, as well as reduced variables related to consistency and portion control. One example is the use of a nutritional shake mix to soften standard monkey chow. The problems are product waste as excess shake mix is discarded once chow has softened, time lost for technicians that are preparing this within individual work areas, and cleaning time. Using the ingredients to form a soft cube diet we are able to use all of the ingredient, control ration size, and reduce tech time significantly. Providing a large scale assembly line approach to diet supplementation provides fresh and palatable choices for the primates as well as ideal options, simplicity, and efficiency for clinical and husbandry staff.

P155 Quality of Special Water Stored in Carboys over a Period of 14 Days

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For rodents in laboratory animal facilities, drinking water is typically provided to animals by automatic watering systems, water bottles, or bacteriostatic gelatin packs. In our vivarium, water provided by bottles is replaced at a maximum of 7 d and bottles are washed prior to refilling. An investigator working in diabetes requested that a compound be added to the animal drinking water. The additive was added to deionized water in sanitized carboys and stored for approximately 14 d, with water bottles being replenished over the 14 d as needed. Due to the cost of the compound being added, there was a desire to determine if the water could be used longer than 7 d to help reduce unnecessary costs. We looked at the quality of water over a 14-d period to make sure the water did not contain harmful contaminants. Microbiological samples were taken from several different sources at set intervals and sent to an outside diagnostic laboratory for testing. Animals were checked daily for overall general health. The first set of tests showed unacceptable results so upon further investigation, it was discovered that the tubing from the water faucet was contaminated. The tubing was replaced and the tests were repeated. The results improved, however, contaminants were still present. The faucet was retrofitted with a quick disconnect water coil and this was removed and sanitized weekly. Subsequent water testing, which had acceptable results, allowed us to set up an appropriate time table for replacing water with added compound.

P156 In Search of 1-Size-Fits-All Nesting Material for the Laboratory Mouse

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Natural nest building by laboratory mice is becoming widely recognized for its substantial benefits, including improved thermoregulation, enhanced breeding performance, and as a general indicator of health and wellbeing. However, for the nesting material to be biologically relevant, it must enable mice to build high-quality, woven, dome-shaped nests. We undertook this study to develop and implement a universitywide, environmental enrichment program that will provide a consistent amount of commercial nesting material to all cages that mice can use to build quality nests. Singly housed CD-1 and FVB males and group-housed CD-1 females were rotated through a 2-wk cage change on corn cob bedding with 9.5g of bulk crinkle paper, 9.5g mini-rolls of paper, and 8g or 4g compact, paper strip pucks. Using a published, naturalistic nest scoring system of 0-5, the nests were scored on 6 separate days over the 2-wk periods between cage changes. For the singly housed males, the bulk crinkle paper and 8g compact crinkle paper pucks allowed all mice to build cupped or dome-shaped nests with average, mid-study scores of 4.2 and 3.8, respectively. The majority of these nests were completed within the first 24 h following introduction of the nesting material. Lower quality, flatter nests with average, mid-study scores of 2.2 and 2.9 were built using the mini-rolls of paper or 4g compact, paper strip pucks, respectively. These lower quality nests failed to be improved substantially in quality over the 2-wk period. The scores for group-housed females were similar to those for singly housed males. Based on these results, the university is implementing an enrichment program that will provide all mice, singly or group-housed, with a minimum of 8g of crinkle paper or paper pucks.

P157 Optimal Method of Warming Neonatal Mouse Pups after a Cage Flood

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Hypothermia resulting from cage floods is a concern for any mouse, but is particularly devastating for neonatal mice. Altricial mouse pups have no fur, are unable to ambulate towards or from warmth, and are inefficient at regulating their own body temperature. These factors put them at risk for hypothermia, but also increase their susceptibility to hyperthermia during the warming process if warmed above thermoneutral (32-34°C) temperatures. They are also at an increased risk for thermal burns due to their lack of fur and inability to move away from the heat source. This study attempted to determine the optimal methods for warming these pups with special consideration for the temperature of the bedding which would be in direct contact with the skin of the pup. We evaluated an infrared heat lamp, a reusable chemically activated pad, a circulating water blanket, and an electric heating pad. Temperatures were recorded every 60 s for 20 min using an infrared thermometer to measure the surface temperature of the bedding and the skin temperature of a recently euthanized 1-2-d-old mouse pup and a data logger to measure the ambient temperature in the cage. Heat lamps posed the greatest risk for hyperthermia and thermal burns with a high bedding temp of up to 40°C, a high ambient temp of 43°C and a high skin temperature of 42°C. The reusable chemical heating pad produced a high bedding temperature of 34°C and an ambient temperature of 36°C The hot water blanket warmed

bedding to 30°C and ambient temperature to 30°C. The electric heating pad set to high warmed the bedding to 32°C while producing an ambient temperature of 30 °C. Our conclusion is the safest means of warming neonatal pups is either a reusable chemical heating pad or a circulating hot water blanket. Electric heating pads may produce temperatures in the correct range but are difficult to sanitize and are known to develop hot spots as they age. The heat lamp was deemed unsafe for the warming procedure and is not recommended.

P158 Employee Recruitment and Retention

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Hiring qualified employees is essential to maintain the daily operations of an animal research facility. As significant time and effort are spent in training employees, retention of employees is also vital. Our department faced significant challenges in hiring employees that met minimum qualifications for animal husbandry position, and employee turnover within this position was high. In an effort to improve recruitment, our department created a new entry-level position in which the only minimum qualification was a high school diploma. Changes were made to the interview process to provide improved clarity about the animal husbandry position. Efforts to improve employee retention included a pay increase negotiated by the Bargaining Unit, providing consistent training to new employees, developing opportunities to improve employee morale, and creating a career path for employees. Each new animal husbandry employee was paired with a trainer who was familiar with the position duties and responsibilities. Opportunities for all employees to further expand their knowledge and support the mission of our department were created. Employees now have the opportunity to advance their careers while staying within the department, through the creation of 3 additional positions that allow for advancement at several levels. Since 2014, 4 employees have advanced to 1 of these newly created positions. These efforts led to an 18.2% decrease in employees leaving the department within 6 m of their hire date from 2015 to 2016. In addition, the number of animal husbandry employees our department has hired has decreased every year over the last 3 y. Feedback provided by employees has indicated that employee morale has improved.

P159 A Novel Enrichment Program for Etruscan Shrews (Suncus etruscus)

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The Etruscan shrew (*Suncus etruscus*) is the smallest terrestrial mammal by mass (adults average 2g) and one of the fastest tactile hunters in the world. Their small size allows them to be readily used for multiorgan analysis and makes them an ideal model for functional live brain imaging. Breeding the Etruscan shrew for biomedical research creates unique challenges and presents opportunities for innovation in husbandry, enrichment, and handling. The success of these mammals in a lab animal setting is dependent on the ability to mimic their natural habitat as closely as possible while allowing adequate monitoring of their health and daily activities. In the wild, Etruscan shrews live breed in a series of tunnels attached to a burrow. In addition to PVC pipe, plastic igloos, and egg cartons, we have implemented the use of molded concrete habitats that serve as a tunnel and nesting system for each breeding pair of shrews. The design of the system allows the husbandry and veterinary staff to clearly view the adults and pups without causing disruption. We have noted a marked increase in litters born and pups raised to weaning age since the implementation of the concrete habitats. Overall the enrichment program has resulted in an increase in appetite, improved hunting ability, an increase in breeding success, and more natural socialization and explorative behaviors in our shrew colonies.

P160 Observations Regarding the Use of Buddy Barrier Systems to Provide Tactile Access to Singly Housed Rats

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Social housing of research animals of nearly all species has become common practice over the last several years. In some behavioral rat studies such as drug discrimination, animals are placed on a restricted diet in order to facilitate the training of lever pressing in an operant chamber for a food reward. An attempt to pair house animals on food restriction produced disparate bodyweights (in a few cases, by over 100 g) between cage mates, who generally maintain dominant/submissive bonding. In order to minimize the effects of dominance and hoarding within a social dyad, a buddy barrier system was adopted which allowed for separation of food rations, as well as visual, auditory, olfactory, and limited tactile access to a cage mate via a metal barrier with small holes placed in the center of the cage. The system was used on 9 studies over the course of 3 y. All 9 studies were approved by IACUC as part of GLP compliance. While the bodyweights of the barrier rats showed less variation between cohorts, the caging presented other issues. Approximately 4% of the animals on these studies were evaluated by veterinary staff for injuries to the mouth, feet/digits, or tail (30/715). It is unclear if these injuries were a direct injury from barrier design, aggression through the barriers, or simply coincidental. Although the barriers allow for tactile housing and prevent food dominance, the IACUC determined that they did not provide social housing per the *Guide*, therefore their use has been suspended based on these injuries.

P161 Pigeons: Flying to New Heights

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When pigeons are singly or pair-housed in cages, their repertoire of species-specific behavior is limited, relative to what is seen typically in natural settings. We removed the cages from our pigeon holding room and converted it to separated "flight rooms" for breeding and study animals. The original room was divided in half with mesh netting, separating the 2 groups, while allowing visual, auditory, and olfactory contact between them. Construction costs for this project were minimal and included PVC tubes, poultry black mesh netting, domed covered dishes for food, water and grit, rubber bowls for bathing and foraging, nest grills and bowls for breeding pigeons, wall-mounted perches, and ceiling mounted swings. An ante space was created that allows caregivers and veterinary personnel to assess the flock without entering their living space. Over the past 6 mo, the pigeons have demonstrated a much larger repertoire of species-typical behaviors than they had when housed in cages. In their flight rooms, pigeons are seen walking, flying, stretching their wings, perching, foraging, cooing, and rocking on swings. Some animals demonstrated aggressive behavior during the first 2 d, but they appeared to settle into their pecking order quickly, given the multitude of perching options and increase in floor space. No abnormal behaviors have been observed in these animals in their new housing arrangement and egg production has increased in the breeding group. Housing pigeons in flight rooms with the space and complexity to support a larger repertoire of species-typical behaviors appears to have improved animal health and wellbeing, relative to housing them in cages.

P162 Getting Water Valves Clean: Sanitation Methods and Verification

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The *Guide for the Care and Use of Laboratory Animals* states that attention should be given to routine sanitation of automatic water delivery valves. Standard cagewash methods were evaluated for effectiveness to sanitize or sterilize rack detachable and fixed cage rodent water valves. Automatic water valves were treated with a commercial glow powder, which fluoresces under black light and processed using the commercial rack cagewashers. Fluorescence was detectable on all valve types after washing using either cagewasher. To determine the ability of cagewash systems to sterilize water valves, autoclaved valves were inoculated with $2x10^{13}$ *Pseudomonas aeruginosa* (ATCC 15442) or *Escherichia coli* (ATCC 12014) diluted in sterile RO water. Valves were allowed to stand 24 h in a

biosafety cabinet and then processed using a standard cycle in either the rack or tunnel washer. Detachable water valves were placed in a valve-washing tray in either the up or down position to test the impact of placement on sterilization and account for variability between staff. Cages with integral valves were always processed facing down (tunnel washer) or out (rack washer) as per manufacturer recommendations. Water valves were cultured after cagewash by rinsing with sterile RO water and incubation in BHI broth for 24 h along with a positive and negative control. Culture results were negative for both strains of bacteria after processing using the rack washer, or when valves were processed face down in the rack washing tray in the tunnel washer. Thirty percent of valves were positive for either bacterial strain when water valves were processed facing up in the tunnel washer. Placement of detachable water valves is important for effective sanitation using standard cage wash methods. Validation of sanitation effectiveness is recommended when using cagewash systems to sanitize water valves.

P163 External Validation of Exhaust Air Dust Testing by Comparison with Traditional Soiled Bedding Sentinels

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For the past 2 y, routinely monitoring rodent pathogenic agents using soiled bedding sentinels has been conducted at our newly constructed animal facility. Recently, we have started systematically validating an alternative method for our modern caging systems (both rat and mouse) using filters for the individually ventilated cage (IVC) exhaust air dust (EAD). We have tested multiple sampling locations for racks connected to different blower models and have run rat and mouse pathogen panels to compare with results from serology, microbiological cultures, and index animal direct sampling PCR. EAD testing was performed at 3 mo intervals and we identified MNV, *Helicobacter* sp., *Pasteurella* sp., *Entamoeba muris,* and *Trichomonas muris* which were not detected consistently in our soiled bedding sentinels. Adding direct animal testing can increase the sensitivity for certain pathogens (for example, *Helicobacter*sp. and *Staphylococcus aureus*). We found the design of the IVC exhaust blower/filter holder may impact the sensitivity of EAD sampling. The outcome of this study will allow us to implement a more sensitive environmental health monitoring program to limit the need of sentinel testing. Furthermore, our findings will externally validate this method for other facilities with similar IVC caging systems.

P164 Assessment of Chemical Disinfection as a Means of Decontaminating Biohazardous Materials

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One of the most common methods for decontaminating biohazardous materials from ABSL 2 housing is the use of an autoclave. While autoclaving is an effective method, it may not always be compatible with equipment or available. The *Biosafety in Microbiological and Biomedical Laboratories (5th edition)* indicates there are several other acceptable decontamination methods. Given that a few of our vivaria have limited access to autoclaves, we investigated the use of chemical disinfection as an alternative to autoclaving. Levels of sanitation after chemical treatment were measured using an adenosine triphosphate (ATP) bioluminescence assay and contact plates (Tryptic Soy Agar with Lecithin and Polysorbate 80) for bacterial colony count. Dirty bedding was removed from 24 cages originating from 2 separate ABSL 2 housing rooms using a biological safety cabinet. Cages were treated with a quaternary ammonia detergent prior to mechanical processing, where they were exposed to 82.2°C (180°F) water and a nonphosphoric acid cleaner. Samples for ATP readings and contact plates were collected after processing and compared with baseline (prior to chemical treatment). ATP readings and contact plates were consistent for 45/48 samples tested. All baseline samples (24/24) had high levels of ATP and greater than 300 bacterial colonies per plate. After chemical treatment and mechanical processing, 21/24 samples demonstrated no ATP or bacterial colonies. Of the remaining 3 samples with either a positive ATP reading or presence of bacterial colonies, there was a greater than 95% reduction in contaminants observed. Due to the similarity between ATP and contact plate results, we conclude that the ATP bioluminescence assay provides an accurate indication of disinfection and can be used independent of contact plates when assessing sanitation. Based on these results, we determined that chemical disinfection is an effective method of decontaminating biohazardous cages.

P165 Creatively Meeting the Standards: Taking Rabbit Housing to the Next Level

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The 8th edition of the *Guide for the Care and Use of Laboratory Animals* clarified its language regarding housing social species, specifying that, "Single housing of social species should be the exception." This is a challenge for many institutions in the United States, as often budgets for new housing need to be planned far in advance. Further, while rabbits are social by nature, not all rabbits engage in positive and mutually beneficial behavior with conspecifics. Ensuring their social needs are being met has inspired creativity and the use of technology. Our team has implemented multiple solutions: monitoring with internal limited-range baby cameras (bunny cam), securing play space by using canine exercise pens situated in the animal housing room, and modifying current equipment by fabricating stainless steel tunnels designed for use with current caging. We have also found that modifying the introduction of our female rabbits in social pens increased our group retention from 30% to 100%. We no longer look for dominant animals or compatible pairs. Instead, we use our social pens, releasing newly arrived cohorts as a single housing group. Incorporating our bunny cam allows us to monitor the groups prior to intervening with their social interactions. Once pairs are established, we use in-cage tunnels to extend social time upwards of 6 h per day, 7 d per week. If pairs are not practical for a specific project, we maintain the groups in the social pens for a minimum of 4 h daily. These solutions have allowed us to meet the requirements set forth in the new *Guide* in a resource effective and environmentally friendly manner while minimizing disruptions to the rabbits.

P166 Optimizing Pairing Practices for Female New Zealand White Rabbits (Oryctolagus cuniculus)

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Our university manages a breeding colony of nearly 400 transgenic New Zealand white rabbits (*Oryctolagus cuniculus*), the majority of which are pair-housed upon weaning. Postweaning, many dams become singly housed upon lab necessity. Historically, these adult females were not repaired. After consulting with other institutions and reviewing the literature, we developed a method for pairing newly arrived, adult female rabbits purchased from vendors. We leveraged their shared stress from shipping to enhance bonding and used buck urine marking to signal a shared social grouping. Thus far we have had 100% success with this pairing method. However, as the singly housed females from the breeding colony were already living in the vivaria, we could not capitalize on the shipping stress experienced by the new arrivals and found it more difficult to create pairs from this pool of rabbits, with only 50% of pairings being successful. As part of our pair maintenance program, we identify which rabbits are dominant and which are subordinate within each pair. We applied a simplified temperament test to 31 existing pairs and found that the majority of dominant rabbits approached a novel enrichment item in 35 s or less, where as the majority of subordinate rabbits took over a minute to approach the item. Using this data as a benchmark, a subset of the singly housed female rabbits slated for transfer to a new lab across campus were first tested for latency to approach a novel object, then transferred to their new facility and immediately paired with a rabbit that scored opposite to them on the temperament test. We predicted that coupling temperament testing results with our previously established social introduction process for new arrival females would result in higher pairing success. Thus far, 100% of pairs created with this modified method were successful. We, therefore, recommend institutions creating a rabbit social introduction program consider a combination of capitalizing on stress-bonding on the day of pairing, marking with buck urine, and if pairing animals already in the facility, explore temperament testing to identify likely compatible pairs.

P167 Novel Individually Ventilated Ferret Cages Designed for Use in Biocontainment Facilities (ABSL2-4)

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Ferrets (*Mustela prutorius furo*) have become increasingly valuable models of viral pathogenesis, accurately reproducing many aspects of human disease associated with such high impact pathogens as influenza, several paramyxoviruses, and most recently, filovirus infection. The study of these agents must be carried out in biocontainment facilities specially equipped to safe guard both research staff and the environment. The type of caging selected for housing animals is of great importance. Use of individually ventilated cages (IVC), supplied and exhausted separately from room air are ideal. However, there are few options for IVC cages specifically designed to house ferrets. Caging is frequently adapted from other species and forces compromise on key aspects pertaining to their use by staff and animals. We worked with a commercial vendor to design a novel IVC cage which would meet many safety and welfare needs when working with ferrets housed under biocontainment conditions.We identified important deficiencies in a number of caging systems used to house ferrets both in biocontainment and conventional facilities. These included size, visibility, accessibility, feeder type and positioning, flooring, cleaning methods, squeeze mechanism presence/absence, and operation. We evaluated each aspect according to impacts on safety and welfare and prioritized design factors to decide on a novel configuration allowing excellent visibility of animals, ease of restraint for injection procedures, and space for animals to exhibit normal behaviors such as hind limb rearing and play. Cages have large transparent windows on each side of the cage rack and are optimized for 2-person handling via strategically placed handles. A novel squeeze mechanism mobilizing the top of the cage allows for easy restraint and multiple cage access points facilitate sedation and removal of a single animal from a group without reaching into the cage. The floor is a transparent autoclavable plastic rather than a metal grid. Overall the cages are a great success. In particular, the safety of accessing animals for procedures has been enhanced and is less stressful for animals. Further work is needed to refine the latching and squeeze mechanisms.

P168 Promenading with Purpose: Providing an Enriched Environment for Technicians

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In the spring of 2016, a team of husbandry supervisors focused on how to enhance animal care technician optimism and drive, while minimizing institutional cost. To accomplish this task, it was essential to assess the husbandry staff's working environment. While discerning the surroundings, the team observed low-level fluorescent lights in the animal housing rooms, hallways, and break rooms. In addition, the lack of windows and dark, drab wall coverings were a contributing factor to the lack of visual stimuli. Finally, the technicians were overwhelmed with clatter. The standard animal sounds, noise from construction projects, HVAC system, and rack exhaust/supply were a source of auditory fatigue that the technicians were experiencing on a daily basis. To help combat the day-to-day monotony, the supervisory team devised a plan to include a brisk walk directed toward a work-related location. To avoid frivolous use of institutional resources, the walk was limited to 20 min and staff members were encouraged to talk among themselves and get to know co-workers with whom they might not otherwise spend time. To keep the staff engaged in the walk, we asked that electronic devices be put away and encouraged team members to participate by asking questions and sharing information on things they might not know about the university's hospital and campus. In addition to fun facts, information also included the whereabouts of secure building access points, security stations, employee occupational health, and parking garages. After several weeks of walking, we noticed a distinct change in the demeanor of team members. People truly looked forward to the walk and it appeared as though they were energized by the activity. Becoming more familiar with their surroundings also aided in preparing our team for needs such as a visit to the hospital's occupational medicine department to receive the yearly flu vaccine or use a different entrance in the event of a hospital-wide lockdown. This increased knowledge, in turn, created a strong sense of ownership and responsibility for our supporting satellite facilities and the university as a whole.

P169 Sorting the House of Slytherin: Not All Pythons Are the Same

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Snakes are an uncommonly used species in research, and the care and husbandry of these reptiles is vastly different from that of more traditional mammals used in biomedical research. Recently at our facility, we have housed 2 different snake species for viral research studies and we will outline here the successful husbandry and veterinary management that was implemented. We sought to limit their stress by housing them in a species-appropriate manner, with adequate spacing requirements and environmental conditions. We will focus on the differences in housing (enclosure, heating, lighting, and humidity) and basic veterinary care techniques (handling, physical examination, deworming, feeding) between green tree pythons (*Morelia viridis*) and ball pythons (*Python regius*). Green tree pythons are an arboreal species and ball pythons are a terrestrial species which leads to variability of their housing and feeding. Both species are housed in similar environmental temperatures (75-80°F), however, green tree pythons should have areas of the cage that fall a bit lower (~70°F) and ball pythons should have a heating source at a higher temperature (~100°F) available to preferentially bask at higher temperatures. Green tree pythons are housed in cages with perches to accommodate their tree-living habits, while ball pythons are housed in a repurposed rodent cage and given ground-level hiding areas. As green tree pythons tend to be more aggressive and strike from an elevated position, handlers feed mice using long forceps held in an elevated position, whereas ball pythons were fed using tongs and mice were held closer to or on the ground. Parasitic treatment and prevention of both species were given fendbendazole and metronidazole via an orogastric tube. It is important to know each species' specific husbandry, medical, and behavioral needs in order to provide the healthiest, most humane, and stress-free environment for captive snakes in a research environment.

P170 Evaluation of Hay Feeding in Pregnant Laboratory Rabbits: Use of an Appetite Suppression Paradigm to Evaluate Effectiveness in Decreasing Veterinary Consult Events

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In reproductive toxicology studies, and per ICH S5, maternal toxicity may be evaluated by measuring daily food consumption values in pregnant rabbits. Pregnant animals demonstrating low food consumption values, particularly early in the course of a study, may indicate poor health and the need for veterinary intervention. Intervention, if unsuccessful, may place safety study objectives at risk. When on veterinary consult (VC), rabbits may be given edible enrichment, such as hay, in addition to their normal pelleted diet to stimulate appetite. This study was designed to evaluate potential appetite stimulant properties of hay in different contexts. Thirty pregnant New Zealand White female rabbits were selected for study and placed into 4 different treatment groups to compare days on VC following an appetite suppressing event. Rabbits in Group 1 (control) were supplemented with hay ad libitum upon arrival. Group 2 rabbits were also given hay ad libitum upon arrival, and then experienced an appetite suppressing event on gestation day (GD) 7. In contrast, Group 3 rabbits were not provided hay before the appetite suppressing event on GD 7. Group 4 rabbits were dosed daily with water via oral gavage beginning on GD 6 to simulate routine research activities and were not supplemented with ad libitum hay. Rabbits in Groups 3 and 4 were provided with hay once dietary intake fell below 40g, a trigger for placement on VC. Rabbits in Groups 1 and 2 spent significantly fewer days ($P < 0.0001$) on VC due to inappetance compared to rabbits from Groups 3 and 4. Based on these results, it is recommended that rabbits in a research setting are provided with hay ad libitum, if possible, to reduce the amount of time spent on VC, enhance animal welfare, and provide quality study data.

P171 Provision of Treat Does Not Negatively Affect the Body Weight or Pellet Consumption of Rabbits

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The nutritional requirements of rabbits are based largely upon their gastrointestinal physiology. The rabbit's well-developed large cecum creates hindgut fermentation, which digests fiber and starches. The composition of the rabbit feed must include adequate levels of fiber to maintain intestinal health. Properly formulated diets that are manufactured specifically for the laboratory rabbit should be used. Abrupt changes in their diet, including the offering of too many treats, can cause dramatic digestive and metabolic disturbances. However, it is also recognized that food enrichments can improve the wellbeing of captive animals, especially those housed singly. Four long-term New Zealand white rabbits (*Oryctolagus cuniculus*), 2 males and 2 females, that are used for training protocols reside at our life science center. To determine if the provision of treats, including apples and dried fruit, would influence the consumption of food pellets or the weight of these rabbits, a brief study was performed that tracked theses parameters of the rabbits while being fed pellets only and pellets with scheduled treats. The rabbits were singly housed in standardized rabbit caging. Weights (g) and pellet consumption (g) were taken daily during 3 trial months: during month 1, all 4 rabbits were fed pellets only; during month 2, 2 rabbits were fed pellets and treats; and during month 3, the groups were reversed. Both males had a decrease in pellet consumption when fed treats, but they remained the same weight. The females had a slight increase in body weight with consistent pellet consumption, but not during the same trial month. One gained weight while on treats while the other gained weight during the month with no treats. It was noted that the females were younger than the males and thus were not at their full adult weight. These results demonstrated that the provision of a measured amount of treats neither benefitted nor harmed the rabbits.

P172 Housing Modification to Prevent the Ingestion of Bedding Materials in Rats Exhibiting Pica Behavior

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Pica is a well-documented side effect of buprenorphine administration in rats. However, due to its potency, it is one of the most commonly used analgesics. It is believed that the pica phenomenon is a response to the stimulus that induces emesis in species that have an emesis reflex. Since rats lack this reflex, it is thought that the pica behavior is analogous to emesis. If the use of buprenorphine is justified due to its analgesic effects, modifications should be made to the housing of the rats to prevent the animals from consuming bedding or any other items that can be harmful if ingested. A literature search revealed many references about the pica behavior but minimal details on the modifications made to the home cage of the rats. An essential element of proper housing is the prevention of the animal being in contact with its urine and feces. After trying a variety of cage modifications at our life science center, it was finally determined that the use of a floor grid in combination with removable portion of the filter top of rat cage bonnets placed over a layer of aspen bedding allowed for the absorption of urine and collection of the feces while preventing the animals from ingesting the substrate. The rats were also offered approved chew toys to provide them an outlet for their desire to chew. Once the post analgesic was discontinued, the animals were returned to a cage with contact bedding.

P173 Use of Veterinary Rounds as a Staff Communication and Educational Tool

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Effective communication in a vivarium is imperative in order to achieve high standards in animal welfare. This can be challenging in an environment where the technical operations staff (tech-ops) who perform nonclinical safety assessment study activities, including test article administration, clinical observation, and sample collection, are a separate group from that which provides veterinary care. Equally challenging is the rapid growth and onboarding of staff in these areas, as they need training and experience with the species-specific signs of normal clinical health, behavior, and normal experimental animal manipulations that are routine in a preclinical toxicology facility. An increase in the number of questions from tech-ops that turned out to be related to study protocol design, study goals, expected outcomes, treatment plans and humane endpoints was noted by the veterinary staff. The tech-ops staff members have a large amount of contact with the animals but have limited involvement in veterinary care and their schedule limits the opportunity for direct communication with the veterinarians. The veterinary staff determined that it was necessary to institute a communication format that not only passed information along to staff and veterinarians, but also served the purpose of building relationships between the tech-ops staff and the veterinary staff. In response the veterinary staff established weekly veterinary rounds during which the tech-ops leadership, any interested tech-ops staff, and veterinary services staff meet to focus on active protocols in which animals may present with unusual symptoms, have unexpected adverse outcomes, or require a tailored approach to veterinary care. The opportunity for face-to-face communication has allowed these 2 teams to improve their working relationship, encouraged more frequent and direct communication, and facilitated a more proactive approach to animal care.

P174 Use of Automated Feeders to Monitor Group Stability in Captive Breeding Colonies of Rhesus Macaques (Macaca mulatta)

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Captive breeding colonies of rhesus macaques (*Macaca mulatta*) commonly live in large multimale, multifemale social groups. The matrilineal dominance hierarchy of these social groups functions to maintain stability. Fighting within the same matrilineal family or between different families due to social instability can result in trauma and mortality. Thus, a primary goal in the management of these groups is the collection of behavioral and clinical data to determine the presence of social unrest before the onset of significant fighting. Changes in social dominance and the frequency of trauma are commonly used to monitor group stability. However, psychological stress has been associated with food intake reduction in other animal species; therefore, inappetence in key individuals or groups of monkeys could be used as another indicator of emerging instability. An incident of intrafamily fighting occurred recently involving the fifth-ranked family of a large breeding group (n=126, 8 families) at our facility that resulted in 4 cases of rhabdomyolysis or moderate female-inflicted trauma and 6 cases of male-inflicted trauma. Because this compound was equipped with automated feeders that quantify individual calorie intake via RFID microchips implanted in the hands of each animal, feeding data were analyzed retrospectively to determine if any significant reduction in daily kcal consumption occurred prior to the onset of fighting and baseline values (previous 30-d average). No significant differences in total kcal intake for the whole group and individual families were observed between baseline and previous 24-h values; however, the affected fifth-ranked family (n=16 adult females) exhibited a nonsignificant ~20% reduction in total kcal intake from baseline to previous 24 h ($P = 0.06$). Most notably, the targeted subfamily (n=4 adult females) showed a marked ~57% reduction in food intake in the 24 h prior to the fighting incident ($P < 0.01$), while the remaining subfamilies showed no significant changes in appetite. The alpha and beta females, as well as the alpha male, also exhibited marked decreases in food intake during the same period. These findings indicate that automated feeders can assist management staff with monitoring group stability in rhesus macaque breeding colonies.

P175 Using Flash Card Booklets to Prepare New Animal Care Staff for their First AAALAC Site Visit

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At our institution, animal care staff and animal technicians are often hired into entry-level temporary positions with a union-based probationary period prior to permanent employment. This has resulted historically in a low-level of turnover and an influx of new animal technicians on a routine basis. With AAALAC site visits every 3 y, it is not uncommon to have animal care staff with very few members that have been through an accreditation site visit. In 2016, 65% of our animal care and cage wash staff (15/23) had never experienced an AAALAC accreditation site visit and both of our facility supervisors had never experienced a site visit while in a leadership role. In order to help prepare our staff in a way that provided on-the-ground resources, we designed and created a set of practice questions and answers that we published into flash card booklets. Each staff member was

given a laminated flash card booklet, held together by a ring clip, so that staff could readily study standard operating procedure expectations and animal husbandry tips while working in facilities. In the month leading up to the AAALAC site visit, daily emails were sent to staff with a topic of the day, including items like how often to clean the feed barrels and how best to ensure recording of all room tasks. The staff were more comfortable and more prepared to answer questions posed by the site visitors, and the visit resulted in full accreditation. The newest technician hired, with only 4 wk on the job, successfully answered rabbit housing questions from the AAALAC team, who then complimented the technician's knowledge base, given the short time in animal care. The booklets were easy to prepare, inexpensive, and will be updated and edited readily as SOPs and reminders may change for husbandry staff over time.

P176 Using Your Enrichment Program to Promote Employee Engagement

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Animal enrichment programs are well-known in the lab animal science community both nationally and internationally. Enrichment, as we all know, is an ever evolving and always innovative aspect of lab animal science. Enrichment programs are now multifaceted in order to execute the best program for each animal species. Many of these facets often incorporate the husbandry or operations staff for execution. Whether they are key stakeholders in the program or just the end users/providers of enrichment they tend to always play some part in the program. So how can animal facilities engage their husbandry or operations staff to retain or build their talent by using their enrichment programs? Using various aspects of our facility's enrichment program such as enrichment preparation, enrichment scheduling, and behavioral management, we promoted employee ownership, enthusiasm, and innovation. By working closely with management staff we were able to afford the time for employees to participate in the various aspects of the enrichment program on a rotating basis. Additionally we were able to allow employees the opportunity to volunteer for different enrichment teams to promote growth and leadership. We detail our experiences with the various mechanisms used to engage our husbandry staff in order to provide some ideas to engage your staff at your home facilities.

P177 Laboratory Animal Institution Census Project: Verifying the Number of Weaned Mice per Cage

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Animal census is an important, foundational part of any laboratory animal institution's husbandry and management practices. In addition to ensuring accountability for principal investigators with regards to IACUC-approved animal numbers, accurate tracking of animal numbers can help ensure proper supplies and workforce personnel and hours are allotted appropriately. This can be challenging for large institutions such as ours with over 70,000 sq. ft. of animal housing in more than 14 on- and off-campus buildings. Moreover, accurately deducting animal numbers from PI protocols can present a significant challenge as hundreds of mice are weaned each day. Therefore, our institution has implemented an approximation of the number of weaned mice per cage (WMPC) based on historical data in addition to regulatory guidelines and census numbers from comparatively-sized institutions (2.7 WMPC). The purpose of this project was to assess the accuracy of our WMPC number with a manual census count over 2 wk in both small- and large-sized, rodent-specific vivaria. Using raw data from our electronic census count of adult mice, new cages of weaned mice were identified. Then, manual counts of newly weaned mice in each of these cages were conducted daily with average numbers determined per vivaria to calculate an overall institution average. We discovered that in both small and large vivaria, we were underestimating our WMPC with a deviation ranging from +0.2-0.8 for the former and +0-0.95 for the latter. Overall, we calculated 3.1 WMPC, which was an increase of +0.4 from our current standard. An increase in our WMPC can translate primarily into an increased deduction from PI animal allotment numbers: this can directly impact n-numbers in individual research studies and lead to a reassessment of future IACUC approvals with regards to animal numbers for all protocols submitted.

P178 Rotating Environmental Enrichment Is Most Effective in the Reduction of Stereotypic Behavior

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Environmental enrichment (EE) is an effective tool in the mitigation of stereotypic behavior in laboratory animals. However, there are many possible options, and not all EE is equally effective. Over a 12-wk period, 18 female Swiss-Webster mice were monitored between 0000-0300, during high activity, to assess the frequency with which stereotypic behaviors were exhibited. These behaviors included excessive wiping, twirling, and route tracing; however, by far the most commonly observed stereotypic behavior was bar mouthing. The mice were divided into 6 groups, which included both positive and negative controls, and 4 EE treatment groups. The negative control did not receive EE, and the positive control received only nesting material. The 4 treatments either had access to manipulada, vertical climbing rings, food foraging tubes, or all 3 forms of EE on a rotating schedule. Each mouse was observed for a 5-m period during the first 4 wk, the middle 4 wk, and the final 4 wk. Interestingly, the prevalence of stereotypic behavior was not highest in the negative control as hypothesized, but rather in the group with access to manipulada, which demonstrated stereotypies during 22.5% of observation time; compared to the controls, which exhibited stereotypic behavior 16% of the time. While the mean observation of stereotypic behavior was lower in mice with access to enrichment than in the controls, stereotypic behavior accounted for at least 10% of observed behavior in most groups, with one notable exception. Mice with access to enrichment on a rotating schedule only engaged in stereotypic behavior for 0.1% of the time they were observed. These data suggest that EE given on a rotating schedule, maintaining a sense of novelty, may be more effective at reducing stereotypic behaviors than the addition of a single type of EE.

P179 Student Standard Operating Procedures Writing Program for an Animal Care and Use Program to Benefit Students and Facility

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Standard operating procedures (SOP) are essential for animal care and use programs. The SOP review process in veterinary teaching facilities often involves input by faculty and animal care staff but rarely incorporates students. A university chapter of the American Society of Laboratory Animal Practitioners (ASLAP) collaborated with our IACUC to develop a novel program to give students the responsibility of becoming first authors of SOPs. The SOP writing program paired students with a writing mentor knowledgeable in the subject matter. The writing mentorship enabled the student to gain an understanding of the specific SOP and related policies and procedures. We hypothesized that after completing the program student participants would self-report improved writing skills, superior understanding of the purpose of SOPs, and familiarity with the development and writing of SOPs. To determine if students benefited from the SOP writing experience, a survey was conducted with participants before and after completing a SOP. Students were asked to select a numeric value to reflect current writing skills, familiarity with SOP writing, and knowledge on key aspects of SOP development. Students were also asked to identify the most important roles of the veterinarian, primary investigator, and animal care staff in SOP development. Participants completed an identical survey after their SOP was finished and approved for use within the animal care program. As hypothesized, after completing a SOP, students expressed greater confidence in writing skills, increased familiarity with SOPs, and more accurately identified specific roles in the SOP writing process. Students also reported that the act of generating a SOP improved their critical thinking and problem-solving skills with an important emphasis on animal care. The SOP writing program has proven to be a valuable adjunct to the SOP review process of our animal facilities and shows that writing SOPs with a mentor contributes to a veterinary student's skill set.

P180 Creating a Safe and Comfortable Space for Timed Pregnant Female Mice

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Breeding and maintaining mice can be challenging due to variables such as light, noise, odors, diet, and vibrations. Any of these factors can cause a decrease in breeding production and impact research animal numbers. We present a case where a customized cage environment and modified husbandry plan helped solve a difficult breeding situation. An investigator required timed pregnant females for study and was experiencing significant difficulty with both cannibalization and maternal neglect with a resulting loss of pups around day 1-2 postparturition. In order to keep up with research needs, we explored various options to create the perfect breeding environment. Initially, the macroenvironment of the room was confirmed to be within normal limits and the cages were moved from a ventilated rack to a shelf rack in a low traffic location in their original animal room. The parameters of the timed pregnancies were also adjusted with the vendor to ensure that first time mothers were not being delivered and arrival was earlier in their gestation. Unfortunately, these changes did not improve the situation. Cages were then moved to a room away from the animal facility main entry door. To decrease vibration and light variables, cages were placed on a filter pad and red screens were placed in front of each cage. Two different shelters were added within the cages to provide additional nesting options. This breeding colony was also given a dedicated animal care technician who was trained on the unique caging environment. By making some simple changes to the cage environment and husbandry plan, we were able to decrease cannibalization, decrease pup mortality, minimize further research delays, and maximize animal welfare.

P181 Identifying IACUC Efficiencies in an Effort to Reduce Regulatory Burden

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Comparative Medicine (CM) continues to make every effort to identify efficiencies to reduce regulatory burden. A global regulatory group and IACUC administrators collaborate frequently and focus on ways to continuously improve and increase the effectiveness of processes and procedures across sites. Some of the areas that we have focused on include: (1) eliminating the need to perform literature searches on animal use protocols (AUP) that do not include potentially painful or distressful procedures, (2) evaluating AUPs that have little to no activity in order to determine whether it may be beneficial to terminate or consolidate these protocols to help decrease the local IACUC burden that is spent on reviewing inactive protocols, (3) simplifying the process for Environmental Health and Safety (EH&S) review and approval of biohazardous, chemical, or radionuclide substances proposed to be used in conjunction with protocols by vetting this procedure through our electronic protocol repository system as opposed to maintaining paper trails of these documents, and (4) implementing a longer renewal cycle in the electronic protocol repository system for nonregulated species' AUPs in order to reduce the burden spent by both principal investigators and IACUC reviewers. Lastly, in order to continue to profit and expand on the improved process above, the Global Regulatory group and IACUC administrators have been consolidated into 1 team. This unified group of regulatory subject matter experts now serve as a conduit for information exchange and brainstorming on practices to continue facilitating reduction of regulatory burden going forward.

P182 Novel Diet Regimen for Lagomorphs in a Compromised Oral Wound Healing Study

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It can be notoriously difficult to get rabbits to eat postoperatively. Adequate pain management and avoiding gastrointestinal stasis are always a concern. These concerns, combined with an animal model for which a normal rabbit diet could pose hazards to a successful study, led to the creation of a specialized rabbit diet regimen. Four male New Zealand White rabbits were part of a pilot study to establish a new animal model for compromised oral wound healing after radiation treatment. Four wk after receiving radiation, a circular bone defect was created on 1 side of the mandible, with removal of an overlaying tooth root. In order to avoid further trauma or fracture at the surgical site, the rabbits needed to be maintained on a soft diet with restricted access to enrichment devices. A novel dietary approach was taken to ensure the rabbits maintained their appetite and weight throughout the study. The rabbits were acclimated prior to surgery to a specialized diet, which included modifications to otherwise standard rabbit dietary products. A variety of fruits and vegetables were also modified to retain the rabbits' interest and provide enrichment. Daily health checks and weekly weights were used to monitor the rabbits' overall condition. Our specialized diet and enrichment plan allowed us to successfully achieve our goal of supporting rabbit weight and condition while preventing mandibular trauma or fracture at the surgical site.

P183 Black Lights Facilitate Vaginal Plug Detection in Time-Mated Mice

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Vaginal plugs (also termed coagulation, copulation, mating, and sperm plugs) are a commonly used index for timing conception in mice. Plug detection can be combined with other timed-mating techniques such as estrous cycle synchronization, vaginal cytology, and postmating body weight measurement to improve pregnancy determination and outcomes in mouse breeding colonies. Some mouse strains generate large opaque, well-formed plugs while others have small, almost translucent, indiscrete plugs that are difficult to detect. Difficulty in plug detection can have significant effects on timed breeding protocols and negatively impact research results. To help improve plug identification in difficult cases, we devised a simple method for vaginal plug detection, using a black light (UV LED bulb, 395 nanometer wavelength), purchased for less than $10. Black lights are commonly used for detecting the presence of semen on materials and fabrics in human forensic investigations. Although mouse coagulation plugs are not identical to human semen, we hypothesized that UV light would assist in plug recognition, particularly for animals that produce plugs which are difficult to detect. Time-mated female mice were lifted onto a wire cage fitting, their tails lifted with 1 hand while a cotton tipped applicator separated the vulvar lips with the other, and that same hand shone UV light onto the vulva. Coagulation plugs fluoresced bright blue-green under the UV light. There was no fluorescence in non-plugged animals, and the black light facilitated identification of plugs that were difficult to discern without UV light. Several labs in our animal facility have been routinely using the black light for plug detection and believe it has improved their timed-mating results. The use of a black light for vaginal plug detection is a simple, inexpensive method that could lead to more successfully identifying conception and refine timed mating protocols.

P184 The Behavior of Ammonia after Opening the Cage Lid and after a Water Bottle Leak

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All conventional ventilated cages employ the same principle of ventilation, circulating air above the bedding and removing it at high air changes per hour. A new alternative has emerged where bedding is placed on a raised, perforated floor and air is passed through the bedding at much lower airflow rates. Wet bedding is the source of ammonia inside the cage. Measuring ammonia inside a cage requires either opening the cage lid or modifying the cage wall to insert a probe into the closed cage. We examined a 21-d cage-change interval and measured ammonia using 3

types of devices. Cages were modified to have a sampling port so that ammonia could be measured via a handheld device. Measurements were taken on the following schedule: day 0, 3, 7,10, 14, 17, and 21. Novel wireless sensors were also used to measure ammonia inside the cage. Every cage received a wireless sensor that took a measurement every 2 h for the duration of the 18-wklong study. Additional wireless sensors were designed to take a measurement every minute and were placed in select cages on day 17 after opening the lid of the cage. Finally, color change sensors were placed in select cages on day 17 after opening the lid of the cage and allowed to remain until cage change on day 21. Wireless sensors (2 h) allow researchers to identify the inflection point of when a water bottle begins to fail. Ammonia curves will model the release of an entire water bottle and then as airflow dries bedding, the ammonia concentration will fall. The rapid wireless sensors (1 m) give a picture of how ammonia behaves after the lid of the cage is opened. Generally, ammonia returned to equivalent levels from before the cage lid was opened within 4 h. Color change sensors lack sensitivity or any real trend data capabilities but do present a low cost option for measuring ammonia, and 1 min wireless sensors corroborate the idea that ammonia concentrations return to previous levels if the source (wet bedding) of ammonia is not removed. Color change sensors did not return to 0 and therefore are not as reusable as claimed. Wireless and handheld sensor data confer that the Lenderking cage can safely extend the cage change interval to 21 d.

P185 Performance of Various Bedding Types in Novel IVC Design

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There is a new individually ventilated cage (IVC) design that employs a radically different method for ventilating a mouse cage. Conventional designs place bedding on a solid-bottom cage and pass air over the top of the bedding, whereas the new design places bedding on a perforated false bottom and passes air through the bedding. This design change has important ramifications for research parameters and facility operation costs, as well as safety considerations for technicians who must interact with these systems every day. Functionally, the addition of a perforated floor piece means that not all beddings will perform equally in this caging system. If the bedding is too soft, the metal floor will act like a sieve and the bedding will crumble and fall through the holes designed for airflow. If the bedding size starts out too small, it will immediately fall through the floor rendering the airflow design ineffective. Seven different beddings were evaluated in a commercially available cage. Ammonia was measured using a handheld sensor and wireless in-cage sensors. Control cages were run simultaneously which contained bedding, food, and water, but no mice. Parameters gathered include ammonia, carbon dioxide, temperature, relative humidity, and moisture content of the bedding. Moisture content was measured in general bedding and at the latrine spot. Certain beddings demonstrate background levels of urease bacteria by the presence of ammonia after the addition of water in control cages. Cellulose-based beddings performed the best in terms of low ammonia, relative humidity, and allowance of the airflow to dry the latrine spots. Combination corn cob and cellulose bedding performed well although not as well as cellulose only bedding. Pelletized corn cob bedding proved to be too soft for the metal floor and crumbled and fell through the airflow holes. Virgin wood-based bedding in both pellets and chips displayed background levels of urease bacteria as demonstrated from nonzero ammonia concentrations upon the addition of water in control cages.

P186 Lean within the Laboratory

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Communication is extremely important within an animal research environment, especially when it comes to maintaining animal colonies. However, when communication is lacking, it can have effects on the overall wellbeing of the animals and waste time and resources of the lab and care staff. Using the lean management principle of maximizing customer value and minimizing waste, communication has greatly improved between all members of the facility. There were 2 specific issues that were able to be improved with the use of this technique, consistently identifying animals that have been infected with a BSL2 agent and reduction of missexed mice at weaning time. The concepts that were chosen from lean management principles to address these issues are a standardization of work and the problem-solving cycle. These principles were chosen because they help in streamlining work and increasing communication. Due to a lack of personnel entry control as well as new investigators, the use of new BSL2 signage was needed. The creation of BSL2 signage allowed staff to identify which cages were infected with specific agents and the date that they were infected. Along with the signage, a presentation was sent to all members of the staff with instructions on how to fill out and place the identification signs. This follows the standardization of work principle and allows everyone who uses the BLS2 suite to understand and follow all experiments taking place inside the suite. The problem-solving cycle principle was used to help identify problems within the breeding colonies and allow us to create a plan, use the plan, measure the results, and allowed changes to the methodology, if necessary. Implementing existing stock and breeder cards, we were able to create a plan for documenting litter histories, saw how well it worked, and added the number of mice per litter (and number of each sex). Training was provided to research staff as to how to use the stock and breeder cards to create a more uniform system. This has improved procedures within the BSL2 suite and reduced unwanted litters from miss-sexed weanings. Both lab and care staff have noticed significant improvements in communication and efficiencies within the animal vivarium by using the lean management principles.

P187 A Guide to Managing Mouse Trio and Harem Breeding Colonies

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The 8th edition of the *Guide* set forth floor space requirements when establishing cage density criteria for breeders. Even though performance indices and litter sizes allow for interpretation, trio and harem mouse breeding schemes can quickly exceed cage density requirements when not managed appropriately. Based on the recommended housing space of a mouse breeding pair + litter, the birth of a second litter from an additional female would exceed the recommended floor space requirements when using a 75 in.to 82 in. standard mouse cage. Consequently, maintaining continuous mouse trio and harem breeding cages is a difficult task. The process of separating pregnant females and recombining after weaning has operational disadvantages and makes record keeping difficult. Hence, a system is needed to appropriately maintain trio/harem breeding schemes to not exceed floor space requirements and ensure the wellbeing of the animals. Our program has developed a method for labeling and managing trio/harem breeder cages that can easily be implemented in any facility in order to streamline the process of daily breeding practices and record keeping. It promotes conformity with floor space requirements and helps communicate the current breeder cage status to the animal care and research staff. Previous systems using handwritten sticky notes were replaced with "generic female breeder cards" and custom reusable cling-on stickers. Identically color-coded stickers labeled with trio/harem breeder number clearly identify the original and affiliated breeder cages created for separated pregnant females. "Generic female breeder cards" are generated per individual female ID to record each litter and transferred each time a female is separated or reintroduced to the male's cage. Cages containing separated pregnant females are flagged with reusable "pregnant" and "trio/harem" stickers to prevent females from being recombined prior to giving birth and ensure recombining after weaning. Upon implementation, we have effectively streamlined the identification of affiliated trio/harem cages and established a clear breeder cage status system and recordkeeping process which in return allows quicker completion of daily breeding tasks and a decrease in cages exceeding density requirements.

P188 All for One! A Focus on Strengthening Culture and Relationships in the Workplace

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Our comparative medicine (CM) team is made up of a large and diverse group of individuals. For the past several years, our company has conducted a culture survey and, upon analysis of our group's results, we directed our attention to 5 specific areas of opportunities for improvement. These areas included staff engagement, trust, communication, re-

tention, and transparency. In early 2017, multiple focus groups were formed empowering technical staff who were motivated and committed to improving these key areas. Many ideas were put forth resulting in the formation of various committees (for example, Team Building, Infusing Fun, Culture Teams, and others) and the creation of a formal CM mentoring program. These groups continue to meet regularly, ask for feedback, and present novel ideas for improving overall morale and trust within the department. Although there will not be a culture survey in 2017, we feel the activity of these groups is a driving force for positive overall staff engagement and the cohesiveness of the department. A more in-depth description of each teams' contribution to improving our culture, as well as other initiatives born from town hall meetings discussions, will be illustrated.

P189 The Challenge of Consolidating and Modernizing Animal Facilities at an Academic Institution in South America: A Transitional Facility Model

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Our university launched a major effort to consolidate and improve its animal facilities within a decade. The main goal was to build a state-of-the-art biomedical research facility in the central campus. Engineering studies determined that the best place to build was the same location holding the old facility. At the start of 2014, the challenge was to build a transitional animal facility (TAF) to maintain rodents during the period of dismantling and building the definitive facility. This TAF would be implemented as a model to improve operation standards towards institutional AAALAC accreditation, with parallel training for the staff and users at a higher level of compliance. A significant amount of planning was needed for site selection, design, building, validation, equipment purchase, staff hiring and training, animal transfer, and procurement. Finally, 3,000 sq ft was remodeled, making housing rooms for rats and mice with HVAC, autoclave and cleaning area, warehouse, procedure rooms, barrier-restricted access, and security. TAF was furnished with new IVC racks to house 5,500 mice and 1,400 rats. The newly built TAF allowed us to enforce centralized operations (previously spread across campus), reorganize personnel and management, and establish clear objectives and monitoring to ensure that research protocols and daily operations were accomplished efficiently, according to IACUC requirements. We successfully moved 5,000 mice and purchased and established a new colony of rats, while minimizing the impact on ongoing research. An accurate breakdown of expenses, including staff salaries, consumables, energy and equipment depreciation, allowed us to set realistic charges for housing and production. After 2 y of operation, the TAF has shown improved animal conditions; acceptance and compliance of stricter rules by researchers, students, and staff; positive animal production and financial figures; and importantly, recognition by researchers that lesser biological variability reduced the number of animals required to accomplish experimental scientific goals. These goals required work as a team with stakeholder involvement at every step along the way.

P190 Using Qualitative Animal Behavior Scores to Assess Technician Training

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An important part of any facility's socialization program is to track colony behavior to see how the animals are acclimating to the various demands placed upon them. A single collection of qualitative data points can show you where your colony is, and consecutive scores can show you if any trends are developing (for good or bad). However, when you have multiple technicians, can you tell whether personal bias is affecting your results? Even though all technicians in a facility are trained using the same SOPs, a qualitative assessment is inherently subjective. Our dog colony's records management software does a good job of holding all of the data collected, but getting colony trends was difficult, and extracting information about individual technicians was impossible. We began exporting the colony socialization lists into a spreadsheet software, using those spreadsheets for our data collection (behavior scores), and entering the data into the spreadsheet software before importing it into our colony records database. Charting functions allow us to observe overall colony behavior and make it easier to identify changes/trends. We are able to sort and chart the data by individual technician to see if there are any irregularities between colony averages and technicians' scores. A technician who scores the animals significantly different than the colony averages is a flashing beacon identifying a need for observation and perhaps retraining. Using this method we were able to identify a single technician whose misunderstanding of a new score was resulting in an apparent surge of undesirable behavior within the colony. Overall this approach increased the visibility of how our colony is doing, highlighted when changes were happening to colony averages, and made it easier to identify when additional focus is needed to get technicians back to SOP standards.

P191 A Multidisciplinary Approach to Reducing the Incidence of Nonhuman Primate Diarrhea

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Diarrhea in captive nonhuman primates can create unwanted scientific variation when encountered on drug safety assessment studies. Supply and use of cynomolgus macaques (*Macaca fascicularis*) for these studies have historically involved managing sporadic diarrhea cases during holding at the vendor site and during acclimation and on study at recipient research institutions. To reduce the incidence of diarrhea, a multidisciplinary global working group was formed to execute this project. Metrics related to diarrhea cases were established and collected over several years to track progress. Potential contributory factors were identified and included increased psychogenic stress during shipping/arrival, acclimation, social grouping or regrouping, and during husbandry or handling procedures. Infectious agents, diet, and heredity were also thought to be potential factors. Initial efforts focused on helping the animals better adapt to their environment throughout the period of holding, transport, and use. During holding at the vendor, the caretaker teams focused on behavior assessments to ensure social pairs or groups were compatible and stable. Caretakers also worked consistently with specific groups of animals and strengthened the environmental enrichment program. The management team reviewed sanitation standards and identified and implemented changes to enhance sanitation. The research teams at the recipient institution also worked to ensure the animals were familiar with their caretakers and changed restraint and study procedures. As a result, the first case trend rate at the research institution decreased from 1.05% at the beginning of 2012 to 0.81% at the end of 2016 and the chronic case trend rate decreased from 0.118% to 0.024% during the same timeframe. We conclude that our approach through multiple actions at the vendor and research site decreased the incidence of nonhuman primate diarrhea in a large contract research setting.

P192 Improving the Survival of Hatchlings and Fledglings in a Colony of Zebra Finches (Taeniopygia guttata)

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Zebra finches (*Taeniopygia guttata*) in the wild lose a large percentage of their young offspring, mainly as a result of predation; however, the level of mortality that should be expected or tolerated in a captive breeding setting is unknown. Over a few years at our institution, a seemingly high number of hatchlings and fledglings (averages above 20% per year) were found dead. Potential causes such as extremes in temperature and changes in research/husbandry staff were ruled out. Although nest box-related infections were considered, *Aspergillosis* sp. was rarely cultured from nesting material, and no pathology associated with aspergillosis or other infectious agents was found when necropsies were performed. Nevertheless, an outstanding concern was of overabundant nesting material in some nest boxes, which placed hatchlings near the level of the box opening and increased the likelihood the birds would fall out. Another deficiency was that in overcrowded breeding cages, juveniles from 1 clutch sometimes attacked their younger siblings. Poor parenting behavior is an additional factor that clearly impacts brood survival. A cooperative effort was initiated to clean nest boxes between clutches, relocate juveniles out of the home cage as early as possible, and break up

unsuccessful breeding pairs after 3 failed clutches. Increased communication between veterinary, husbandry, and research staff, including a photo gallery of finch age classes to clarify when a bird can live without its parents, is part of an ongoing endeavor to increase the survival of young zebra finches. As a result of these changes, the tally of immature birds found dead was gradually decreased, often to less than 10% per month.

P193 Comparison of Temperature and Humidity Levels between Cages and Rooms in an Animal Facility from 2010– 2017 Located in the Southwest United States

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During our 2010 AAALAC site visit, we received a suggestion for improvement due to the site visitors' concern about the wide range in humidity levels at the room level in the animal facility. Subsequent to the visit, 6 new humidity control sensors were installed in animal rooms in June 2010. The sensors were programmed so the mean reading was used to modulate the humidifier valve. Weekly, we monitored the 6 rooms using a building automation software program. In addition, quarterly from fourth quarter of 2010-2012 and then semiannually from 2013-2017, we placed hobo units at the cage and room levels, respectively, to monitor the temperature and humidity levels in the cage and room environments. Hobo units at the cage level consistently showed temperature readings 2-3°F greater than those at the room level, and humidity levels 3-6% greater than those at the room level. We concluded that cage-level temperature and humidity levels more accurately reflect the ambient temperature and humidity being experienced by the mice than those recorded at the room level.

P194 Assessment of Acidified Autoclaved Water in an Immunocompromised Laboratory Rodent Colony

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Unexplained high mortality was observed in a severely immunocompromised strain of mice. One possible explanation for this was that the drinking water provided to mice was contaminated or would become contaminated by the animals when accessing water via the sipper tube. The use of acidified and/or autoclaved water to control bacterial growth is common practice within the laboratory animal industry and is the standard for immunocompromised rodents housed at our facility. We endeavored to determine whether our husbandry practices would allow for water bioburden and pH levels to remain at levels that would prevent risk of exposure to a potential source of infection. To determine the duration of set pH and bioburden of prepared but unused water bottles and bottles placed at the cage level over the course of a weekly cage change cycle we 1) measured pH internally on a daily basis and confirmed levels at predetermined time points at an external diagnostic laboratory, 2) assessed visual microbial burden by macroscopic observation of water paddles on a daily basis, and 3) assessed specific microbial content at predetermined time points at an external diagnostic laboratory. The use of acidified autoclaved water was shown to adequately control microbial growth in water supplied to immunocompromised mice over a period of 7 d. pH levels were found to be within an acceptable range of the standard 2.5 to 3.0 required in order to be considered to be acidified. Microbial burden was observed only in the positive control sample. All test paddles from cages with and without animals were negative for bioburden by visual assessment. Additionally, no specific microbial content was identified by an external diagnostic laboratory in any test sample. The results indicate that the current husbandry practices employed for controlling pH and bioburden can accommodate a 7-d cage change cycle for severely immunocompromised mice. Through this evaluation, we were able to determine that water source was not the cause of the unexplained high mortality rate observed.

P195 Ventilated Rack and Air Handling Unit Decontamination Using an Active-Closed Vaporized Hydrogen Peroxide Exposure

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Vaporized hydrogen peroxide (VHP) decontamination is an effective method of eliminating potential pathogens from surfaces in clinical and research settings. As part of our efforts to develop safe and effective VHP decontamination of murine housing rooms and portable equipment used in housing, the hydrogen peroxide (H_2O_2) cycle, programmed in the air handling unit (AHU), was selected after the AHU was connected to 2 individually ventilated cage (IVC) mouse racks with plenums appropriately sealed. This equipment and its room were VHP decontaminated using a VHP generator and aeration system. This active-closed VHP exposure ensured that the housing room and all interiors of IVC rack plenums, connecting hoses, and AHU prefilter chambers and ducts were appropriately VHP exposed and decontaminated, a process confirmed using biological and chemical indicators. In addition, as the housing room air dissipated the H_2O_2 levels to <1 ppm, deemed safe for reentry, we assessed whether H_2O_2 uptake and later off-gassing had occurred in the materials that comprise the IVC, AHU, and/or connecting hoses. To evaluate the duration of H_2O_2 off-gassing within VHP exposed equipment, measurements of H_2O_2 levels (ppm) were made using an H_2O_2 concentration monitor with levels present in the supply and exhaust connecting hoses, and within newly introduced, empty IVC-docked microisolators recorded over the course of 2 d post-VHP active-closed exposure. Our data suggests that despite a <1ppm room H_2O_2 level, sufficient additional time is required for proper aeration of housing secondary supporting equipment post-VHP exposure to ensure H_2O_2 levels do not exceed the recommended 1ppm limit within the microisolator, IVC rack plenums, connecting hoses, or AHU prefilter chambers or ducts prior to IVC rack repopulation with murine inventories.

P196 Development of Naturally Aged Rats and Elucidation of Aging Mechanism

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It is well known that laboratory animals can be easily influenced by their breeding environment. Therefore researchers need to focus on that in case they keep animals for a long period as animal models of aging. We examined the effects of breeding environment made by humans, such as handling, cage change, and restraint on longterm breeding of rats, and also evaluated various characteristics as senescence biomarkers. Sixteen to 35 male rats (F344/NSlc, 4-wk-old, approximately 200 g body weight) were obtained from Japan SLC every 3 mo. As a routinely handled group, we tried to touch the whole body of rats gently (approximately 1 min/ animal) in the monthly measuring of their body weight. Rats were allowed free access to a commercial standard diet and reverse osmosis (RO) water with ≤ 1.0 ppm chlorine. Physiological (measurement of body weight, food/water consumption, and survival rates), morphological (autopsy, histological examination) and hematological (WBC morphology) analyses were performed. Body weight peaked at 13 mo and then declined. There was no significant change in food (15.5±1.4 g/day) or water (20.2±3.7 mL/day) intake with aging. At 18 mo, the average life expectancy of rats handled routinely (54.5±16.8 %) tended to increase compared to rats without sufficient handling (21.1±7.0 %). Lung congestion, cecal enlargement, and fundal gastritis were often found in dead rats at autopsy. Our results show that good handling may have a positive influence on animal health and be an effective way to prolong its lifespan. We will continue to search for novel biomarkers and analyze their age-related changes.

P197 Improved Identification, Assessment, and Care of At-Risk Mice in Flooded Cages

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Flooded cages are a common occurrence in most animal facilities. Depending on the severity of the flood and the type of caging, morbidity in rodents can be documented due to hypothermia, reduced mobility, and dermatitis. A large rodent population, overseen by multiple staff members mandates that husbandry and veterinary staff are trained to handle these cases using the same protocols. In severely flooded cages, mice will often have a reduction in mobility due to moistened pelage that accumulates dust from chewed food pellets. This inability to easily move around can quickly lead to hypothermia. Two methods were tested for effectiveness in returning warmth, comfort, and mobility while minimizing time spent attending to the wet mice and reducing the risk of unwanted chemical and microorganism contaminants in the cage. We have developed a straightforward protocol that achieves animal wellness goals, reduces the introduction of potential contaminants and, decreases the time spent cleaning the mice. The first method tested used a warm damp paper towel to wipe down the mouse. The second method consisted of a warm water bath using heated water from the drinking water supply. A baby bottle warmer was used to heat the water to an appropriate temperature (90°F-100°F). The mice were then dried with a clean gauze pad. Eco-bedding was also added to assist in nest production and passive drying of the affected mice. We determined the warm water bath, drying with gauze, and supplemental bedding to be the most efficient and also appeared the least stressful to the mice.

P198 Reevaluation of the Validity of Collecting Multiple Urine Predose Samples from NHPs in EU-Standard Social Housing for Safety Assessment

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In a sustained commitment to 3Rs animal welfare standards for drug development studies, EU-standard social housing is now the default housing configuration for our institution's nonhuman primate (NHP) drug development studies. This group housing arrangement provides a safe, comfortable accommodation enabling the animals to engage in species-specific behaviors and greater opportunities for socialization with the ultimate goal to reduce animal stress. Because not all CRO EU-standard housing configurations provide the option to collect urine samples within the main cage, there may be instances when animals must be separated to obtain samples, with the accompanying potential to induce stress. Acknowledging this potential, as well as the realization that urinalysis findings have only rarely identified an important safety signal on our studies, we initiated a reevaluation of the standard practice of collecting 2 predose urine samples traditionally thought required to best detect test article-related findings in light of the intra and interanimal variability inherent in some urinalysis parameters (for example, urine volume). To this end, we have performed a 5-y retrospective analysis of urinalysis data generated on 68 NHP studies and compared the reference intervals derived from 1 versus 2 predose samples on each study for 10 standard urinalysis parameters. Preliminary results suggest no meaningful impact on the study reference intervals and resultant safety assessment by sampling predose urine only once. Impact on trend analysis and determination of the correlation between individual predose values will be investigated next and included in the data presentation.

P199 A Company's Global Effort to Refine Enrichment: Exercise Pens and Group-Housing for Laboratory Rabbits

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Avoiding abnormal behaviors is a challenge in laboratory rabbit welfare. Most standard enrichment seeks to encourage rabbits' behavior, but does not ease issues seen in captivity. *The Guide* states single-housing social species should be the exception. Research rabbits are often singly housed due to fear that grouping may lead to aggression. Our company used 2 different approaches in the USA and Switzerland. U.S. authors provided enrichment and regular daily exercise in large pens. NZW and DB rabbits are housed in European-style cages with shelving and small toys. In a pilot test, 5 rabbits were placed individually in playpens, 5 d/week, 30 min/day. Two 60 ft^2 galvanized metal pens were built next to each other, allowing partial contact through a divider; had enrichment items plus rubber floormats so animals will not slip. Rabbits are constantly supervised by the vet staff. Swiss authors performed a pilot test with DB females in a toxicology study housed in groups of 3, ages 13-25 wk. Rabbits were randomly assigned to groups on arrival, marked with male rabbit urine to lower aggression and housed in 3 connected cages per group (total floor area 20,100 cm^2.) Each cage had resting boards and a hay box. Additional enrichment included chewing sticks, balls, jingle balls, and hemp rope. Body weight was checked twice per week; thorough physical check done once a week. Behavioral observations happened up to 5 d a week, SID or BID, 5-10 min. each time. Regularly exercised rabbits displayed increased social demeanor, species-specific behaviors, and showed less fear response towards staff. At the other facility, 2 groups out of 8 had no incidents; 2 groups were eventually separated and 6 had incidents requiring mostly topical treatment. Most showed positive interactions. Issues appeared more often postpuberty. Rabbits in both programs were easier to handle and less fearful of staff. Playpen program gives opportunity to positively interact with rabbits in a rewarding environment, reducing risk of compassion fatigue. Group-housing females may be feasible if study is not impaired by skin wounds or systemic NSAID treatment, and if animals are not aged. Urine marking, regular checks and detailed behavior observation will help determine if groups may be kept together or not.

P200 Animal Allergens: What's Your Exposure?

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Laboratory animal allergens (LAA) can pose a significant health risk to those who come in contact with different mammalian species. Animal occupational allergen exposure is common, often causing acute and chronic allergic diseases. The sources of these allergens consist of dander, hair, bodily fluids, and soiled bedding material. However, LAA can be controlled and reduced through a variety of engineering controls and PPE. At our facility, we employ back draft tables and HEPA-filtered equipment such as hoods, ventilated caging systems, and dumping stations to reduce the exposure of airborne animal allergens. In addition to the engineering controls, our required PPE consists of N-95 respirators, safety glasses, lab coats, gloves, and shoe covers to further reduce exposure. Two independent assessments were conducted to evaluate employee exposure to rodent allergens during work activities. Calibrated air sampling pumps measured exposure to particulates during bedding changes, cage dumping, dosing, and necropsy. The air samples were compared to the mouse and rat allergen occupational exposure limit (OEL) of 5 ng/m^3. Our results indicate that engineering controls alone reduce the exposure to LAA. This has led to an evaluation of our current standards for the use of N-95 respirators and engineering controls.

P201 Comparing In-Cage Ammonia Measurement Techniques

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Measurement of individual cage ammonia concentrations is important because high levels have been shown to cause nasal subclinical degeneration and inflammation, rhinitis, and olfactory epithelial necrosis. One approach to limit ammonia levels is performance-based cage changing, where cages are changed as conditions require, as opposed to calendar approaches which use fixed-time intervals. However, measuring ammonia levels is often cumbersome, inaccurate, or high cost, thus limiting performance-based changing. We investigate and quantify 3 measurement approaches spanning a range of cost/performance: (1) paper chemi-

cal strips combined with computer vision, (2) a handheld environment sensor from RKI, and (3) small, battery-operated wireless sensors from a commercial vendor. We developed a paper strip combined with a computer-vision approach, where the strips' color component to ammonia-level function was computed by sending a strip's digital image and color scale to a computer, potentially improving accuracy over a human reader. In the wireless sensor approach, small ammonia-reading wireless sensors were placed in each cage and relayed the ammonia levels into the cloud. The handled sensor is a more standard approach where a gas sample is placed in a commercial reader. We found that although strips had the lowest cost, their accuracy was limited and the ease of use was low. The hand-held sensor, while having high accuracy, required manual intervention in each cage, and requires a large up-front cost. For both methods, the measurement period was thus limited to the scale of days. We found that the wireless sensor's automatic readings and high-frequency, every 2-3 h, made a qualitative improvement in the level of monitoring, with a cost level in between strips and traditional hand-held readers.

P202 Biologic Testing and Evaluation Methods of Germ-Free Gnotobiotic Isolators

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Constant testing and evaluation are needed over time when using germ-free isolators to maintain the highest degree of confidence of being truly axenic. The more testing methods used, the higher degree of confidence results as contaminations can be more visible in incubation from differing media. Our gnotobiotic animal facility uses 4 different types of testing and evaluation. Fresh fecal samples from germ-free mice are combined with water from rodent drinking bottles in an isolator to create the testing medium. The fecal medium is tested for growth on sterile sheep blood agar plates (SBA) and sterile tubes of thioglycollate (TGT) for growth. A gram stain slide is also created for stain and review of visual microbial agents. Lastly, a PCR is run on the remaining sample against a control of bacterial DNA and compared. Use of the animals within the germ-free isolators can only proceed with confidence in the record. Our records show that in 2014 uninterruptible tests were 19.95% for PCR, 1.85% for gram stain slides, 16.32% for SBA, and 0.41% for TGT. In addition, false positives were found for PCR (3.63%), Gram stain slides (0.12%), SBA (0.13%), and TGT (0.12%); and false negatives for PCR (0.26%), SBA (0.44%), and TGT (0.76%). Since inconsistency is common in the evaluation of testing of all kinds due to a number of possible reasons, it is important to regard all testing types as a whole instead of relying upon a single test. Thus, multiple testing is required in the evaluation of germ-free isolators in both type and quantity to maintain confidence of being truly axenic.

P203 Husbandry, Care, and Enrichment of Diabetic Sinclair Swine in the Laboratory Environment

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Diabetic swine are often used to study diabetes and its effects on bodily systems, as well as to test treatments and medical devices used to manage diabetes and minimize its deleterious effects. Diabetic swine require unique care and husbandry. However, published literature on the care and husbandry of these animals is limited. Diabetic swine require daily insulin injections to control blood glucose, frequent blood glucose checks for insulin adjustments, specialized feed, close monitoring for any dietary, health, or wound abnormalities, and diabetes appropriate enrichment. We detail husbandry and care requirements for diabetic Sinclair swine. Specifically, the treatment regimen used in these swine included a single long acting glargine insulin injection following the consumption of the first of 2 equivalent daily meals, frequent blood glucose checks, and timely adjustments of insulin dosages to compensate for any changes in the diabetic state of the animal. This regimen helped to maintain a herd of Alloxan-induced diabetic swine in good health for over 3 y. The swine maintained healthy weights, had no nonexperimental procedure associated hypoglycemic events and had 0 observations monitoring, vascular access and maintenance, anesthetic requirements, and enrichment methodologies for of extended wound healing. Specific attention is given here to glycemic control, diet and nutrition, health stimulation and expression of natural behaviors. The procedures developed and described for Alloxan-induced, diabetic swine helped produce a successful long term research population.

P204 Comparison of Commercially Available Nesting Materials in Mice

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The opportunity to nest is an important form of enrichment for the laboratory mouse. Nesting material is essential for breeding cages, fulfills a behavioral need, and is important for thermoregulation, particularly in individually ventilated caging (IVC) systems. Our objective was to evaluate 3 different commercially available nesting materials for mouse preference as determined by nest quality and durability between cage changes. Thirty Swiss Webster mice were used for this study, divided into 3 groups of 5 per sex. The study was conducted in 3 phases, each lasting for 2 wk, and each group was given a different nesting material for each phase. Material A was dense cotton fibers, material B was a softer cotton texture, and material C consisted of hemp fibers. Prior to study start, mice were singly housed and other enrichment items were removed as nesting material was placed. At the start of each phase, initial weight and location of nesting material within the cage was recorded. The following day, nests were weighed and scored for quality. They were placed back into the cages in the same location. The shape of the nest was maintained as much as possible during handling. After 2 wk, at scheduled cage change, nests were scored for quality as well as durability and were again weighed. These steps were repeated for each of the 3 phases. Material A was preferred by the mice, as it was used almost immediately and the resulting nests sustained quality and durability. Mice made good quality nests with Material C, but took longer to use it. Material B was not used well. Therefore, Materials A and C would be acceptable materials for enrichment. Further studies would evaluate a broader range of nesting materials with different mouse strains, and would also investigate nesting material usage in group-housed mice.

P205 Germfree Isolator Gloves Inspections: If It's Not One Pinhole, It's Another

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Isolators containing germfree animals require an environment that is free from all bacteria, viruses, and parasites. General maintenance of the isolator is required to ensure that the isolator unit operates efficiently while still maintaining the sterility. The frequency in which general isolator maintenance is performed may vary based on the durability of the isolator parts. In this case, we are focusing on the isolator gloves, which are 1 of the major components of the isolator. Pinholes can be a source of entry for unwanted contaminants within the germfree isolator so it is important to inspect the gloves for breaks. Initially, we set the frequency of changing the isolator gloves at intervals of every 6 mo. However, we noticed that during glove inspections, small pinholes were found before the next scheduled glove maintenance change. We began to question if the frequency of scheduled glove maintenance changes were too long and if the procedures of inspecting the gloves needed to be modified or completely changed. Alternatively, we explored whether something changed in the way the gloves were being manufactured that we were unaware of. We discuss how our evaluation of gloves, frequency and methods, has changed, along with reassessing the type of gloves, as we search for the best glove to use in our isolators. Forty-two isolators were used to monitor the daily inspection of both the left hand and right hand of the gloves for approximately 6 mo. A total of 5 different types of gloves from the manufacturer were used to test which gloves would be more resistant to developing pinholes. In conclusion, the time frame for a technician to locate a pinhole in the gloves ranged between 30 s and 90 s. No results showed the length of time a glove could remain in tact before a pinhole developed. However, the majority of pinholes detected in the gloves occurred less than 40 d after the new pair of gloves was installed. As a

result, we have changed the frequency of glove observation by requiring both the left hand and right hand of the isolator gloves be examined each time hands are inserted into the gloves and after removing hands from the gloves. One more glove is currently being tested for resistance to developing pinholes in a short period of time.

P206 Interdigital Cysts and the Effect of Flooring

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A 5-year-old, 8.1 kg male beagle socially housed in accordance with the *Guide for the Care and Use of Laboratory Animals* and USDA regulations on suspended, diamond-shaped plastisol coated steel flooring in indoor kennels repeatedly presented with interdigital cysts on one or more paws. Physical examinations also revealed hyperkeratosis of the digital and metacarpal pads resulting in cracks, and interdigital dermatitis. The scope of treatment for interdigital cysts at our facility varies based on severity: 10-min soaks of the affected paws in chlorhexidine solution once (SID) or twice (BID) a day for approximately 14-30 days; 250 mg Cephalexin, PO, BID for 3 d; or 125 mg amoxicillin, PO, BID for 10 days. For associated lameness, we administer 25 mg carprofen PO, SID until resolution. Despite responding favorably to treatment, the overall condition of the dog's feet did not improve and the cysts would recur. We suspected that the chronic poor condition of the feet was a contributing factor to cyst formation and hypothesized that rough-coated flooring could increase wear and improve the condition of the feet. In August 2016, we replaced approximately 30% of the flooring with flat, fine grit coated Fiberglas panels to allow for natural wear of the pads and minimize contact between the flooring and the interdigital space. Prior to the switch in floor panels, the dog presented with interdigital cysts 4 times between October 2015 and August 2016 with clinical care required for 14–30 d per episode. Since implementation of the flat, fine grit coated fiberglass panels, the dog has had 1 recurrence of cysts in December 2016 with resolution in 14 d. Additionally, there was a substantial reduction in hyperkeratosis and interdigital dermatitis on all 4 paws.

P207 Unlock Your Local Talent: The Use of Postgraduate Students as Casual Animal Attendants

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Many institutions have a common problem of how to adequately staff animal facilities over weekends and public holidays or when there are changes in demand for services. In Australia, Enterprise Staff Agreements and legislation require full-time employees receive an increased pay rate on overtime days. Such increases place pressure on management to meet forecasted budgets. In addition, working weekends and public holidays has a negative effect on employees work-life balance. Extended overtime work can cause fatigue, muscle soreness, and can increase an employee's risk of major health problems which include cardiovascular disease, depression, and lack of motivation. At our institution, with a new animal facility being opened and demand for services increasing, we needed to address how to adequately staff all the facilities especially during the weekend and public holidays. We investigated several options: 1) apply for an increased salary budget, 2) change staff to a 7-day instead of a 5-day roster, and 3) employ casual staff. A staff budget increase was not possible as budgets are locked in a year in advance. Changing staff hours was not possible as staff numbers were too low to effectively cover all facilities. We decided to hire talented postgraduate students as casual animal technicians because they were already working in the animal facilities, had completed all required training, were competent with animals and needed extra money. Each casual employee went through competitive hiring, and agreed to be available on weekends and public holidays. All casual employee were extensively trained by senior animal technicians. Once competent these casual employees were added to the weekend roster and fulltime staff were removed. Many advantages were found when employing talented postgraduate students as casual employees, which included economic benefits, such as a more flexible workforce for the same budget and thus a better operational model; increased staff moral and health benefits; and boosted productivity from all employees.

P208 Calcified Cage Residue Buildup: Your Autoclaves, Not Your Washers, Are the Likely Culprits

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Many animal facilities grapple with a white, cloudy residue build-up on their rodent caging, causing an opaque appearance which cannot be removed, even after hand-scrubbing with standard cleaners. The build-up is aggravated with every cage processing, ultimately impairing animal care staff from performing effectual health checks. To determine the cause of this issue, we first looked at what was considered the obvious source: soap deposits from the rack and tunnel washers which were being baked onto the cages via the autoclaving process. We sent in scrapings of the residue for analysis, which conclusively excluded detergent residue. The composition of the material revealed a high mineral content, composed of calcium, magnesium sodium, potassium, chloride, aluminum, iron, sulfate, silica, sulfur, and phosphate, which are all minerals found in hard water. These results redirected our investigation to our autoclaves; in particular, to potential steam condensate carryover from our boiler's water into the steam piping feeding our autoclaves. At this point, we reached out to our on-site system matter experts on boilers and an external chemical specialist to determine why our steam condensate tested so high for mineral content. Finding the initial cause of the carryover in the steam condensate led us to implement several steps to reverse the negative impact on the quality of the water feeding our boilers. It took over a year to troubleshoot the entire residue issue and almost 2 y before all of the negative factors were successfully addressed. The quality of steam from your boiler is vital since it plays a critical role in your autoclave operations. Remaining steadfast in evaluating and funding key preventative maintenance measures will provide vital cost savings for your facility.

P209 Signage: Simplifying Tech Life

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Compassion fatigue can be defined as the cost of caring for animals in biomedical laboratories. Compassion fatigue is something all too familiar for laboratory animal technicians. They understand the importance of laboratory animal research and recognize this field is accompanied with physical and emotional sacrifices. Technicians offer compassion and unparalleled care in the form of daily husbandry tasks, health checks, providing medical treatment, social interaction, and enrichment to the animals used in a biomedical setting. They develop bonds with and grieve the loss of these animals. We rely heavily on the technicians to be our eyes and ears for compliance, health, and animal welfare for our animal care program, adding to their burden. At our facility, we have numerous extensive protocols across a decentralized animal care system and have found it troublesome to locate specific procedures within a protocol or what the humane endpoints for a protocol may be. In order to reduce the burden on the technicians and avoid wasting valuable time flipping through protocols and countless amendment forms, we implemented protocol-specific signage for approved animal procedures and humane endpoints in each animal holding room. These signs contain the IACUC approved protocol number, bulleted approved procedures (for example, tail vein injections or submandibular bleeding) or humane endpoints for that protocol; these signs are located on the inside door of each of our animal holding rooms. We have found that by implementing these quick reference signs, our technicians and animal care staff have been able to catch noncompliance issues and ensure the welfare of the animals by determining when humane endpoints have been reached in more timely manner. This has relieved some of the burden on the technicians by making their vital jobs easier and has been immensely beneficial to the animal care program as a whole.

P210 Creating a Short-Term Stable Environment for Rabbits in a Cargo Van

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A stable macroenvironment is prerequisite to perform animal testing. We faced a problem when conducting an investigation into rabbit ocular damage by millimeter wave exposure using a gyrotron, a high-power linear-beam vacuum tube. There is no available animal facility at the gyrotron facility and the nearest one is 6.3 miles away. Since rabbits are prone to transportation stress, it is vital to keep rabbits onsite during testing. To create a stable macroenvironment, we used a cargo van for short-term rabbit housing. The cargo van was rented from a local rental agency and was parked inside a large, air-conditioned room at the facility. To control the interior environment, a window air conditioner, humidifier, dehumidifier, air deodorization device, and a LED lamp were set inside the cargo area without changing the original state of the car. Six male Dutch rabbits (10–11-wk old, or retired breeders) were individually housed in their cages for up to 6 d. Food and water were given daily. Pet pads were changed daily. We evaluated microbial contamination in the air by a passive sampling method. After 70% ethanol sanitation of the interior, settle plates were exposed to air for 30 min and cultured. Average numbers for bacteria and fungi were 0.2/dish and 4.7/dish, respectively. These results indicate that the van was as clean as a nonbarrier animal facility. The temperature was stable (average 20.2°C) and ranged from 17.1 to 22.6°C. Average relative humidity was 50.7% (range 33.1–70.8%). Ammonia concentration was below the detection limit of 0.5 ppm. CO_2 concentration, illumination, and noise were within appropriate levels. Relative weight loss was 6.1% ± 2.2% (n = 22) during an 8-h transport and weight was recovered and stabilized by 48 h after arrival. We conclude that a cargo van can be a choice for short-term rabbit housing.

P211 Increasing Veterinary Technician Productivity through Murine Ulcerative Dermatitis Treatment Schedule Optimization

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Ulcerative dermatitis is one of the most common health concerns in C57BL/6 mice and background strains. As per our facility's veterinary care procedures, primary ulcerative dermatitis treatment is topical. Finding an effective topical treatment, while maximizing veterinary technician productivity, is essential. Our hypothesis proposed that a dilution of sodium hypochlorite would outperform the current standard treatment, povidone-iodine/silver sulfadiazine cream, when each is administered at a frequency of 3x a week rather than daily. In this study, we compared the effectiveness of 2 topical treatments administered to available clinical cases: 10% povidone-iodine solution followed by 1% silver sulfadiazine cream and a dilute (0.005%) sodium hypochlorite solution administered daily or 3x a week. New cases were randomly assigned to 4 topical treatment groups (povidone-iodine/silver sulfadiazine cream 3x a week, dilute sodium hypochlorite 3x a week, povidone-iodine /silver sulfadiazine cream daily, and dilute sodium hypochlorite daily) with all groups receiving an initial nail trim. Successful treatment was defined as resolution of clinical signs within 14 d. Currently, 188 mice have been enrolled in the study (February 2017 to May 2017). However, due to other research needs, only 90 mice completed the assigned treatment trial. Time-to-resolution data was analyzed via 1-way ANOVA and no significant difference was noted among all treatment groups ($P = 0.2728$). Based on our current analysis, we can conclude that providing treatment 3x a week increases technician efficiency while maintaining the facility's animal care standards. Consequently, we recommend both povidone-iodine/silver sulfadiazine cream and dilute sodium hypochlorite 3x a week as an effective treatment for resolving ulcerative dermatitis. We continue to enroll animals into each treatment group and with increased numbers, differences may be noted providing better insight into animal care and technician time management.

P212 Novel Humidified Ventilated Rack Caging System

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Maintaining temperature and humidity are vital to rodent health, breeding performance, and are defined by the *Guide for the Care and Use of Laboratory Animals*. Fluctuations can result in changes in animal behavior and may have impacts on research variability. Many factors can affect breeding performance and animal behavior, including preweaning mortality from excessively high or low humidity. Several methods are available to control humidity, including costly building-level humidification systems and portable in-room equipment. Retrofitting these systems into an existing facility is costly and often requires extensive renovations and troubleshooting. These also are poor at conserving resources and excessive conditioning of air is required, much of which is exhausted through room ACH before animals are in contact. As a potential solution, we evaluated a novel individually ventilated cage system (IVC) that delivered controlled humidity at the cage level to breeding mice in an arid winter environment. Cages of similar strains were split between the humidified rack and static controls. Housing methods, handling, and cage components were identical. We evaluated macro and microenvironments, water hardness, noise, organic loads, and breeding performance (number, size, born-to-wean ratio, and frequency of litters). Required modifications for water and drainage were minor. However, a step-up transformer was added to offset low water conductivity level (100us/cm). Ambient noise was minimal, less than the animal change station. Rack humidity was maintained between 33-53%, while room humidity swung greatly from a low of 10% to peaking at 42%. No significant differences in cage ATP or ammonia and no adverse behavioral or health issues were detected in the IVC vs static cages. The humidified rack performed well and was easy to service and manage. It offered a low-cost, portable, and customizable solution to meet *Guide* humidity levels especially for retrofitting facilities without centralized humidity control.

P213 Confused Beetles or Confused Identification? A Case of Misidentification and Management of a Minor Drugstore Beetle (Stegobium paniceum) Infestation

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Beetles are an infrequent pest of research animal facilities. However, when identified, beetle infestations are a nuisance to manage and to eliminate due to their high fertility rates, long life spans, and resistance to common pesticides. Adverse effects of beetles are uncommonly reported in rodent studies but are nevertheless a concern for scientific control and validity. A beetle infestation was identified and localized to a biological safety cabinet within a mouse housing room in a primarily rodent facility. Initial identification suggested a confused flour beetle (*Tribolium confusum*) infestation, a common pseudoparasite in animal facilities linked to grain and bedding stores. However, further investigation correctly identified the pest as a drugstore beetle (*Stegobium paniceum*). While drugstore beetles also infest food storage, they are less often identified in laboratory animal environments. The most likely source of the infestation was thought to be a specialized scientific diet that was stored in a laboratory separate from the facility's main food and bedding storage. A mixture of pest mitigation strategies was used to control the infestation. We intend to educate the laboratory community on beetles common to food storage and provide guidelines on proper identification. Furthermore, we will discuss the probable epidemiology of this infestation and strategies for pest control. Finally, recommendations will be provided for prevention of similar scenarios.

P214 Environmental Enrichment in the Home Cage: Determining Optimal Nesting Conditions for Common Laboratory Mice

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In an effort to create a more enriched environment, caregivers often include items in an animal's home cage that entice them to engage in species-specific behaviors such as foraging or exercise. For laboratory mice, shredded paper and compressed cotton squares are 2 such items that encourage them to display one of their most common behaviors—nesting. The key to this process though is striking a balance between the quantity of what is being given and the quality of the enrichment that it is providing to the animals. Shredded paper and compressed cotton squares were introduced into the cages of 6 commonly used strains of laboratory mice at specific ratios. Three days after the introduction, the nests were measured using a scoring system ranging from 0-5. This system assigns values to each side of the nest based on its construction. In addition to the

nest score, the inside and outside temperature of the nest was measured. The change in temperature demonstrated how different enrichment items affected not only a nests construction but also how it would aid in the thermoregulation of the mice that were using it. Based on the comparison of the data generated by both the nest scores and the nest temperatures, a determination was made in regard to which ratios of enrichment would create the most ideal nesting scenario for the mice. Ultimately the data showed a clear distinction between the qualities of nests created using shredded paper, compressed cotton squares, and combinations of both together with 2 such combinations distinguishing themselves as providing the most beneficial nesting scenarios for the animals. Optimizing the enrichment that is introduced into the home cage of the mice allows them to not only reap the practical benefits of a robust nest but also live in an appropriately enriched environment.

P215 A Simple Labelling System for Zebrafish Tanks

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Zebrafish tanks present an obstacle in tank labeling as rodent cage card holders are not available for most commercial zebrafish systems. Some users use a vinyl film label which may peel off leaving the tank unlabeled. Some users use specialized label printers with water resistant ink. Many users turn to mailing label style identification which is difficult to remove and leaves a residue on the tank. With a variety of strains of zebrafish and different hatch dates we looked for a solution to our labelling needs. We developed a system using suction cup hooks, hanging laminated cards, and a cross-labelling method so if the cards are knocked off we can easily identify which tank the card should be placed on. The new system allowed tanks to be washed with the information easily transferred to the new tank. This low cost solution has proven successful in improving our tank labelling and eliminating the incidence of label residues.

P216 Implementing an RFID Census System at a Large Academic Institution

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When moving from a manual census to an automated RFID system, we wanted to minimize any additional burden on our researchers, while ensuring our animal care processes were efficient. We discussed the proposed RFID system with several users (animal care supervisors and researchers) for input on what worked well versus challenges that were encountered with their implementation. Based on this input as well as processes in place at our institution, a novel system for handling RFID census was developed.Specifically, the research team uses a standard cage card holder and "tree" cage card which allows for recording of basic information. The card has a Buckeye tree on one side which discourages the research team from recording information on top of the tree image. When the animal care staff enter the room, it is easy to determine how many tree cards need to be added to the automated system. Using a handheld RFID reader and mobile printer, the animal caretaker enters the census unit into the database and replaces the cage card holder with an RFID imbedded card holder. Census units are automatically uploaded into the database, and the research team can verify the number of cages present in the room from any web-assessible device. When census units need to be removed from the database at the time of euthanasia, we have implemented a drop-box approach to this check-out. At the exits to our vivaria, we have installed a mail box drop box. At the end of the day, the animal care staff remove the RFID cage card holders in the dropbox, and check them out of the database. Research staff have been trained that any cage checked out of the "vivarium hotel" before 3 pm will be checked out that night and not incur an additional day per diem charge. When the transition was made to this system, both in person and online training was made available. Our training emphasized that the RFID card holders should be treated as cash and not left laying around in the lab. We have been using this system for several years, and have been extremely pleased with the efficiency gained as well as the increase colony information that is available to the research team. Overall, it was a seamless transition and well accepted among our research users.

P217 Towards Standardization of Welfare Assessment in Cephalopods: The Case Of Octopus vulgaris

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Cephalopods are the only invertebrate taxa regulated in Europe in a similar way to all vertebrate laboratory animals. According to Directive 2010/63/EU, animals should be checked daily to ensure that welfare and health status are kept according to species-specific requirements. To our knowledge, there is no indication on daily welfare assessment for any cephalopod species used for research purposes in a laboratory setting. In our case, *Octopus vulgaris* is housed individually in tanks with appropriate substrate and den as enrichment and fed every day with live crabs. Animals are housed in natural seawater within a flow-thorough system. In order to be able to check whether animal health and welfare are secured following European legislation and the *Guidelines for the Care and Welfare of Cephalopods in Research*, we designed a form so that for each tank and on daily basis is possible to collect information on animal data (sex, arrival date, weight, indicators of physical state, posture, locomotor activity during attack, and exploring activities and position and conformation of the den (for example, if the 2 bricks are in the same position of previous day or it has been disrupted and latency to prey, thus to evaluate normality of predatory behaviour). Environmental conditions are also recorded for each system, which include temperature, dissolved oxygen, and water flow. Each animal caretaker is trained to perform necessary tasks and to report correctly all information included in the form. In our experience, daily records of environmental conditions and animal welfare have been successfully implemented, providing the tool to check daily welfare of octopus and to standardize how animals are kept in the lab setting.

P218 Improving Welfare and Compliance by Using Metal Washers to Measure Tumor Burden in Mice

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Tumors can grow rapidly and compromise the health and wellbeing of research mice and rats by interfering with the rodent's locomotion, grooming, and/or ability to access food and water. One responsibility of the care staff at our animal research program is to identify tumors that approach the maximum size criteria delineated in the IACUC tumor burden policy. While tumors are typically measured with a pair of calipers, providing calipers ($12.33 per mechanical set) for the department would prove costly with a husbandry staff of ~60 members. Additionally, staff training for the use and maintenance of the calipers would be time consuming. Instead, a fast, simple, and inexpensive solution was developed: using flat metal washers with central diameters of 1.5 and 2.0 cm, which allow tumors to be identified as they approach or reach the IACUC's maximum tumor diameter for mice (2.0 cm). This allows animal care staff to then communicate with researchers and veterinary staff to increase the frequency of tumor monitoring and intervene before an animal welfare or compliance issue occurs. The metal washers are inexpensive (~$4.76 per metal clip and 2 rings) and easy to use as they can be set directly against the tumor to measure its size. Tumors that are too large or fit snuggly in the ring are easily identified. These rings can be quickly sanitized between animals and do not have the risk of breaking as compared to calipers. All animal technicians now receive a pair of metal rings upon entering the program. Implementation of the rings has resulted in increased animal welfare and compliance as tumors are being identified in a timely fashion and allowing for appropriate intervention.

P219 Development of Sling for Turkeys (Meleagris gallopavo) Postorthopedic Surgery

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Turkeys are an emerging biomedical research model for deep flexor tendon research. They have similarities to humans in anatomic, biome-

chanic, and response to injury and healing related to the flexor tendon. A challenge for using turkeys is that postoperative cares have to mimic clinical scenarios. In this study, we developed a sling system that can be used for these applications. Turkeys have unique attributes that required special consideration in sling development. They are bipedal, have a unique respiratory system, and delicate bone structure. The textile component had to be a material that was strong enough to hold them upright permitting air sacs to expand easily yet firm enough to restrict movement that could possibly fracture their hollow bones. The frame segment had to be wide enough to socially house two turkeys and tall enough to remained nonweight bearing on their surgical digit. The frame was manufactured using 1 in. polyvinyl chloride (PVC) pipe. Knit mesh material ripped and stretched easily, and button socket snaps for attachment opened during abrupt movements. Wings needed to be restrained with a secondary device; a cotton fabric sleeve running from the base of the neck to the tips of the wings with drawstring closures. A strap nestled over their back secured them to the sling by buttons, and a 4-inch leg chute with drawstring closures prevented them from removing their legs. Successful completion of the sling was accomplished using upholstery fabric to attach to the frame with sewn loops and mesh fabric near the body of the turkey along with the secondary restraint sleeve and attachment using a back strap. A week of acclimation prior to surgery yielded turkeys sitting comfortably in the sling without bearing weight, allowing therapy and observations to be performed on either surgical foot.

P220 A Novel Design to Promote a Natural Walking Gait of the Vervet (Chlorocebus sabaeus) for Dynamic Measurements and Observations in a Controlled Space

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We needed to make numerous measurements of different aspects of the gait of a freely walking vervet (*Chlorocebus sabaeus*) monkey. The measurements required a controlled, natural pace without running, using quadrupedal locomotion, and without climbing. Commercially available exercise caging provided too many uncontrollable variables and visual obstacles. A solution was achieved through the development of clear-cast acrylic ambulation chamber. The chamber needed to be clear to make the animal comfortable and allow for observations and digital video recording. Our specific application of the ambulation chamber was to enable measurements on a computer-monitored plantar pressure surface. The actualized ambulation chamber measured 8 ft long x 2 ft wide x 4 ft tall. The chamber was fitted with a main access door for the monkey to enter and leave naturally, multiple air vents, and openings on each end for the provision of enrichment treats. Braces across the inside top added stability but were positioned high enough from the base and close enough to the lid to discourage the monkey from swinging from them. A full-length, piano-hinged lid allowed for posttest cleaning of the interior. Using the ambulation chamber entailed a transfer cage to relocate the monkey from its home cage to the chamber, where once inside it could freely explore the full scope of the unit. Enticing the animal from one end of the chamber to the other by technicians stationed at both ends with fresh banana slices promoted very agreeable participants. Once adapted to this new environment, the monkeys demonstrated no reluctance to participate in the desired procedures irrespective of the presence of any unfamiliar human observers.

P221 Employee Engagement: Improving Morale, Work Relationships, and Efficiency

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At our facility, a diverse group of employees from several departments works together to execute animal-based research projects. Animal technicians, managers, veterinary technicians, veterinarians, supervisors, trainers and researchers support and assist one another on a daily basis. During times of stress or when there is an increased workload, colleague relationships can weaken and become strained. Forming strong, lasting bonds and encouraging positive work relationships can lead to increased morale, increased efficiency, and an overall better work environment. Three years ago several employees at our facility wanted to improve relationships at work so they established an engagement committee. At first, the engagement committee focused its efforts on engaging comparative medicine staff, mainly animal technicians, by organizing activities outside of work. Over time, the scope of the engagement committee changed to include oncology and vaccines researcher colleagues. Since these 2 groups work closely with comparative medicine staff, improved relationships and communication would greatly benefit the research activities. Several oncology and vaccines researchers were asked to become a part of the engagement committee to figure out how to create positive interactions among all of the groups. After several meetings, it was determined that comparative medicine staff would feel more invested and engaged in the projects if they knew more about them. Therefore, the engagement committee began organizing lunch and learn events with vaccines and oncology investigators. The engagement committee has morphed into a collection of employees from several different departments and now runs numerous activities monthly both inside and outside of work. Some of our engagement activities included a picnic, pumpkin and ornament decorating contests, and a workshop that gave animal technicians the opportunity to shadow a vaccine assay procedure. The activities offered by the committee improve work relationships among the different departments, increase morale, and promote positive group dynamics. Employees are more engaged in their work and with one another leading to increased efficiency and an overall happier work environment for all.

P222 The Anesthetic Flow: Streamlining Calibration Service

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Our facility has evolved into coordinating anesthesia calibration services for investigators, support staff, and in-house units. Calibration is necessary because it ensures that all anesthetic machines are in proper working order, safely delivering the appropriate mixture of gas and oxygen to the patient, and ensuring the equipment is safe for human use. Service is also necessary to remain in compliance with IACUC policy. We have 2 campuses, campus A and campus B, which are serviced yearly. Approximately 10 machines were being serviced per day over 2 d on campus A, and 10 machines on campus B over the course of 1 d. Machines were scattered throughout the facilities in centralized, decentralized, and satellite areas. With the demand for servicing growing yearly and new PIs joining, our processes quickly became time-consuming and inefficient. As a result, a new system for scheduling and servicing anesthesia machines was developed. Two months prior to servicing, emails specifically tailored to campus A and campus B research staff are sent, which includes a service request form. This form is filled out in its entirety by laboratory personnel and sent back to a designee. Deadline for forms is 1 mo prior to service. Forms are reviewed, sorted by servicing date, and confirmation emails are sent. In order to accommodate more machines per day, we designate a central drop off location for servicing portable machines. As a result, we can now service 30+ machines per day with flexibility for growth. An additional challenge we face yearly is accommodating the original service date requested. If we are not able to accommodate the date requested (for example. due to machine location), we apologize for the inconvenience and suggest alternative options. We also remind lab personnel that the service is provided as a courtesy and the pros for participating. We designate a single point person per campus to communicate with the laboratories regarding their request. We find this helps for laboratories with questions prior to servicing and allows the point person to develop a relationship with laboratory individuals. Providing these insights to our struggles and triumphs will help other institutions streamline their own facility processes for similar services.

P223 Using Innovation and 3D Technology to Problem Solve in the Vivarium

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We are often presented with challenges in obtaining the precise equipment needed to support animal studies due to limited availability. Recently, the use of innovative technology, specifically 3D printers, has become an efficient, economical, and practical approach to meeting these needs. We formed a team of innovative thinkers and partnered with a business technology vaccines benchtop lead and a drug safety scientist to

streamline the process of brainstorming unmet needs, prioritizing these needs, and implementing solutions. Some were solved by using 3D printer technology and actually printing devices that uniquely solved the problems. For example, commercially available tube holders held multiple tubes allowing for debris to collect in the unused wells. These tube racks held the tube low to the benchtop and did not allow the technician to visualize the volume of blood in the tube during collection. One facility had printed a 3D tube holder that held the tube at an angle and height that was ideal for submandibular blood collection. Technicians at another site used a different blood tube and slightly different technique. A scientist was able to modify the design to print custom tube holders for the technicians at each site. Another problem we encountered was that commercially available mouse restrainers for IV dosing are expensive ($400 each) and do not hold up to needed cleaning and disinfection procedures. Restrainers routinely need to be replaced due to warping, cracking, and breakage. Working with a vaccines benchtop lead allowed us to design a restrainer that is more ergonomic to use, more durable, can be disinfected between dose groups, and cheaper to produce. Also, we came across problems when technicians were performing rodent procedures using gas anesthesia. Technicians were challenged with keeping the nose cone in place. Historically, the cones were held in place using lab tape. The tape routinely needs to be repositioned or reinforced throughout the procedure. In addition, the adhesive on the tape has potential to compromise the asepsis of the area. The innovation team brainstormed ways to secure the nose cone to the work surface. A clamp was designed that would connect to the work surface and hold the nose cone in place. Prototypes were printed and then tested and refined until all nose cones fit snuggly into the clamps.

P224 A Cyno Love Connection: A Novel Pairing Strategy for Aged Male Cynomolgus Macaques (Macaca fascicularis) with Ovariectomized Females

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Aged male cynomolgus macaques (*Macaca fascicularis*) are notoriously difficult to successfully pair, especially after an extremely long period of single housing. We brought in a small colony of aged males from another facility where they had previously been successfully paired with juvenile males for a brief duration of time, but they had then been single housed since ~2011. These males are considered to be senior citizens and are of wild-caught Mauritian origin. Knowing the history of these animals helped us to formulate a plan to attempt pair housing with retired ovariectomized (or possible hysterectomized) Mauritius females. Some of the females we used for this pairing method had been previously ovariectomized/hysterectomized for health-related or study reasons and were due for retirement; they therefore made ideal potential partners for these males. Others were specifically surgerized for the sole purpose of becoming potential partners and all would be assigned to a retirement protocol for pairing purposes only. A strict regimen was followed for each planned pairing and, within the span of 9 mo, we have successfully created 9 compatible male/female pairs that we feel are very stable. Since the pairings have taken place, we have seen a decline in established stereotypies in the aged males and a decrease in aggression as well.

P225 Implantation of Radio Frequency Identification Transponders in Neonatal Mice

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A previous study in our animal facility revealed that adult mice implanted in the scapular region with a next generation radio frequency identification (RFID) transponder experienced minimal transponder migration, loss, and tissue reactivity. With a 60% reduction in transponder size from the previous generation, our facility explored the feasibility of using microtransponders for permanent identification of neonatal mice on day 1. A less invasive method of identification than toe clipping, the transponders provide permanent reliable identification and increase efficiency of long-term neonatal to adult dosing. A small pilot study was conducted postmortem on mouse pups to determine if implantation was feasible. Repeated practice on dead animals was performed to improve skin handling technique, rostral versus caudal implantation approach, and technician hand positions. Once a suitable method was developed, microtransponders were implanted subcutaneously into 1-d-old, 61 BALB/c mice (25 males and 36 females) under cryoanesthesia. Surgical glue was used in closing the implantation site wound to prevent transponder loss. Mice were monitored daily and weighed weekly for signs of rejection from the mother, transponder loss, or unexpected complications, until weaning. Weekly body weights and daily health monitoring of mice occurred until their scheduled endpoint at either 30 or 90 d post-implantation. At the planned endpoint, transponders were collected along with the surrounding skin and subcutaneous tissue for histopathology. Overall, results indicated that there was no maternal rejection or death of any pups post implantation, minimal migration of the transponder, and mice tolerated transponders with minimal tissue reactivity for the duration of the study. Implanted mice grew at the same rate as control animals. The use of these microtransponders appears to be a well-tolerated method for permanent electronic identification of neonatal mice as early as day 1.

P226 Removing Variables during Mucosal Exposure of SHIV in Rhesus Macaques (Macaca mulatta)

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Eight rhesus macaques of approximately 7 y of age were used as models to mimic virus transmission risks observed in humans, particularly in human mucosal HIV transmission. To recreate the risk of viral infection via oral mucosa, 4 macaques received 6 x 30ul doses of 10% acetic acid into the submucosa of the middle right cheek via a micro syringe. On day 4 after the procedure, a lesion appeared. Initially, approximately 300ul of virus was dripped onto the lesion site and let sit for approximately 5 min then the remainder was absorbed with gauze. Concerns of variables, such as other mucosal inflammation or teething, arose while doing this procedure. In an effort to eliminate these variables, it was decided to use the hard plastic casing from a 3cc syringe and needle as a barrier to localize the area of induced inflammation that the virus came in contact with. This casing was effective because it had an opening at both ends and the larger end was correctly sized to surround the inflammation. After placing the casing around the acetic acid induced inflammation, 300ul of SHIV virus was slowly dripped into the top of the casing and held in place for approximately 5 min. Before removing the casing, gauze was wrapped around the base to soak up the excess virus. Punch biopsies in the dose site confirmed virus was, in fact, present and follow up blood work confirmed SHIV infection. Localization of the virus was achieved and yielded positive results.

P227 Behavior Modification of Personnel through the Use of Operant Conditioning

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One of the more challenging tasks that we all face in an animal facility is the modification of longstanding practices by our personnel. Although difficult, such changes are often necessary to improve efficiency, enhance animal welfare, or improve safety. Yet, to achieve this, we have to overcome a natural resistance to change that is embedded in behaviors that are comfortable and familiar; furthermore, maintaining the new behavior can be especially daunting, as old behaviors become engrained and preferred regardless of how inefficient or unsafe they may be. We recently encountered this circumstance when we determined that 2 different improvements were necessary in our facility: the need to consistently use protective eyewear and to discontinue the practice of overfilling rodent cage feed hoppers. To achieve the desired changes involving these different and unrelated concerns, we employed a key concept in operant conditioning used to alter behavior in both human psychology and animal behavior: positive reinforcement. Tailoring this approach over a 12-wk period, we were able to modify the behavior and culture of our personnel. In our approach to modifying the behaviors, we initially provided staff with a description of the problem and benefits of change in both a group and individual setting. On a weekly basis, we then met one-on-one

with each individual, cageside during change-out, to provide positive feedback on any observed improvement until all had achieved the intended change, and finally provided a group reward upon successful completion of both improvements. We have achieved near 100% compliance with the use of safety eye protection. And, we have discontinued the practice of overfilling feed hoppers throughout the facility. Using the thoughtful application of positive reinforcement techniques with our personnel resulted in a smooth, painless, and lasting change in practices and demonstrated that the culture of a team can change while realizing desired improvements.

P228 Rapid Identification of Pathogenic Bacterial by 4-Plex Polymerase Chain Reaction Assay in Laboratory Animals

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Disease prevention is important in animal experiments for animal welfare, scientific quality, and safety. We were trying to find an objective, rapid, and accurate identification method for important pathogenic bacteria, such as *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Staphylococcus aureus,* and *Streptococcus pneumoniae.* In this study, conventional PCR assay was used for rapid identification. As a result, we established a species-specific 4-plex PCR assay for directly detecting pathogenic bacteria associated with diseases of laboratory animals. Under optimal PCR analysis conditions, the 4-plex PCR assay simultaneously yielded a 220 bp from *K. pneumoniae*, a 410 bp from *S. pneumoniae*, a 616 bp from *S. aureus,* and a 890 bp from *P. aeruginosa*. The 4-plex PCR assay detection limits were 10pg for *K. pneumoniae*, *P. aeruginosa*, *S. aureus,* and *S. pneumoniae.* Also, 4 pathogenic bacteria through our 4-plex PCR assay were successfully identified in the lung of experimentally infected mice. As well, *K. pneumoniae*, *P. aeruginosa*, *S. aureus,* and *S. pneumoniae* are major pathogens causing nosocomial infection in humans. Our 4-plex PCR assay will be applied as a useful method for detecting pathogenic bacterial infections in laboratory animals as well as economic animals and hospitalized patients. In addition, rapid diagnosis through our 4-plex PCR assay will improve quality control in laboratory animal facilities.

P229 Where Will the Mice Go? Our Journey in Retrofitting Vivarium Space for Gnotobiotic Use

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Germfree mice serve as research models for investigators interested in studying microbiota under various experimental conditions. When an institution decides to establish a gnotobiotic component to their animal research program, multiple factors must be considered to ensure a successful startup. Flexible film isolators were purchased to support breeding and housing germfree C57BL/6 mice. Following identification of space, a consultant provided recommendations on infrastructure changes that would make the space more conducive for gnotobiotic procedures. Rooms were designated as a supply prep, supply storage, breeding/experimental room, and biocontainment. Some of the challenges encountered included developing biosecurity procedures including restricted access, reconfiguring of electrical outlets, accessing a water source, and providing an autoclave for dedicated use by staff. We describe the challenges encountered when repurposing small and large animal space into a dedicated gnotobiotic work area, and the solutions devised to address those challenges.

P230 Transitioning to Electronic Animal Health Records

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Comparative Medicine (CM) strives to identify innovative strategies to foster continuous improvement and progressive advancement in a technologically adept world. With this in mind, paper-based animal health records can prove to be very labor intensive, extremely time consuming, and a potential fomite. For example, records may be illegible due to poor penmanship, misplaced due to a poor filing system, tedious to archive, or can potentially hinder timely communication of important alerts to appropriate staff. In December 2015, CM transitioned to paperless animal health records to reduce paper burden, increase efficiency, enable easier analysis of these records, and streamline the scheduling of animal care and veterinary events. This transition began with the inception of system that would globally harmonize electronic animal health records and associated documentation. The goal was to enable more efficient clinical tracking, generate cross-site information sharing capabilities, improve upon biosecurity, aid in regulatory compliance, ease the accessibility of animal records, and simplify associated archiving processes. To support creation of electronic animal health records, a number of veterinary management software products were evaluated and the business processes for animal health record keeping were standardized across multiple sites. Partnerships with technology colleagues and a commercial veterinary management software company were formed to develop a program configured to meet diverse requirements based upon our standardized process flow. Implementation of electronic animal health records (eAHR) came with both challenges and successes; however, we were able to successfully attest to demonstration of meaningful use of the software. CM continuously evaluates its goals to ensure improved workflows and software alignment while leveraging the functionality of the electronic health records.

P231 Establishing an IACUC Grant Protocol Congruency Review Team

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The National Institute of Health (NIH) requires that each institution ensures the research proposed in the grant is congruent with the protocols approved by the IACUC (NIH Grants Policy Statement section 4.1.1.2) and that confirmation of congruency be documented. The added responsibility of documenting congruency has shifted to the IACUC office. An approved protocol is necessary prior to grant award, demanding that the grant-protocol congruency review occurs before or during the standard protocol review process. Given the duration of the protocol review process, this stands to be a logistical strain on IACUCs and a potential delay to experimental work as researchers await the approval of their protocol and grant award. To address this need, this institution assigned congruency review, documentation and follow-up to the grant-protocol congruency review team. The team effectively managed this review process by using the institution's electronic protocol management system to ensure that potentially noncongruent items are brought to the PI's attention during the protocol review process, thereby reducing the review and approval times. The protocol management system effectively doubles as the means by which potentially noncongruent items are communicated to the PI, as well as a form of documentation. In addition, the team tracks the results of all congruency reviews to gather metrics on common congruency issues, which in turn can be used to educate investigators as they write grants and protocols, ideally reducing the incidence of disparities that would negatively affect rigor and transparency.

P232 Qualitative and Quantitative Behavioral Measurements to Assess Pain in Axolotls (Ambystoma mexicanum)

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Effective pain relief in animals relies on the ability to discern pain and assess its severity. Commonly used pain assessment tools in mammalian species include the grimace scale or behavioral changes which are difficult to apply to amphibians. Analgesiometric tools such as von Frey fibers and the acetic acid test have been used to evaluate analgesic efficacy in mammals and amphibian species. Unlike the wide body of literature in laboratory rodents, few objective measures exist to assess the presence and severity of pain in amphibians. Furthermore, no publications exist regarding appropriate analgesic doses and assessment of pain in axolotls (*Ambystoma mexicanum*). Our lab sought to evaluate behavioral tools for cageside pain assessment and to develop a reproducible and reliable method to evaluate analgesic efficacy in axolotls. Animals were divided into control and treatment groups (n=6 per group), with the treatment groups receiving buprenorphine injection (50 mg/kg every 24 h for 48

h by intracoelomic route) or butorphanol immersion (0.50 mg/L or 0.75 mg/L every 24 h for 48 h). Qualitative and quantitative behavioral assessments from other amphibians were adapted for axolotls to develop cageside tools for behavioral assessment and the impact of analgesic administration on behavior. Qualitative tests include tapping the home tank, directing water jets, or physically touching specific anatomical points, and placing a novel object in the home tank. Quantitative testing relied on the acetic acid test (AAT). The significance of treatment effects at each time point are assessed by Kruskal-Wallis 1-way analysis of variance for unrelated samples; Kruskal-Wallis testing across operators demonstrate interuser consistency of observations. Treated animals did not demonstrate a significant difference when compared to controls during behavioral assessment. The AAT testing is reproducible and demonstrates a dose-dependent response. However, a significant difference between the AAT response of control and treatment groups were not observed.

P233 Successful Sedated Pairing of Cynomolgus Macaques (Macaca fascicularis)

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Our institution houses approximately 150 adult female cynomologus macaques for long periods of time for pharmacokinetic and imaging support. Maintenance of this colony requires replacing ~30 to 40 animals per year. According to the *Guide*, social animals must be socially housed unless there i s a veterinary or study exemption. At our institution, long term cynos are housed in pairs or quads. Many pairs remain together the entire length of their stay at our institution, but some are separated due to incompatibility or study purposes which often necessitates re-pairing of animals. Historically, cynos were paired in an introductory period by allowing access to each other through a mesh divider followed by full contact if initial interactions appeared positive. This pairing method was not optimal, as it required a significant time commitment from staff to observe for compatibility behaviors and many pairing attempts resulted in animal injury. Adult female cynos are notoriously difficult to pair (and re-pair) once established groups/pairs have been broken up for study or aggression reasons. Since 2011, our institution has had great success with sedated pairing in a novel environment. In this approach, once potential partners are identified (ideally without previous contacts), they are sedated (ketamine/midazolam) and allowed to recover together in an unfamiliar housing room. They are then observed frequently throughout the day but without a need to closely monitor for behavioral signals of compatibility. However, in situations where the 2 cynos know each other before being brought to a novel housing room, additional behavioral observations for compatibility are almost always needed. In our experience, compatibility problems arise when cynos are familiar with each other and/or former partners remain in the same room. To date, we have had over 150 successful pairings and recovered technician time using the sedated pairing in a novel environment method.

P234 Postoperative Warming in Sheep

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Postoperative sheep that have undergone an open chest procedure often cannot maintain a normal body temperature, despite the use of warming agents such as hot water blankets, forced-air patient warming, and warm fluids. These sheep can come into recovery with temperatures as low as 35°C (95°F). Warming the sheep quickly can aid in a faster and smoother recovery, minimizing time intubated and restrained. When recovering in a sling, the sheep's legs are exposed and have IV fluid lines, arterial lines, and ECG cables attached, making the legs difficult to wrap for warmth individually. By constructing a tent out of surgical blankets to drape over the sheep and placing a forced-air patient warming blower under the blanket, the entire underside of the sheep is able to be warmed without disturbing the various lines and cables. Using this method, sheep temperatures returned to normal levels and average of 50% faster than without the warming tent. Average recovery times also improved from 4-5 h to 2.5-4 h.

P235 Outreach by Engaging Children of Employees at a Contract Research Organization

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Often employees of research facilities are required to sign a confidentiality agreement when hired. We are advised that public opinion with regards to animal research is low and it may be simpler to avoid conversations that engage. Nevertheless, in order to help improve public perception, we must speak the truth and show our love and compassion. What better way to start this discussion than with our own families. A committee of passionate employees worked together to create a day in which children of fellow employees would be invited to come and learn more about our work and the tasks we do every day to help bring safe treatments to the world. A Saturday near the National Take Your Child to Work Day was selected and the event Journey Into Research was created. When families arrived at the event, each child was given a name badge and provided a passport to have stamped at various stations along their learning journey. They are also given a bag to collect handouts and tokens to remember the day. The day begins with a brief introduction about our CRO and an opportunity for questions and answers. Then the adventure begins. Each specialized department within the CRO was invited to host a station during this event. The participating departments were then tasked to develop creative ways to educate the youth that attended. For example, Small Animal Toxicology created a kid-sized rodent cage that the children could explore and learn about the needs of a research rat. Success of the Journey into Research can be seen from the smiling faces of participants, discovered through positive feedback received, and ensured by increased participation over the 3 years it has been offered. A positive impact on the public's perspective will be realized because the families of employees were given the opportunity to ask questions and receive the truth about CROs and see first-hand what each department does.

P236 A Simple Solution to the Scavenging of Waste Anesthetic Gases from Rodent Masks

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The majority of rodent surgeries in the research environment are performed using isoflurane. Scavenging of waste gas can be incomplete because the adapted face mask on the animal is not sufficiently tight around the snout. Some surgeons experience headaches, nausea, fatigue, and dizziness when exposed to isoflurane as a result of exposure to waste anesthetic gas. To alleviate this impact, some surgeons have used face masks, but the mask is uncomfortable to wear, must be fitted to each user, and is expensive. We sought another method to reduce exposure. We connected refurbished suction equipment to the external evacuation. We then attached "Y" shaped tubing to the suction equipment and positioned the tubing so that the ends of the "Y" were on either side of the rodent face mask just above the animal's ears. When using the modified device, surgeons reported reduced symptoms of anesthetic exposure. To verify the success of the newly developed device, using an unbiased method, our health and safety officer assessed waste gas exposure while the device was in place. The results found that the isoflurane exposure was now undetectable. We have since implemented this procedure in our operating room and have received positive feedback from many users. Although we have used this exclusively for isoflurane, it is likely that this solution could also be applied to other anesthetic gases. Overall, we have developed and validated an inexpensive way of capturing exhaust gas.

P237 Illumination Device for Mouse IV Injections Using Computer Aided Design and 3D Printing

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Robust IV injections in preclinical models can be critical for therapeutic dosing and introduction of pathogens. However, injections can be particularly challenging for animals with poor vein to skin contrast, such as C57BL/6 mice. Current technologies exist to illuminate the tail, but they pose several challenges. These devices illuminate beneath the tail and can interfere with user vision when the tail is thinner than the light or when the tail is lifted during the procedure. Moreover, the devices often intro-

duce awkward injection angles and positions. We developed a novel tail vein illumination device using computer aided design (CAD) and rapid prototyping with 3D printing. The device consists of a cylindrical animal restrainer, a tapered slot for tail placement, and dual collimated lateral LEDs as the illumination source. The illumination device was designed using CAD. Parts were prototyped using 3D printing. High-density SMD3528 LEDs were used for illumination. Tail vein injections with saline were performed on C57BL/6 mice using the illumination device (4 mo, n=8) and a solid brass mouse restrainer (4 mo, n=8). Success is determined by the number of animals injected on the first attempt. All animal cages were placed on warming pads. With the illumination device, 7 out of 8 mice were injected over 27 min on the first attempt. With the brass restrainer, 2 out of 8 mice were injected over 21 min. The tail vein illumination device presented here allowed for clear visualization of the entire tail vein in C57BL/6 mice. While injections with the device required more time, first attempt success rate was much higher compared to the brass restrainer. Critical features of this device include the lateral collimation for precise lighting of the tail vein and not reflection of the tail surface. The lateral design also prevents user blinding when the tail in lifted out of the tapered slot. The optimal distance of the LEDs provides some heating and dilation of the tail vein. Together, this device allows for accurate tail vein identification, reduces multiple injection attempts, and increases efficiency and throughput.

P238 Preventing Missed Medications in Nonhuman Primates Using Electronic Verification Methods

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Our national primate research center prescribes approximately 25-100 opioid injections daily in conjunction with clinical and surgical procedures delivered across multiple time points. While medication prescriptions are entered into an internal database, verification of delivery was previously performed using a manual signature on paper, and therefore verification in real time of opioid administration was difficult. Although our percentage of missed analgesic doses was already considerably low, we felt there was still room for improvement. Due to the fact that missing a prescribed pain medication for a nonhuman primate (NHP) bears animal welfare ramifications, the veterinary staff enlisted the help of the information technology (IT) unit to automate verification. A system that provided feedback in real time to key staff even while off campus was created using a digital signature to verify administration for all opioid medications. The digital signature is entered directly into the existing medical record database. Opioids are delivered in most situations at 8 a.m., 12 p.m., 4 p.m., and 8 p.m. A notification email is generated 4× a day indicating whether prescribed injections were digitally verified. This method allows staff to immediately identify medications not verified within the expected time frame for administration, and correct any oversights to prevent patients missing analgesic doses. With our new system, we have eliminated missed opioid doses and feel confident that our NHPs receive all required analgesia, thanks to this process improvement.

P239 Health Checking Mouse Cages: Red Light or White Light?

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Minimizing mouse colony disturbance during routine health checking is one of the most challenging issues faced by animal care technicians on a daily basis. The inside of modern mouse caging can be dark and lead to difficulty in visualizing the animals and cage environment. A hand-held light source is a widely used method to improve visibility inside cages. Historically, we have used either incandescent or white light emitting diode (LED) pen lights to shine into cages. With the daily use of these lights, there was increasing concern about routinely disturbing the mice. Mice have limited ability to see into the long wavelength or red light spectrum. We hypothesized that placing a red filter on a white LED light would minimize mouse disturbance as measured by behavioral response to cage evaluation. To test this hypothesis, 19 cages of pair-housed, sentinel-ICR mice were selected as they are uniformly handled and all cages were located at the same position on ventilated racks. Using a crossover study design, cages were placed into 1 of 2 groups that received either a daily health check with white LED light (broad spectrum wavelength) or red filtered LED light (above 580 nm). At the time of health checking, a uniform scoring system was used to evaluate activity of the mice for 5 s before light exposure and then in response to the light for 10 s. After the first 2 wk of the study, the light was switched to the opposite color followed by an additional 2 wk of health checks. Our results indicated that there was a significant increase in the activity response of mice exposed to white light compared to those that were exposed to the red light ($P < 0.05$). Regardless of title, rank, or purpose, everyone working in the animal facility has a responsibility to minimize disruptions within the environment so research goals can be achieved. Our results demonstrate a refinement to routine health check practices by reducing a daily disruption to their environment while continuing to ensuring animal welfare.

P240 The Art of Compassion: A Celebration of the Human–Animal Bond

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Compassion fatigue in the research environment is a topic gaining a lot more attention as we learn to not only recognize the human–animal bonds that are formed but celebrate them as well. It can be overwhelming to work in such a caring and emotionally charged environment without eventually finding oneself facing challenges in dealing with those emotions. Finding ways for both the employee and supervisor to manage personal feelings about working in animal research has become something that many organizations are now seeing as necessary. Helping employees deal with their feelings ensures a positive impact on the employees, their jobs, and ultimately their animals as well. A small group was established at our company to specifically develop a program that would be effective across the organization in both addressing and raising general awareness of compassion fatigue. One such project was implemented which we call The Art of Compassion. Among our staff we have several very talented artists who, upon request, will provide our technicians with a drawing of an animal which has made a positive impact on their lives. The request process involves the technician writing a small essay explaining how this particular animal has touched their life. Oftentimes, simply formally acknowledging these feelings is a way to help someone who is struggling to recognize the emotional tax our jobs often incur. Celebrating and encouraging those human–animal bonds is just one way in which our institution wanted to help animal care staff combat the negative outcomes that are often associated with compassion fatigue. Showcasing an example of how our institution is beginning to address compassion fatigue is just one way to start the conversation and help others understand the importance of recognizing compassion fatigue.

P241 An Approach to Metrics Gathering in the Absence of a Reliable Database

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Intuition tells us that implementing a comprehensive electronic system to facilitate workflow and system management will result in better performance. Yet, it is often difficult to verify whether business processes have improved and whether researchers are better-served postimplementation. Often, we rely on perceptions with little or no hard data to evaluate the new state against the old. Confounding the situation, changes to overall business processes make comparing the 2 states inconsistent. Prior to beginning system implementation of a new animal census and operations software solution, a separate project was initiated to identify key business procedures which would be affected by the new system. These data were compiled in relation to standard operating procedures. Next key metrics were established, with a criterion that equivalent time points needed to be available from both old source data and in the new system. Finally, data were collected and analyzed using standard statistical techniques to provide an adequate baseline performance. This would enable adequate comparison of the success or failure of the new system to improve performance. As the system was implemented, project managers kept a detailed log of decisions points. This included items for which the implementation team was divided on the best implementation approach. The available metrics were used to justify a principle of simplicity of design. Once the new system was launched, these decisions were revisited in light of the data gathered across all time points. In order for leadership to support further changes to the implementation design, requests needed data illus-

trating the new system was sub-optimized. Despite an apparent lack of access to metrics, a systematic approach can yield meaningful data that can be used to assess the success of a new system and thereby drive further leadership decisions.

P242 How Clean Is that Equipment? A 2-Step Approach to Ensure Proper Sanitization of Principle Investigator-Owned Equipment

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When it comes to specialized equipment owned by individual principal investigators (PI), verifying that the equipment is properly sanitized can be an issue. Multiple campuses and animal husbandry buildings can increase the risk of equipment not being properly sanitized/disinfected. We took a 2-step approach to solving this problem. By combining ideas from the university IACUC team and the comparative medicine center, we were able to identify, approve, and test various standard operating procedures (SOP) developed by the lab regarding equipment sanitation. The new SOP ensures the efficacy of sanitation and disinfection in accordance with the Animal Welfare Act and the *Guide for the Care and Use of Laboratory Animals*. The coordination between the IACUC and the husbandry programs impacts over 300 principal investigator laboratories, 3 campus locations, and 15 different buildings. The 2-step process requires the PI to identify equipment that animals come in direct contact with and that cannot be cleaned using normal sanitation procedures. The IACUC requires the PI to identify this equipment in their protocol and provides instruction on how to create a SOP according to the IACUC policy. The IACUC will review the SOP ensuring that the equipment meets the criteria for the policy and that the sanitation approach is acceptable. Once approved the IACUC informs the cage wash supervisor to schedule a testing date with the PI to test all equipment covered in the SOP using an adenosine triphosphate bioluminescence testing system (ATP). During the IACUC semiannual laboratory inspections, the inspection team will confirm that the lab has a sanitization SOP on file. If an SOP does not exist, the IACUC will work with the lab to develop and submit one for testing. This process ensures that all equipment has been identified, approved, and tested.

P243 Setting Up a Self Help Station to Assist Researchers with Efficient In Vivo Drug Administration Guidelines

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Researchers can sometimes be overwhelmed while handling drugs for in vivo studies, especially when it comes to preparing correct dilutions, calculating dosages, and maintaining accurate record keeping. Not all of them have a medical or veterinary background, so it is a must to provide them with one-on-one training and easy-to-use tools. The goal is to have them feel comfortable using properly identified sterile vials, pharmaceutical grade saline, and administering the drugs after obtaining stock drugs from the veterinary staff. We have placed a "help yourself" station in a conspicuous location in the vet staff office area equipped with the following items: child dilution sheets to add to parent stock drug log given at time of controlled drug checkout; formularies with step-by-step instructions for the most common anesthetics/analgesics; pre-printed labels for dilution vials with area to record lot number, prep date and initials; a highlighted 30 d postdilution expiration statement; analgesic sheets to record pain management regime; postop analgesia labels to write drug given/dose/date/time of day to be applied to cage cards or lab books; red dot do-not-use labels to seal the top of expired dilution vials and avoid dosing animals with them by mistake; sterile empty vials of different volumes with sealed rubber tops; 10 mL pharmaceutical grade sterile saline vials; and ¼ in. color dots to differentiate dispensations from same drug batches. Investigators needing to provide medication per their IACUC-approved protocols receive personalized training by the vet staff to clarify proper methodology, responsibilities, and acknowledgment of good practices/procedures. They are also shown how to use the self-help station. Since its implementation about a year and a half ago, we have noticed the station needs to be replenished biweekly, since users visit our office area frequently to collect what they need. Semiannual IACUC inspections confirmed the majority of the labs are using these supplies, and are complying with requirements. In fact, we have not found any nonsterile tubes with unidentified drug solutions during lab walkthroughs. Users have expressed their satisfaction with the convenience of this help yourself supply station.

P244 Rat Thunder Jacket—A Zen Experience

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Germ-free rats are fairly uncommon, relative to germ-free mice, and restraining these animals safely and effectively for compound administration and blood collection can be challenging. There are many commercially available varieties of restraint devices, but most of them are not optimal for use in isolators because they occupy a lot of space and present puncture risks due to their rigid and bulky design. To address these challenges, a sock restraint system was developed for use during compound administration (for example, intraperitoneal [IP], subcutaneous [SQ], intranasal [IN], oral gavage [PO]) and blood collection from the maxillary (facial) vein, common in germ-free settings. The sock is compact, soft, and easy to sterilize. One hundred and fifty Fisher-344 rats of both sexes, ranging from 2 wk to 12 mo of age, were acclimated fairly quickly to sock restraint, enabling safe and efficient technical manipulations within germ-free isolators.

P245 Optimization of Final Rinse Temperatures in Rack Cage Washers

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To optimize efficiency and reduce maintenance costs, a study was conducted to see if cycle times could be reduced by lowering the final rinse temperature without impacting the cleaning effectiveness of the cagewash machine and meet regulatory requirements. For the experiment, the cagewash machine's programing was changed to allow a 165°F guarantee on the final rinse, however the set point for delivered water temperature was not changed (190° F). This allowed the incoming water to meet the USDA requirement for 180°F final rinse temperature and achieve the time/temperature requirement for sanitation recommended in the *Guide for the Care and Use of Laboratory Animals*. To ensure proper contact temperature was being achieved, a tri-tape (160°F/170°F/180°F) temperature tape was placed inside of the outlet of the water fill pipe, thus ensuring the incoming water was above the desired 180° F. We then placed a 165° F temperature tape on each piece of equipment that was being washed, ensuring that it was reaching at least 165° F. Adenosine triphosphate (ATP) microbial ATP testing was performed prior to and after the equipment was washed. This experiment was repeated in each of our rack washers, for a total of 74 cycles. Our results showed that the temperature lowering results (mean 4.96 RLU's) was not statistically different than achieved with a 180° F guaranteed rinse temperature (mean 1.24 RLU's). Due to these results, all of our rack style cagewash machines have been re-programmed to the 165° F final rinse guarantee with water delivered at 190° F. It is estimated that we are saving 15% (7 min) on a normal cycle compared to running our rack washers at 180-degree final rinse.

P246 Assessment of Root Vegetables as a Food and Fluid Source for Live Pest Traps Placed in Laboratory Animal Facilities

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Pest control programs are deemed to be an essential component in research animal housing environments, per the *Guide for the Care and Use of Laboratory Animals.* However, daily assessment of humane live traps in room/housing areas can become labor intensive, particularly in facilities with extensive room numbers, loading dock and support areas, and expansive farms areas that may be visited sporadically. Currently, the practice at our institution is to place traps at the intersection of the floor and wall and load traps with a solitary rodent chow pellet, as an enticement to loose mice. External site visitors to our institution recommended that, in areas where routine trap checks were challenging, a fluid source be added to ensure that confined mice would not dehydrate. Our goal was to investigate a durable food/fluid source, such as a root vegetable, that could provide sustained nutritional support to

trapped mice. We assessed 5 nutritional groups: chow pellet with either a ½ baby potato, whole baby carrot, or water gel cup, or just ½ baby potato or baby carrot alone. Mice (n=40) were housed singly, in static cages without bedding, to emulate trap environments and were randomly assigned to food/fluid group. Mice were assessed for physical condition and body weight (BW) daily. Overall, all animals lost BW and condition within 72 h, with the majority of mice (23/40) removed from study due to >20% BW loss by day 4 and were returned to standard caging with ad lib nutrition. For animals given the pellet and potato, significantly more animals ($P < 0.005$) animals (6 of 8) maintained clinical condition within 72 h, compared to all other groups. Mice provided pellet and potato, lost an average of roughly 12% bodyweight within 4 d, compared to 19-29% loss in other groups. We propose that provision of a baby potato, in addition to a food pellet, in remotely located pest traps will provide practical and economical nutritional support to confined mice, thus allowing for live traps to be checked every 2 to 3 days (for example, Monday/Wednesday/Friday), instead of daily, for pests.

P247 Environmental Conditions and Scientific Rigor in Animal Research: How Do They Matter?

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The NIH's Office of Research Infrastructure Programs (ORIP) improves animal research by modernizing research facilities and supporting development, preservation, and sharing of animal models of human disease. These models must be well characterized to ensure experimental rigor as the NIH strives to support research that is reproducible, robust, and transparent. Environmental conditions like temperature, light, or feed affect animal behavior and metabolism, and thus may modify study outcomes and affect experimental reproducibility. To further explore these topics, ORIP issued a Request for Information (RFI), NOT-OD-17-011, seeking input from researchers, facility managers, scientific organizations, veterinarians, and others who rely on and develop animal models or work in animal research facilities about their knowledge and practices regarding monitoring of environmental conditions, accounting for them in experimental design, identifying their influences on experimental results, and sharing such information with other scientists. The RFI also asked for recommendations for ORIP's priorities on how to improve scientific rigor while accounting for environmental conditions. Responders noted that the effects of environmental factors are complex, involve multilevel interactions that affect physiology and behavior through various, often undetermined, biological mechanisms, and should be kept extrinsic to experimental protocols but must be clearly accounted for. Specifics of what the most pressing issues are and how to best address them depends on the type of animal and level of attention the research community pays to these matters. Responders also suggested that ORIP could raise awareness by organizing workshops and web-based courses, sponsoring lectures at scientific research meetings, working with journal publishers, and facilitating development of species- or research field-specific tools that would aid the process of collating relevant extrinsic variables. Based on RFI findings, ORIP is engaging the community to improve methods for monitoring environmental conditions in facilities and encouraging researchers to account for extrinsic factors in animal research to enhance reproducibility of research through increased rigor and transparency.

P248 Single Cage-Card System to Improve Animal Welfare in Rodent Facilities

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The cornerstone to providing superior health care in rodent research facilities is the ability to identify animals on the study, along with the procedures performed and/or substances administered. This knowledge enhances animal welfare by allowing veterinary care staff to concentrate their observations on the experimental animals. A cage-side method to identify these animals is ideal as the information is available in real time during animal observations. Cage cards have traditionally been the method of communicating this information to the animal care staff. Cage-card systems can be cumbersome and time consuming for both research and animal care staff, often requiring multiple cards to relay pertinent information. We developed a single-card system that identifies animals on experimental studies, provides legible information including dates of activities, procedures performed, and/or substances administered to the animals. These cards are preprinted by the veterinary technicians with information provided by research staff on a "rodent study form" which is submitted electronically and requires minimal time to complete. The study cards are placed on experimental cages by the researchers when they begin the study. The use of these study cards has resulted in earlier detection of clinical signs associated with experimental procedures. This will provide more timely intervention, improved animal welfare, and legibility of cage cards. We recommend the use of study cards to improve the animal welfare and compliance in rodent facilities.

P249 Efficient Production of Chimeric Rats Using Vitrified Blastocysts

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Genetic modification of rats is often carried out using rat ES cells. However, large numbers of blastocysts are required to produce chimeric rats. In addition, a great deal of effort is required to tune the blastocysts with the time schedule of the ES cells injection. This study was performed to produce ES cell chimeric rats using cryopreserved blastocysts. We compared IVC-BL group (collection of 8-cell embryos and cultured to blastocysts in vitro) and BL group (direct collection of blastocysts from uterus) for harvesting of blastocysts. Vitrification procedure was performed according to the method described in our previous report. The percentage of morphologically normal blastocysts after vitrified-warmed in IVC-BL was significantly lower than BL (63 vs 91%, P <0.05). There was no significant difference in fetal development between cryopreserved groups and fresh control (IVC-BL, 51%; BL, 49; and fresh, 36%, respectively). Vitrified blastocysts (BrlHan:WIST@Jcl(GALAS), BL group) were injected with CAG/Venus ES cells (Crlj:WI-ES1/Nips, RGD ID: 10053737) to confirm the potential for generation of ES-derived chimera rats. Majority of the E15.5 fetuses (92%, 46 chimera / 50 fetuses / 62 transfer) and newborn pups (76%, 16 chimera / 21 born / 34 transfer) were chimeric and detected the Venus expression. In additional series, F344 ES cells (F344/NSlc-ES37/Nips, RGD ID: 10054027) which were gene-modified by CRISPR/Cas9 were injected into vitrified-warmed blastocysts of Long-Evans x Brown-Norway F1 rats for generation of chimera (12 chimera/ 34 born/ 58 transfer). We confirmed germline transmission in both injection groups. This is the first report of the establishment of ES cell chimeric rats with germline transmission using cryopreserved blastocysts. The time schedule control of ES cell injection was easier in vitrified blastocysts of host embryos.

P250 Angiographic Assessment of Below-the-Knee Arterial Vasculature in Familial Hypercholesterolemic Swine (Sus scrofa)

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More than 200 million patients have been diagnosed with peripheral artery disease (PAD), a narrowing of arteries in the legs. It presents as limb pain during exercise, but often progresses to ischemic pain at rest and gangrene, necessitating amputation. Above-the-knee vasculature of familial ypercholesterolemic (FH) swine has become well-validated as a model of human vasculature in terms of size and biological response to endovascular therapies. Currently, there is a pressing need for an animal model targeting below-the-knee arteries, yet this territory remains poorly characterized. We have undertaken characterization of this territory in FH swine. A total of 42 below-the-knee vessels (23 anterior tibial arteries; 19 posterior tibial arteries) of FH swine (36±6 kg) were assessed angiographically. Anterior tibial artery segments measured 66±8 mm in length and 2.18±0.31mm in diameter while posterior tibial artery segments measured 85±6 mm length and 3.12±0.28 mm in diameter. While both vessels are comparable to the human anatomy and provide suitable targets, the posterior tibial artery may be preferred for device implantation as the superficial course of anterior tibial artery may be subjected to external trauma. This anatomic characterization of below-the-knee vasculature in FH swine encourages further evaluation to determine if the tibial arteries can provide a reliable model for human interventions.

P251 Characterization of Normal Skin Thickness for Various Body Regions, Ages, and Genders of Yucatan Miniature Swine

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Dermatological studies of miniature swine provide a unique opportunity for risk assessment because of demonstrated similarities to human cutaneous anatomy, biochemistry, and physiology. The Yucatan is a popular miniature pig in use in biomedical research today. Yucatan skin is slate grey-black in color, slight to moderately pigmented, and contains a sparse haircoat (sometimes called the Mexican Hairless Minipig). The physical thickness of miniature pig skin can impact drug absorption during in vivo or ex vivo studies, or affect wound healing or phototoxicity studies. Therefore a good understanding of the relative dimensions of each major skin application or collection site is indicated. We present the results of a histologic skin characterization study in the Yucatan pig. Four skin samples [neck, dorsum or back (lumbar), flank, abdominal] were collected by 8 mm punch biopsy from 18 animals [ages ranged from 5.0-36.5 mo (males) or 3.5 wk-10.3 mo (females)] following euthanasia and immediately fixed in 10% neutral buffered formalin. The number of hair follicles per surface area was first enumerated. The skin samples were then processed, embedded in paraffin, sectioned, and stained with haematoxylin and eosin for histologic evaluation. For site-specific skin quantitation, the layers were measured microscopically using an image analyzing system containing a verified standard micron scale. Each interfollicular measurement was taken at random and in triplicate using a 40x objective and averaged for each sample. Gender, age, and body region specific means (µM), ± SDs, and ranges are presented for 5 interfollicular skin components (full thickness, stratum corneum thickness, epidermis thickness, number epidermal cell layers, and dermis thickness). Relative ratios of strateum corneum, epidermis, and dermis to full thickness measurements were performed. Data are compared to published measurements of adult human skin illustrating similarities or differences. These cutaneous measurement data will aid investigators contemplating a dermal study utilizing the Yucatan miniature swine model.

P252 Astrovirus Detection by Exhaust Air Particles Monitoring in an Enzootically Infected Mouse Colony

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The use of individually ventilated cage systems (IVC) presents new challenges for an effective microbiological monitoring of laboratory mice colony due to the system intrinsic characteristics of biocontainment and bioexclusion. In this context, the traditional approach based upon the use of soiled bedding sentinels (SBS) is being progressively supplemented by environmental microbiological monitoring. In particular, exhaust air particles sampling is being proposed as a powerful tool to increase the sensitivity of detection of a variety of microbiological agents and, at the same time, to reduce the number of animals used as sentinels. This system relies on a dedicated sliding filter placed before the exhaust air prefilter of IVCs air handling units. Relevant size of dust particles coming from all the IVC cages served by the air handling unit are collected and concentrated on the filter that becomes an ideal substrate for PCR testing. The aim of this study was to evaluate the capability of exhaust air particles sampling to monitor murine astrovirus in an enzootically infected mouse colony housed in IVCs. Murine astrovirus is a recently described single-stranded RNA virus, found to be common among laboratory mice. Infection is clinically silent and no lesions have been found so far regardless of the immune status of the animals. The study was carried out monitoring the air handling units of 4 IVC racks within a facility housing several colonies of genetically engineered mice, previously confirmed by serological testing to be enzootically infected with murine astrovirus at an unknown prevalence. Each unit served 2 double-sided racks housing 280 cages. The exhaust air particles monitoring system was able to detect murine astrovirus by PCR analysis as early as 1 wk after the beginning of exposure in all the 4 air handling units and confirmed the positivity at all the timepoints (2, 4, 12, 16 wk) of the 4-mo study. In conclusion, exhaust air particles monitoring using PCR analysis proved to be a sensitive and effective tool to detect and monitor murine astrovirus in laboratory mice.

P253 Early-Life Stress Alters Correlations between IL-6 and Depression Behaviors

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Postpartum depression and anxiety disorders are disabling mood disorders that impact mothers and their children, but most depression research in animals only includes males. This limitation has led to significant gaps in our knowledge of these common disorders. Recent studies from other laboratories indicate the significant role of the immune system in what our laboratory has shown to be transgenerational behavioral effects. Naturally occurring increased levels of IL-6 in mice have been associated with stress susceptibility leading to a depression-like phenotype following social stress. The current study investigated the role of cytokines in the effects of chronic social stress (CSS) on rat F1 offspring behavior. The CSS model of postpartum depression and early-life stress depresses maternal care in F0 generation dams using an intruder male to antagonize the dam, simulating neglect and establishing a cycle of deficient social and maternal behavior in the F1 and F2 generations. We hypothesized that impaired social behavior of F1 juveniles would be associated with increased peripheral IL-6 concentrations. There was a strong correlation (r^2 =.86, $P < 0.001$) between social investigatory bouts and IL-6 for control juvenile F1 females that was absent in their CSS counterparts. Similarly, there was a moderately strong correlation between IL-6 and both self-grooming frequency (r^2 =.46, P =.04) and duration (r^2 =.49, P =.03) only in the juvenile F1 control females. In conclusion, CSS disrupts the robust associations between social and depression associated behaviors and basal IL-6 concentration seen in control F1 juvenile females. This correlation, while not yet a direct cause and effect relationship, further advances the immune system as a reservoir of targets for future therapies for mood disorders and postulates that IL-6 may be involved in stress associated social impairments. Current work is on how transgenerational social stress alters immune-behavior associations and the response to vaccination.

P254 The Role of Platelets in the Pathogenesis of Mouse Cytomegalovirus

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Platelet decline is characteristic of the acute stage of many viral infections, including cytomegalovirus (CMV). Decreases in platelet number can be partly driven by platelet sequestration in associations with other cells, such as monocytes, lymphocytes, or endothelial cells. Infection of myeloid cells such as monocytes and tissue macrophages are essential to the pathogenesis of CMV infection in tissues. When platelet-monocyte aggregates forms, the expression of monocyte surface receptors change, eliciting an activated Ly-6C^{lo} phenotype; this may enhance monocyte infection and invasion of tissues. We hypothesized that platelet decline and platelet-monocyte aggregate (PMA) or platelet-lymphocyte aggregate (PLA) formation would occur during acute infection of CMV in mice, and that platelet depletion would inhibit aggregate formation and decrease viral load in tissues examined. To establish time points of interest, male BALB/c mice were infected with 3 X 10^6 plaque forming units of murine CMV on day 0. Platelet counts were longitudinally tracked every other day over 21 d. A platelet nadir was observed on day 3 postinfection, followed by a rebound thrombocytosis on day 8 (P = 0.04). To determine whether sequestration in PMAs or PLAs contribute to this decline, additional mice were infected and euthanized on days 0, 3, 8, or 21 for blood and tissue harvest to quantify PMA/PLA formation by flow cytometry. PMA and PLA formation decreased rather than increased at all timepoints following infection compared to baseline (P = 0.05), indicating that neither aggregate formation contributed to platelet decline during acute infection. A subset of mice was treated with platelet depleting antibody every 3 d to determine the effect of platelets on viral entry into tissues at each time point; organ viral load was quantified by plaque assay and compared to undepleted controls. Three of 4 undepleted mice demonstrated higher levels of CMV in the spleen than the depleted mice. Contrary to our original hypothesis, platelets may enhance the ability of

CMV to enter into tissues such as the spleen through associations with cells other than monocytes and lymphocytes.

P255 Comparing Methods for Monitoring Diabetes in Mice

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Many methods for monitoring diabetes in the clinic can be applied to mouse models of this disease. Most handheld glucometers are designed for use during treatment plans intended to lower glucose to within target ranges. However, these meters may not be accurate as chemistry analyzers across the glucose range seen in severely diabetic mice. Glucose in whole blood or serum is also affected by the time since the last meal, the time of day, stress levels, and other factors; the abundance of glycated hemoglobin (% HbA1c) is often used clinically instead of glucose measurements because it is a more stable marker of glucose that is less sensitive to these factors. We hypothesized that handheld glucometers, serum chemistry, and HbA1c would have different diagnostic values among diabetic mouse strains that differ in the severity and duration of their disease. We found strong agreement between a commercially available glucometer and a commercially available clinical chemistry analyzer using submandibular blood from severely diabetic BKS *db/db* mice (BKS.Cg-*Dock7*^{m} +/+ *Lepr*^{db}/J; 10 males) and DBA/2J mice treated with streptozotocin (5 males), in addition to a variety of nondiabetic strains (23 males). Using transiently diabetic C57BL/6 *ob/ob* mice (B6.Cg-*Lep*^{ob}/J), we compared HbA1c levels to submandibular blood glucose (glucometer) and serum glucose (clinical chemistry analyzer), during a range of ages (6-8 wk) when glucose values peaked and then declined. We found that HbA1c values clustered by genotype between obese (*ob/ob*, 19 males) and lean (*ob/+*, 15 males) mice, including in *ob/ob* mice that were not hyperglycemic when tested (glucometer reading < 250 mg/dL). HbA1c was stable during this period, while blood and serum glucose values declined as a consequence of strain-dependent compensation. Glucose values measured from submandibular whole blood and serum were comparable in several models despite different analytical methods. The handheld glucometer had great ease of use while the chemistry analyzer was useful in measuring other relevant parameters including lipids. Overall we found evidence to support the use of each of the 3 analytical methods compared.

P256 Comparison of the Use and Effects of 3 Nutritional Supplements During the Pre and Postweaning Development Period in C57BL/6J Mice

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Nutritional supplementation is used in mouse colonies to promote pup survival and maturation. However, supplement selection and application may vary between and within facilities with little attention to effects on pup survival and weight gain. This study compared 3 different nutritional supplements for acceptability and their effects on weight and survival in C57BL/6J pre and postweaning mouse pups and lactating dams. A total of 96 pups were randomized to receive a dough nutritional supplement (DNS), gel nutritional supplement (GNS), or moistened pellet nutritional supplement (MNS) ad libitum from P11-P28 compared to a control group without a supplement. Stool was observed daily for a dye marker indicating supplement consumption. Mice were weighed every other day from P11 to P28, and on P31, P35, P38, and P42. Pups were weaned at P21 and followed until P42. The proportion of pups consuming supplement was 100% for DNS, 100% for GNS, and 96% for MNS. The median (minimum-maximum) time (days) for pups to consume the offered test supplements was 19 (15-23) for DNS, 19 (15-21) for GNS, and 20.5 (17-22) for MNS. All dams consumed the offered test supplements by P12 but had no weight effects. In the P11-P42 period, DNS and MNS pups gained 16.8±3.3% and 15.8±2.8%, respectively. Control pups and GNS pups gained more slowly, 14.6±2.8% and 14.7±2.8%, respectively. Likewise, average daily gain was significantly higher in DNS pups (0.56±0.11g/d) as compared to controls (0.49±0.09g/d, $P \leq 0.05$). There was significantly more weight gain in the DNS and MNS groups than in the GNS and control groups during the P25-P42 period ($P \leq 0.01$). No morbidity or mortality was observed. Supplementation is best applied in a strategic manner during the early postweaning period. Additional factors like cost, supplement composition, storage requirements, and ease of administration should be considered when selecting a nutritional supplementation plan within an animal program.

P257 Using Microdialysis to Evaluate the Effects of Enrichment on Dopamine Levels in Conscious Nonhuman Primates

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Enrichment can be defined as any type of environmental stimuli that enhances optimal psychological and physical wellbeing for an animal. Enrichment, which includes feeding (monkey chow, grapes, and trail mix) and watching television, is typically provided to our rhesus monkeys during our study paradigms. We wanted to evaluate, by quantitative analysis, if enrichment would have an effect on the outcomes of our nonhuman primate (NHP) microdialysis studies. The NHP microdialysis model was developed at our facility with the collaboration of various departments. Imaging analysis, mathematical formulas, and delicate surgeries were performed to obtain a working model. Microdialysis is an in vivo sampling technique used to monitor the neurotransmitters in the interstitial fluid (ISF) of the brain. Neurochemicals, such as dopamine, are collected at timed intervals by using a probe with a semipermeable membrane that is connected to inlet and outlet tubing that is perfused with a salt solution (aCSF). For the central nervous system, dopamine plays a major role in motivation, arousal, emotional response, and reward-motivated behavior. For our studies, we used 3 rhesus monkeys implanted with cisterna magna ports (CMPs) for collecting cerebral spinal fluid (CSF) along with microdialysis cannulas implanted into 4 locations in the striatum using a head cap. Crossover microdialysis studies were performed without enrichment or with enrichment (TV only or TV with food) provided at the fourth hour over a 7-h study period, with vehicle (PO at 3 h) and cocaine dosing (IV at 5 h). An increase in dopamine levels were seen after cocaine was administered in all studies with a reduction in dopamine levels as more enrichment was provided. Peak ISF dopamine levels from baseline values were as follows: without enrichment (+189%), with TV only, (+136%) and with TV and food (+91%) and similar trends were seen in the CSF. This study indicates that enrichment may affect the sensitivity of cocaine-stimulated dopamine release. For future studies, the assessment of supplemental enrichment and the appropriate level of animal habituation should be considered in the initial study design.

P258 Evaluation of Presurgical Skin Preparation Methods in Mice

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Mice routinely undergo surgical procedures for use in research. However, studies of surgical site preparation are rare. The purpose of this study was to evaluate 2 hair removal techniques and to compare 2 surgical skin preparations in C57 background mice. Our hypothesis was that depilatory agents in combination with iodine and alcohol scrub would result in more epidermal damage, leading to an increased susceptibility to surgical site infections. We evaluated 4 different skin preparation conditions (n=15/group): 1) depilatory agent with iodine and alcohol scrub, 2) depilatory agent with iodine and saline scrub, 3) shave with iodine and alcohol scrub, 4) shave with iodine and saline scrub. The dorsum of each mouse was prepared in accordance with the assigned treatment group while under anesthesia. Skin was swabbed for bacterial cultures immediately after hair removal and again following scrubbing to measure reduction in bacterial load. A 1cm, full thickness, skin incision was made and closed with 5-0 polypropylene suture. Incision sites were photographed for visual scoring and sampled for histological scoring at 0, 1, and 7 days postsurgery to assess inflammation, edema, and epidermal damage. Photographs were viewed by 3 persons to obtain a modified ASEPSIS wound score. Prepreparation bacterial load growth score was higher (mean ±SE, 2.7 ±0.53) than post preparation (0.7 ±0.17, $P < 0.0001$), and lower with depilatory hair removal agent (1.15 ±0.39) versus shave (2.25 ±0.79, $P =$

0.0115). Modified ASEPSIS wound scores were not significantly different between hair removal methods ($P = 0.29$) or preparatory agents used ($P = 0.21$). Histopathological lesions were significantly lower with the depilatory hair removal agent (4.24 ± 0.12) compared to shave (5.67 ±0.12, $P = 0.0002$), but no difference due to scrub method was observed. There were no significant differences between alcohol and saline for any parameter measured. We conclude that the use of depilatory agent for hair removal is superior to shaving for hair removal and that when combined with iodine, the use of either alcohol or saline for a final scrub is acceptable.

P259 Comparison of Pharmacokinetics Profiles for Nalbuphine Following IP and SC Administration to C57BL/6 Mice

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Mice undergo a variety of procedures that necessitate the use of analgesic agents. Opioids are often essential to successful pain management plans, but most are controlled substances, and their use requires appropriate federal and state DEA registrations. Nalbuphine is a potentially effective opioid analgesic for mice that is not currently classified as a controlled substance. It has received little attention as an analgesic for mice and standard dosage regimens have not been developed. This study compares the pharmacokinetic (PK) profiles of 10 mg/kg nalbuphine in male C57BL/6 following SC or IP administration. Blood was collected from 3 mice per treatment at each time point of 0.083, 0.167, 0.33, 0.5, 1, 2, 3, 6, 12, and 24 hours postadministration. Plasma concentrations were measured and standard PK parameters calculated. Profile characteristics for each route of administration were similar with significant differences ($P < 0.05$) in plasma concentration time points 0.083, 0.5, 1, and 3 h. Nalbuphine was absorbed more quickly when administered SC (T_{max} = 0.087hr) than IP (T_{max} =0.167hr) while the half life ($t_{1/2}$) was shorter for IP (0.94 hr) than for SC (1.12 hr), but differences were not significant ($P < 0.05$). When administered SC, the $AUC_{0\text{-tldc}}$ and $AUC_{0\text{-inf}}$ are higher than for the IP route with significant differences ($P < 0.01$). The Cl/F and Vz/F were significantly lower ($P < 0.01$) with SC administration. Plasma concentrations were below the level of detection by 12 h. The results of this study suggest that nalbuphine is absorbed and eliminated quickly, making it a possible candidate for acute pain management.

P260 Comparison of the Effects of Etomidate, Benzocaine, and MS-222 Anesthesia in Xenopus laevis followed by Evaluation of Flunixin Meglumine Analgesic Effects

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Alternative anesthetics for exotic species are often limited due to the small numbers of these animals utilized in research. In *Xenopus laevis*, MS-222 remains the gold standard for anesthesia, but due to certain disadvantages, including respiratory irritation when handling the powdered form and the necessity to buffer the solution, other anesthetics may provide alternative anesthetic regimens. In this initial study, we examined the depth and length of anesthesia produced by immersion in 3 doses of etomidate (15 mg/L, 22.5 mg/L, and 30 mg/L) and in 3 doses of benzocaine (0.1%, 0.5%, and 1%) compared to MS-222. Both optimal doses for benzocaine and etomidate, 0.1% and 22.5 mg/L respectively, generated adequate surgical plane anesthesia time of at least 10 min or greater in order to perform oocyte collection. Flunixin meglumine was also further characterized as an analgesic option using 25 mg/kg or 50 mg/kg injected into the dorsal lymph sac. Acetic acid testing was performed to determine effectiveness of the pain relief at 1, 3, 6, and 24 h after surgical oocyte collection. The 50 mg/kg dose of flunixin meglumine was not well tolerated, resulting in the death of 5 out of 12 frogs within 24 h despite uneventful anesthetic recoveries. Histology was performed at 3, 14, and 28 d postoperatively in each study group to determine any pathologic changes associated with administration of the drugs. In conclusion, etomidate and benzocaine offer alternative anesthetic options, and the administration of flunixin meglumine should be limited to the 25 mg/kg dose published for *Xenopus laevis* in a previous study until further dose response studies can be performed.

P261 Refinement of Perioperative Feeding for a Rodent Vertical Sleeve Gastrectomy Surgery Model

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Provision of liquid enteral nutrition (LEN) during the pre and postoperative period is standard protocol for rodents undergoing bariatric surgery. Compared with a pelleted diet, LEN has increased digestibility while decreasing obstruction risk during surgical recovery. Despite the postoperative benefit, consumption of these diets following murine bariatric surgery is consistently low. This may be attributed to the discomfort of rearing to access the sipper tube and coagulation of LEN within the bottle, obstructing outflow. In addition, extensive daily labor is required to measure, fill, and unclog the LEN set up. To address these issues, a commercially available high-calorie dietary gel supplement (DG) was investigated as a sole food source for mice in the perioperative period. C57BL/6 male mice were conditioned on high-fat diet for 8-10 wk prior to surgery. The study groups were: vertical sleeve gastrectomy (VSG) + DG (n=8), VSG + LEN (n=8), sham surgery + DG (n=6), and sham surgery + LEN (n=6). Food and water intake, clinical condition, and body fat composition (nuclear magnetic resonance imaging) was monitored at baseline and throughout the study. Significant differences were found in caloric intake in the pre and postoperative periods across all study groups. There were no significant differences in body weight change per day, or in water intake from the pre to postoperative periods. Between postoperative weeks 3 and 5, VSG mice on DG had an increase in body fat composition as compared with VSG mice on LEN. Differences in body fat composition between weeks 1-3 were not significant in the remaining study groups. Three mice in the VSG + LEN group were euthanized due to clinical illness. The clinical condition of the remaining animals was unremarkable throughout the study. In summary, feeding a high calorie gel diet to mice undergoing VSG surgery is a less labor-intensive alternative to LEN. Differences in caloric intake and body fat composition were noted between diets with no adverse clinical outcomes. Further characterization of dietary gel supplement is recommended to ensure that these nutritional variations do not impact specific disease phenotypes in this murine VSG model.

P262 Response of Bovine and Murine Neutrophils to Stimulation by Interferon-γ in the Context of Infection with Brucella

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Brucella sp. are gram negative, pleomorphic, facultative intracellular bacteria, and the etiologic agent of brucellosis, which remains a significant cause of disease in humans and animals in many parts of the world. Although neutrophil recruitment is associated with the pathology in several manifestations of brucellosis, the role of neutrophils in *Brucella* infection has been relatively neglected. In previous studies, we, and others found neutrophils to be a major target of *Brucella* infection. Here, we infected wild-type and interferon-γ deficient mice (n=4-5 mice/group) intraperitoneally with *Brucella* and found that interferon-γ deficiency resulted in markedly enhanced neutrophil recruitment to the joints and spleen as determined by flow cytometry. In other murine infectious disease models, interferon-γ has been shown to decrease inflammation by decreasing neutrophil viability. Thus, we sought to determine if interferon-γ had a similar effect on bovine neutrophils and investigated the effects of interferon-γ on bovine neutrophil survival via viability staining by flow cytometry, and migration via transwell plate assays. Bovine neutrophils were isolated from peripheral blood via density gradient centrifugation (blood was drawn from 3 cows) and peritoneal thioglycollate-elicited neutrophils were harvested from C57BL/6 mice. For neutrophil survival studies, neutrophils were incubated with 0, 1, 5, or 50 ng interferon-γ/ml and incubated overnight in the absence of infection, or 1.5 h with *B. abortus* before being analyzed. For migration assays, neutrophils were incubated in the transwell with the above-mentioned concentrations of interferon-γ, while chemoattractant was placed in the bottom well. Interestingly, we found that interferon-γ does not significantly induce bovine neutrophil death during *Brucella* infection in

vitro, and in a long-term incubation, actually increased the viability of bovine neutrophils. Therefore, it is unlikely that inducing neutrophil death is a mechanism by which interferon-γ could restrain neutrophilic inflammation during *Brucella* infection in cattle. In addition, this data suggests that interferon-γ has differential effects on the survival of bovine versus murine neutrophils.

P263 Evaluation of PCR and Culture Methods for the Detection of Opportunistic Bacteria in Barrier-Reared Colonies

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Opportunistic bacteria (OB) can be associated with clinical signs of disease in hosts with compromised immune systems, but are typically asymptomatic in immunocompetent animals. OB are challenging to detect, as they are generally found in low copy number in infected animals. In this study, microbiological and PCR test methods were used to screen (n=144) mice and (n=84) rats from endemically infected, barrier-reared colonies. Mouse and rat colonies were selected based on a health history, which included one or more of the following agents: *P. aeruginosa*, *K. oxytoca*, *K. pneumoniae*, *P. mirabilis*, *S. aureus*, *S. pneumoniae*, and *Beta-hemolytic streptococcus.* Male and female animals were sampled from multiple ages, including weanlings, adults, and retired breeders. Bacterial cultures were performed on nasopharyngeal wash, cecum swabs, and colon swabs using multiple media. Real-time PCR screening included feces, body swab, oral swab, and nasal aspirate specimens. Prevalence for culture was low for *P. aeruginosa*, *K. oxytoca*, *K. pneumoniae*, *S. pneumoniae*, and *Beta-hemolytic streptococcus (group B)*, ranging from 0-6% by agent. Isolation of *P. mirabilis* and *S. aureus* was more consistent at 19% and 43%, respectively. Most OB were isolated at similar rates for both sample types, except for *S. aureus*, which was detected more efficiently in the respiratory (43%) versus the gastrointestinal (0.5%) specimens. Overall, PCR provided a larger percentage of positive results as compared to culture. Positive PCR results for fecal samples ranged from 6-79% by agent, whereas detection in oral swabs ranged from 2-50% by agent. *K. pneumoniae* was detected in oral swabs only. Most OB species were found in both rats and mice, although *S. pneumoniae* and *K. pneumoniae* were detected only in rats. Gender did not appear to have a significant impact on agent detection for either host species. Age did not appear to play a large role in detection for most OB, although the retired breeders showed a slight advantage regarding the detection of *K. oxytoca*, *P. aeruginosa*, and *S. pneumoniae*. Neither test method provided robust detection of OB within historically positive populations, therefore successful detection may require the screening of large numbers of rodents.

P264 The Effects of Buprenorphine on Wound Healing Models

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Buprenorphine is a commonly used postoperative opioid analgesic used. A concern was raised by an investigator at our institution when observing mice in their wound healing model, which appeared to have impaired healing during their study. A possible cause of the healing impairment was proposed to be the preoperative dose of buprenorphine used in the surgical procedure. The objective of this experiment was to determine if a preoperative SC dose of buprenorphine has any effect on wound closure in mouse models. To address this question, 40 female group-housed B6-albino mice were split into 4 treatment groups, n=10. Each group was anesthetized with 2.5% isoflurane and received 2, bilateral 4-mm full-thickness skin punch biopsies on their dorsum. Two of these groups received a subcutaneous saline injection, and 2 received a subcutaneous buprenorphine injection. The groups were further subdivided so 1 saline and 1 buprenorphine group each received a daily, topical chlorhexidine solution application to their wound sites to determine if the topical treatment of the cutaneous lesion impaired or contributed to wound closure. Biopsy wounds were imaged and total wound area and perimeter were quantified from images to determine wound closure progression. In addition, to test an alternative wound healing model, a second study compared wound closure progress and rate using a felt wheel abrasion procedure in both buprenorphine and saline groups. As our punch biopsy data showed little variation between the 4 groups we only compared 10 animals across 2 groups, buprenorphine and saline for an n=5 in the second experiment. Mice were anesthetized depilated and then an approximately 2-cm^2 area of dorsum was abraded using a mechanical felt wheel. Our results show no significant differences in progression of wound closure or closure rate across any of the treatment groups in either the biopsy or abrasion procedures. We conclude that there is not sufficient evidence presented here that the buprenorphine dose in our anesthetic protocol inhibits or impairs wound closure in these mice, nor is there sufficient evidence that chlorhexidine treatments impair or contribute to wound closure.

P265 Evaluation on the Efficacy of Palatability Enhancers in Antibiotic Cocktails

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Alteration or clearance of the gut microbial flora is commonly achieved by oral administration of antibiotics in the drinking water. The antibiotic cocktail of ampicillin (1g/L), vancomycin (0.5g/L), neomycin (1g/L), and metronidazole (1g/L) is frequently used. This cocktail is unpalatable to laboratory mice resulting in decreased water consumption which may result in dehydration, decreased food intake, and weight loss. This reduced water consumption results in decreased dosage of the antibiotic cocktail, potentially impacting the clearance of the microbial flora. The objective of this study was to determine whether a palatability enhancer could be identified that would improve the consumption of the antibiotic cocktail. Single-housed male C57BL/6J mice (5 mice per group) were placed on either reverse osmosis deionized (RODI) water, RODI water with antibiotic cocktail only, or RODI water with antibiotic cocktail and a palatability enhancer. For palatability enhancers, 2 different concentrations of sucralose (1.0 % or 2.0%) were tried with and without sweetness intensifiers as well as 2 different concentrations of each of the following: sucrose (60mM or 120mM), saccharin (0.2% or 2.0%), monosodium glutamate (0.09% or 0.18%), or sodium chloride (50mM or 100mM). Water consumption and body weights were recorded daily throughout the course of the study. RODI animals remained stable across the duration of the study in body weight and volume of water consumed. All mice on the antibiotic cocktails had decreased water consumption and weight loss. The tested palatability enhancers at the concentrations used were not effective at masking the unpalatable taste of the antibiotic cocktail in C57BL/6J mice. Future studies will examine other antibiotics that may be more palatable.

P266 Determining the Mouse T Lymphotropic Virus Genome by Next Generation Sequencing

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Mouse T lymphotropic virus (MTLV; murid herpesvirus 3) belongs to the herpesvirus family. Upon infection in neonatal mice, T lymphocytes development is compromised and leads to thymic necrosis. Detection of MTLV antibodies for serological assays requires virus antigen that is produced either by virus propagation in cell culture or inoculation of weanling mice to acquire infected tissue. We hypothesize that sequencing MTLV will allow us to identify a virus protein that could be expressed to simply and improve antigen production. Sequencing of MTLV is the first step in this process. Thymus glands were pooled and homogenized from 5 MTLV inoculated suckling BALB/c mice. MTLV capsids were purified by ultracentrifugation. DNA was isolated and sequenced by using the next generation sequencing (NGS). After sequencing, paired-end reads were generated and debarcoded. The resulting sequences were assembled after mouse genomic DNA filtering. The largest contig identified is 153,830 bps and contains 173-255 ORFs (minimum of 50 nucleotides). MTLV, is most closely related to *Macaca nemestrina* herpesvirus 7, Human herpesvirus 7, and Human betaherpesvirus 6A when compared to other viruses in GenBank. Although MTLV was not present in GenBank at the time we determined the genome sequence, more recently a sequence sharing 100% nucleotide identity with MTLV, described as murine roseolovirus (MRV), was deposited in GenBank. We identified 1 protein consistent with a glycoB protein found in related viruses that will be targeted as a potential antigen for anti-MTLV antibody detection. Since its identity in 1961, the genome of MTLV has gone undetermined for more than 50

years. However, sequencing by NGS was accomplished in a few weeks. NGS is a rapid method that can be used for sequencing both novel and existing viruses for which the genomes have never been determined.

P267 Development of an Internal Control to Evaluate the Accuracy of 16S rRNA Next Generation Sequencing

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With lower cost and higher quality of data, the next generation sequencing (NGS) platform has gained rapid application in microbiome research and clinical diagnosis. The sequence and taxonomy data output resulting from NGS is complex and enormous. The relative abundances of each operational taxonomic units (OTUs) is used for downstream pipeline data analysis. However, the interpretation of the OTU relative abundance data is difficult to interpret without a functional internal control and may vary among populations with different microbial diversity. We identified a rare phylum 16S rRNA sequence to use as an internal control for rodent gut microbiome NGS analysis. Serial 10-fold dilutions of the internal control were included in NGS analysis of fecal samples obtained from germ-free, germ-free contaminated, specific opportunistic bacteria-free, and barrier-reared C57BL/6 mice. We found that the relative abundance of this control is linear only within a dynamic concentration range. A plateau-effect was observed for higher concentrations of internal control suggesting that the relative abundance is not linear at or above this range. An internal standard consisting of 5×10^4 copies, which falls within the dynamic range, can be used to evaluate reproducibility of each NGS sequence run for consecutive testing of the same population. In addition, we found that this internal control can be helpful for our understanding of background and/or contaminating 16S rRNA sequence that may be introduced into a sample via husbandry supplies and environment or sample collection and processing.

P268 AG B6, a C57BL/6 Congenic Strain Highly Susceptible to Zika Virus

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Zika virus (ZIKV), a mosquito-borne virus in the family Flaviviridae, is an emerging infectious disease. Animal models are critical to understanding ZIKV pathogenesis and to test antiviral compounds. ZIKV is noteworthy among flaviviruses in its ability to infect in utero resulting in fetal demise, microcephaly, and congenital hearing/eye defects, its association with Guillain-Barré syndrome, and its ability to be transmitted through sexual intercourse. The AG 129 (129/SvEv-*Ifnar1*^{tm1Agt} *Ifngr1*^{tm1Agt}), AG B6 (C57BL/6J.129-*Ifnar1*^{tm1Agt} *Ifngr1*^{tm1Agt}), A B6 (C57BL/6J.129S2-*Ifnar1*^{tm1Agt}), and G B6 (C57BL/6J.129S7- *Ifngr1*^{tm1Agt}) mouse strains harbor double (AG 129, AG B6) or single knockouts (A B6, G B6) of the receptors for Type I (IFN-a/b) and Type II (IFN-g) interferons. AG 129, AG B6, and A B6 strains were susceptible to the ZIKV strain H/PF/2013 at 10^3 plaque forming units (pfu)/mouse. Among the 3 strains, AG B6 (n=12) was the most susceptible based on Kaplan-Meier survival analysis (median survival time = 7 d postinfection (dpi)) and weight loss when inoculated intravenously by the retroorbital route, euthanasia being performed when mice exhibited moribundity, paralysis, or >20% loss of initial body weight. AG 129 (n=12) and A B6 (n=15) were less susceptible and G B6 (n=14) were resistant. Others have shown that younger mice are more susceptible to ZIKV than older mice. AG B6 (n=22) as old as 22 wk of age still succumbed to ZIKV when inoculated as above (median survival time = 9 dpi). We tested different routes of inoculation using the same ZIKV strain and dose. Retroorbital and intraperitoneal inoculations were equally efficacious (median survival time = 7.0 dpi). In contrast, subcutaneously inoculated mice lived significantly longer (median survival time = 8.5 dpi, $P < 0.0001$). Quantitative RT-PCR suggested that susceptibility of AG B6 (n=8) to ZIKV is due to high levels of ZIKV in its tissues. A histopathology survey of AG B6 (n=13) harvested at 6-8 dpi identified lesions in numerous tissues, including the brain, testes, ovaries, muscle, and cells of the growing incisors. By characterizing this ZIKV model in adult and pregnant mice, this research will elucidate how ZIKV affects the developing fetus and the peripheral nervous system and will provide a small animal model to test antiviral compounds.

P269 Using the Rat Grimace Scale to Identify Pain in Acute Colitis In Adult Sprague Dawley Rats: Preliminary Data

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The rat grimace scale (RGS) is a novel assessment tool that has been effective in identifying pain in acute somatic pain models. Its applicability in visceral pain models has yet to be assessed. Colitis is a widespread and economically important disease in humans. Humans frequently report pain associated with colitis but this symptom has received little attention in rodent models. In such models, disease progression of colitis is typically monitored with the disease activity index (DAI), which generates a score based on the presence of fecal blood, stool consistency and body weight loss. We hypothesized that the RGS would identify pain before the DAI increased significantly and that RGS and DAI scores would be positively correlated. Twelve adult (189-385g) male and female Sprague Dawley rats were given either 4% dextran sulfate sodium in water to create acute colitis, or tap water (control) for 6 d. Rats were assessed with the DAI and real-time RGS daily. Comparisons to baseline for both scales were made with Friedman's test (Dunn's multiple comparison test). Correlation between the 2 scales was examined with a Pearson's correlation coefficient. After 6 d, rats were euthanized. DAI scores increased significantly from baseline on days 5 and 6 ($P < 0.05$, both). In contrast, RGS scores increased significantly on day 4 ($P < 0.05$) and this increase exceeded a derived analgesic intervention threshold of 0.67/2. There was a positive correlation between the DAI and RGS scores ($P < 0.0001$). These preliminary data suggest that pain, as measured with the RGS, is present before the DAI increases. The positive correlation between the DAI and RGS indicates that disease severity is associated with increased pain.

P270 Optimizing Body Temperature Management during Recovery from General Anesthesia in Adult Rats

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Small mammalian species, such as rats, are particularly prone to hypothermia during anesthesia. During anesthesia, external warming is effective at maintaining core body temperature, but hypothermia occurs during recovery when warming stops. Hypothermia prolongs recovery in rats and is associated with numerous adverse effects in humans. Effective strategies for maintaining core temperature in rats during recovery have received limited investigation. Sixteen adult (215-450g) male and female Sprague Dawley rats were block randomized to short (n=8) or long (n=8) postanesthesia warming strategies. Anesthesia was induced and maintained with isoflurane (1.75%) for 40 min. A target core body temperature of 37.5±1.1°C (core temperature ±2SD recorded from previous work) was maintained with a heating pad. Rectal temperatures were measured every 5 min with a standardized thermometer insertion depth, corrected to estimate core temperature. Animals recovered on the heating pad. Once sternal, they were placed in a heated recovery cage (floor temperature 35.7±4.4°C) for 30 (short) or 60 (long) min. Rectal temperatures were taken every 10 min during this period and every 30 min until 120 min postanesthesia. Within group, comparisons were with 1-way ANOVA for repeated measures (Dunnett's posthoc test). Between group, comparisons were with 1-way ANOVA (Bonferroni posthoc test). Normothermia was maintained in both treatment groups during anesthesia without significant differences between groups ($P > 0.99$). During postanesthesia warming in the long group, mean temperatures increased at 90 ($P = 0.03$) and 100 ($P = 0.008$) minutes, though hyperthermia (>38.6°C) was limited to one animal. Mean temperature in the short group did not differ from baseline during postanesthesia warming, with 1 hyperthermic animal (observed to be very active during recovery). After warming, normothermia was maintained until the end of the observation period in both treatment groups. A short warming period of 30 min effectively maintained core body temperature during recovery from isoflurane anesthesia.

P271 Comparison of a Supraglottic Airway Device with Blind Orotracheal Intubation in Rabbits

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Orotracheal intubation in rabbits is generally more difficult than in dogs or cats. Direct visualization of the glottis is limited by a fleshy tongue, narrow inter-incisor space, and small oropharynx. It was hypothesized that a rabbit-specific supraglottic airway device (SGAD) would be faster to place, provide a larger cross-sectional airway area, and cause less airway damage than blind orotracheal intubation (ET). Fifteen adult New Zealand white rabbits were randomized to receive SGAD or ET for general anesthesia. Each device type was placed by a single investigator. Animals were premedicated with IM dexmedetomidine (0.1 mg/kg) and midazolam (0.5 mg/kg) and anesthesia induced with IV alfaxalone (0.3 mg/kg). A CT scan of the head and neck was performed following premedication and after SGAD/ET placement. Outcome measures were time to device insertion, smallest cross-sectional airway area, airway sealing pressure, and histological score of tracheal tissue. Data were analyzed with a Mann-Whitney test. Unsuccessful ET placement occurred in 2 rabbits. Body weights were similar (ET; n = 6, 2.6 [2.3-4.5] kg. SGAD; n = 7, 2.7 [2.4-5.0] kg). Device insertion was faster ($P = 0.02$) with SGAD (33 [14-38] s) than ET (59 [29-171] s). Cross-sectional area was reduced from baseline (12.2 [6.9-13.4] mm^2) but similar between groups (SGAD; 2.7 [2.0-12.3] mm^2, $P = 0.003$, ETT; 3.8 [2.3-6.6] mm^2, $P = 0.001$). CT image evaluation revealed that the SGAD tip migrated into the laryngeal inlet in 6/7 rabbits. Airway seals were higher in ET (15 [10-20] cmH_2O), but not significantly (SGAD; 5 [5-20] cmH_2O, $P = 0.06$). Greater airway damage was present following ET (histological score 3.3 [1.0-5.0]), than SGAD (0.67 [0.33-3.67], $P = 0.03$). The rabbit-specific SGAD had several advantages over blind orotracheal intubation; however, the SGAD did not provide a larger cross-section airway area as expected. This requires further investigation.

P272 An Evaluation of the Effect of a Small, Positive Reinforcement Treat on Serum Chemistry in Long-Tailed Macaques (Macaca fascicularis)

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Serum chemistry is routinely assessed during nonclinical safety studies of experimental medicines. Such assays are typically done in the fasted state in *Macaca fascicularis,* as feeding may influence certain clinical chemistry analytes. At our facility, fasting is continued until after blood samples have been processed to serum and evaluated to ensure they are suitable for analysis. Consequently, technicians cannot provide food reinforcement at the primary collection, critical in maintaining a positive trusting relationship with the animals. This study was undertaken to evaluate whether a small food treat administered after blood collection would impact routine clinical chemistry values. Twenty cynomolgus macaques (10 male/10 female, 4.5-8.7 y) were assigned to study, fasted for 13 h, and had blood collected for serum chemistry while manually restrained. Ten animals (5/sex) were provided a small food treat (4 grapes) and the remaining 10 animals (5/sex) were maintained in the fasted state to serve as controls. All animals had additional blood collections at 30, 60, and 120 min after the first sample. Blood samples were collected into serum separator tubes, processed to serum, and analyzed on a chemistry analyzer for routine clinical chemistry analytes. Changes in mean clinical chemistry values relative to baseline values for both groups included decreases in glucose (statistically significant for males in both groups) and phosphorus (statistically significant for males in the treat group only) by 120 min, although decreased phosphorus was generally greater for animals in the treat group compared to controls. The glucose decrease often occurred earlier for control animals compared to animals given a food treat, suggesting possible delayed fasting-related glucose decreases associated with the treat. However, glucose differences between the groups were not significant. These results suggest that providing a small food treat to cynomolgus monkeys has no clear impact on most routine clinical chemistry parameters within 2 h and a negligible impact on glucose concentration that would be unlikely to affect the interpretation of this analyte.

P273 Proper Clipping and Marking of Dose Sites in Rats

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The proper clipping and marking of dose sites for certain dose routes, such as subcutaneous injections and dermal applications, are often critical for proper evaluation of dose response. Clearly identifying the dose site allows for injection or application of a test article to the desired location. Additionally, marking the dose site ensures the proper area is harvested for necropsy and histology purposes, as well as subsequent microscopic evaluation. This study was designed to assess the optimal frequency of clipping and method of marking to allow for concise harvesting of the dose site. We hypothesized that the most effective clipping and marking combination would be clipping and marking twice weekly while using a permanent marker with high-contrast ink. Two different shaving schedules and 2 different marking tools (standard permanent marker and a high contrast permanent marker) were compared using 8 rats/sex. Rats were shaved with a size 30 blade. The methods of marking the rats were drawing a cross on the rat's back to indicate quadrants and also adding a circle in one of the quadrants to indicate a bleb (subcutaneous application). The rats were photographed and observed over a 2-wk period using a scoring system for hair growth and visibility of marking to ascertain the ideal combination and yield the most effective result. However after the 2-wk period, while the twice weekly regimen was the most effective, the marking tool we used made no significant difference.

P274 Quantification of Aflibercept and Ranibizumab Efficacy in DL-2-Aminoadipic Acid-Induced Retinal Neovascularization and Vascular Leakage in Nonhuman Primates

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We sought to define the optimal strategy for grading and quantifying the retinal neovascularization and leakage induced by intravitreal (IVT) injection of DL-2-aminoadipic acid (DL-AAA) in the green monkey and to further validate the model by evaluating the comparative magnitude of response to IVT aflibercept and ranibizumab treatment. Both anti-VEGF drugs are current standards of clinical care for retinal neovascularization. DL-AAA was administered intravitreally at 1 dose of 5 mg bilaterally in 6 adult St. Kitts green monkeys (*Chlorocebus sabaeus*). Weekly ophthalmic examinations including slit lamp biomicroscopy, color fundus photography, fluorescein angiography, and optical coherence tomography were conducted up to 18 wk. Aflibercept, ranibizumab, or normal saline were injected intravitreally at 8 wk post-DL-AAA following randomization of eyes to treatment on the basis of week 8 leakage. The area and fluorescence intensity of retinal vascular leakage were quantified from the 1 and 3 min angiograms. A manual segmentation tool was used to identify the leakage area. Once the total leakage area was identified using the brush tool, we measured the area and fluorescence for the cumulative sum of the highlighted regions of interest within each of the angiograms. Both aflibercept and ranibizumab treatments attenuated the area and fluorescence intensity of retinal vascular leakage, starting from 1 wk, peaking around 4 wk, with gradual reoccurrence of leakage to a level less than the pretreatment condition at 10 wk post-administration. Treatment with aflibercept resulted in a 75% reduction in average leakage area in 3 min angiograms while a 50% reduction was observed in response to ranibizumab. The changes in area and intensity were similar between 1- and 3-min angiograms. But a complete inhibition of leakage was only observed at the 1-min angiogram interval in eyes treated with aflibercept. Measurement of the area and fluorescence intensity of DL-AAA-induced neovascularization and leakage provides an additional reproducible method for quantitative analysis of retinal vascular leakage. Both aflibercept and ranibizumab treatments significantly reduced retinal vascular leakage, with aflibercept exhibiting a more pronounced effect. Reference to the established behavior of these control articles in the DL-AAA test system and application of these quantitative methods will allow more robust screening of candidate anti-angiogenic compounds.

P275 Comparison of Anesthetic Effects of Alfaxalone and Isoflurane on Functional Connectivity in Rhesus Macaques (Macaca mulatta)

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Alfaxalone is a neuroactive steroid molecule registered for use in dogs and cats for both the induction and maintenance of anesthesia. It is suggested to be an optimal anesthetic in animal studies and has been increasingly used in nonhuman primates (NHP). We hypothesized that total intravenous anesthesia with alphaxalone would alter functional connectivity in the adult rhesus macaque (*Macaca mulatta*) brain (n=4, 9-13-y-old) compared to isoflurane inhalant anesthesia using a default-mode network (DMN). Alfaxalone was first given as an IM injection (5 mg/kg) followed by IV infusion (0.125 or 0.156 mg/kg/min) for 1 h. Anesthesia was then transitioned to ~0.8% isoflurane for an additional 1.25 h. Resting state functional MRI (rsfMRI) data were acquired using a multiband EPI sequence (TR/TE=1090 ms/25ms, spatial resolution= 1.5×1.5×1.5mm3). Resting state fMRI data were processed by FSL and AFNI. The averaged time course of rsfMRI signal in the posterior cingulate cortex (PCC) was used for seed-based correlation analysis separately. *Z* transformation was applied to the individual correlation maps to show normalized correlation maps. The averaged *z* values of connectivity between the PCC and the anterior cingulate cortex (ACC) or dorsomedial prefrontal cortex (DMPFC) were examined for statistical differences. The results demonstrated that high-dosage alfaxalone significantly reduced functional connectivity in the dominant DMN of the PCC-ACC ($p<0.05$) and the PCC-DMPFC ($P = 0.06$) compared to 0.8% isoflurane anesthesia. Also, high-dosage alfaxalone showed a stronger inhibiting effect than low dosage alfaxalone by significantly reducing functional connectivity in the PCC-DMPFC ($P = 0.05$) but not in the PCC-ACC. These findings reveal that alfaxalone suppresses neural activity more dramatically than isoflurane anesthesia in animal studies in a dose-dependent manner, suggesting such dose-dependent effects should be considered when choosing anesthetic protocols that examine neuronal function in neuroimaging.

P276 Campylobacter jejuni in Laboratory Zebra Finches (Taeniopygia guttata)

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For well over a decade at our institution, zebra finches (*Taeniopygia guttata*) have been used as models for research into song learning, as well as for development of transgenic finches. When birds arrive from commercial vendors and other academic institutions, they are placed in quarantine and tested for various viral, bacterial, protozoal, and ectoparasitic agents. Semiannual testing for enteric pathogens and ectoparasites is also performed on birds in the resident colony. Historically, animals in quarantine and in the colony have been colonized with *Campylobacter jejuni*, a gram-negative, microaerophilic bacteria commonly associated with both domestic and wild birds. Although the zebra finches are not clinically affected by *C. jejuni*, it is a known zoonotic pathogen that causes gastroenteritis in humans worldwide. Human transmission is predominantly foodborne and associated with consumption of contaminated poultry; however, humans can also become infected with *C. jejuni* from contact with reservoir animal species. Because *C. jejuni* carrier finches pose a risk to researchers and husbandry staff, a study was undertaken to investigate the epidemiology of *C. jejuni* strains present in the colony. Thirteen Campylobacter-like organisms were isolated by microaerobic culture. 16S ribosomal DNA sequencing was performed on 6 colony isolates from 2010, as well as on 4 from the current colony and 3 from quarantine animals from 2 different vendors. All isolates were confirmed *C. jejuni* based on 16S rRNA sequencing, and biochemical testing was performed on 12 of these 13 isolates. Biochemical analysis revealed that all 12 of the isolates tested were negative for gamma glutamyl transferase, but 11 were hippurate and esterase positive, 3 were urease positive, and 4 had the ability to produce hydrogen sulfide. Further molecular characterization using whole genome sequencing of these *C. jejuni* isolates and comparing them with *C. jejuni* human isolates will increase our understanding of the epidemiology of *C. jejuni* in this avian reservoir host.

P277 Intraperitoneal Injection of 70% Ethanol as a Method of Euthanasia in Zebra Finches (Taeniopygia guttata)

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Euthanasia methods for avian species as outlined in the 2013 AVMA *Guidelines for Euthanasia of Animals* are extrapolated from methods used in mammals. However, avian euthanasia requires special considerations due to anatomical differences from mammals. Further, these methods pose challenges to field researchers due to lack of equipment and limitations imposed by federal regulations. We determined if intracoelomic (IP_C) injection of 0.5mL of 70% of ethanol is an efficacious euthanasia method in zebra finches (*Taeniopygia guttata)*. Twelve cull zebra finches were block randomized by sex to receive an IP_C injection of either 0.5mL of 0.9% sodium chloride (saline), or 0.5mL of 70% ethanol. Saline was chosen as a control to assess parameters due to injection alone. Finches were video recorded for 5 min pre and postinjection and were observed until cessation of all movement (CAM). If CAM was not achieved by 300 s postinjection, finches were euthanized with CO_2, and control birds were euthanized with CO_2 at 300 s postinjection. Blinded and randomized retrospective analysis of multiple behavioral parameters, blood glucose measurement, gross necropsy, and histological analysis were performed. There was no significant difference in the time to loss of the righting reflex for finches that received ethanol (85s ± 8.0s) compared to those that received saline and then euthanized with CO_2 (73.8s ± 6.4s). Only 50% of the finches in the ethanol group achieved CAM by 300 s postinjection. Additionally, ethanol substantiated more negative behavioral outcomes. Blood glucose was significantly higher in finches that received saline (446.3mg/dL ± 27.0mg/dL) compared to finches that received ethanol (248.2mg/dL ± 10.7mg/dL, $p < 0.0001$). Gross necropsy and histological analysis revealed no observable artifact from either substance. Overall, we conclude that IP_C injection of 0.5mL 70% ethanol is not an efficacious method of euthanasia in zebra finches. Further studies are required to refine the dose and volume of ethanol to induce euthanasia in zebra finches.

P278 Multimodal Analgesia for Mice Undergoing Surgical Procedures

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Most analgesics used to treat mice postsurgical procedures are either nonsteroidal anti-inflammatory drugs (NSAID) or opioids such as carprofen (5mg/kg) and buprenorphine (0.05 mg/kg), respectively. We assessed the efficacy of combining these drugs by analyzing standardized pain facial expressions and nesting behaviors of 56 FVB/J female mice before and after ovariectomy surgery. Mice (n=8) were assigned to the following groups: control (isoflurane only); NSAID or opioid; NSAID or opioid plus a local anesthetic (bupivacaine (8mg/ml)); or NSAID, opioid, and local anesthetic. Welfare and analgesic efficacy was estimated by recording body weights, time-to-integrate to nest (TINT) tests, nest complexity, and mouse grimace scores (MGS) before and after surgery. The MGS measures physical characteristics including orbital tightening, nose bulge, cheek bulge, ear position, and whisker changes in mice, while TINT tests analyze innate nesting behaviors that are indicators of mice wellbeing. Nest complexity is measured by the height and composition of nesting material 24 h after being provided to each mouse pair. Each side of a nest is measured as if it were a square, and scores are given based on wall height with 0 representing undisturbed material, and 5 representing walls taller than ½ the height of a dome. Behavioral tests were used to score and monitor mouse wellbeing throughout the study. Our results showed mice treated only with an NSAID had significantly higher MGS for 24 h following surgery ($P < 0.0001$) compared to all other groups. There was no statistical difference in MGS or TINT scores of mice receiving various combinations of multimodal analgesia. In addition, there were no significant differences in nest complexity, TINT test, or body weight between groups. The results suggest that a multimodal analgesic approach can provide greater pain relief than carprofen alone during postsurgical recovery. Exclusive administration of buprenorphine can also provide similar pain control compared to multimodal analgesic regimens. Further investigation is recommended to validate study results.

P279 The Effects of Isoflurane on Behavioral and Stress Response in Mice: A Comparison of 3% and 5% Induction Concentrations

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The use of modern inhalational anesthetics compared with injectable agents enables animals to regain consciousness more rapidly, reducing stress and risk of injury. Isoflurane (ISO) is the most commonly used method of general inhalational anesthesia for mice in biomedical research. However, a wide range of recommendations from institutions and IACUC guidelines suggest various induction concentrations at 3%, 4%, and 5%. Suitability of low and high concentrations for induction of anesthesia in mice was investigated to determine if 1 concentration is more or less stressful. Male and female mice (n=128) in 4 strains, B6, NSG, BALB/cJ, NU/J, were randomly assigned to the exposure of 2 of the concentrations: 3% and 5% with 1L of oxygen flow rate. Mice were individually placed into the anesthetic induction chamber and ISO gas was piped into the side of the chamber until a surgical plane of anesthesia was reached. The latency to and duration of ataxia, time to recumbencey, number of jumps or pawing of the face, pattern of respiration, and the time to a loss of response to a noxious stimulus (toe pinch) were recorded. Blood samples were taken to evaluate catecholamine levels as a biochemical marker of stress. Induction times were found to be shorter with a higher induction rate at 5% as compared with 3%, with no significant differences in behavioral and biochemical responses in mice overall. However NSG male mice had significantly higher levels of norepinephrine compared to other strains regardless of induction percentage despite no significant differences in behavioral response. This study demonstrates that ISO at 3% and 5% are both suitable rates of inductions for mice. Higher induction rate was tolerated among the four strains with the exception of the NSG male mice. In summary, the study indicates that in general, lower induction rate at 3% ISO is better tolerated in mice.

P280 The Longevity of Sterility and Stability of Diluted Carprofen in a Multidose Vial in the Laboratory Animal Setting

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Using compounded multidose vials (cMDV) is a common practice in the laboratory animal setting, where medications often are diluted to provide appropriate doses to rodents. However, bacterial contamination of MDV has been well established in both the human and veterinary medical literature. For this study, we created 14 cMDV by diluting carprofen into sterile water (dilution, 1:10) and stored 6 cMDV each at 5 and 24 °C. The stoppers of the cMDV were not cleaned with alcohol, and all were punctured twice daily for 28 d. The sterility of the diluted carprofen was evaluated by assessing bacterial growth on days 0, 7, 14, 21, and 28 and by testing for bacterial endotoxin on days 0 and 28. We used liquid chromatography–tandem mass spectrometry to assess the stability of 2 cMDV, with each cMDV being divided into the 2 storage-temperature subsets for days 0, 7, 14, 21, and 28. Neither bacterial contamination nor endotoxin was detected, and drug stability was stable over the 28 d. We suggest that with pragmatic techniques, such as secondary containment and consistent use of new needles, the contents of cMDV can remain sterile and stable for 28 d.

P281 A Systematic Approach to Improve Superovulation Yields in C57BL/6NCrl mice

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Three to 4-wk-old C57BL/6NCrl (B6N) mice are often observed to have lower oocyte and zygote yields compared to published results for other C57BL/6 substrains. This study aimed to determine which controllable variables in our superovulation protocol are associated with changes in plug rates, oocyte, and zygote yields. We compared our 3 to 4-wk-old resident B6N (n=83) and imported B6N (n=62) protocol to both 4 to 5-wk-old resident female B6N (bred no longer than 2 generations from vendor founder colony, n=8) and 4 to 5-wk-old nonresident B6N mice imported at 3 to 4 wk of age (n=5). All mice receive either 5 or 7.5 IU pregnant mare serum gonadotropin (PMSG) by IP injection followed 47 h later by 5 or 7.5 IU of human chorionic gonadotropin (HCG) IP. Immediately after HCG injection, females were placed either with a single-housed (commercial cage, n=15) or duplex-housed (commercial cage with a metal divider) housing a proven B6N stud male on each side (n=83). Copulatory plugs were checked the following morning (17 h after HCG injection). Between these 2 experimental groups of mice we compared plug rates, oocyte, and zygote yields, Plug rates were significantly higher in resident B6N mice (62%) compared to imported B6N mice (55%), and in all females that were mated with singly housed (76%) males compared to males in duplex housing (62%). All other performance parameters were not statistically significant. The decreased yield from recently imported mice is attributed to recent shipping stress. In conclusion, although C57BL/6NCrl mice might have lower average egg yields compared to other strains, shipping stress was the variable that has negatively affected our superovulation yields the most.

P282 Comparison of 2 Blood Sampling Methods in Mice to Increase Animal Welfare and the Reliability of Experimental Results

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There is a strong need to gain systematic knowledge about the potential impact of routine procedures on laboratory animals to ensure animal welfare and reliability of experimental results. Blood sampling is a frequently used procedure within laboratory animal research. Two of the most common anesthesia-free techniques to obtain blood in mice are the saphenous venipuncture and submandibular bleeding. It is common practice that these methods are applied repeatedly to 1 individual. The impact of both techniques, either performed singly or repeatedly, on the animal's welfare is not fully known yet. We directly compared the effects of both sampling methods on physiological stress responses, behavior, tissue damage, and procedure duration in 2 frequently used mouse strains: C57BL/6 and BALB/c mice (n=45/strain). The mice were randomly assigned to 3 treatment groups (saphenous venipuncture, submandibular bleeding, control) of 15 mice/strain and were sampled weekly (80 ul/ blood sample) during 4 wk for the experimental groups and 5 wk for the control group. To evaluate the acute physiological stress response, plasma corticosterone levels were determined. As an indicator of chronic stress, body weights were recorded once a week and the thymus was weighed after necropsy. Behavior was recorded using video cameras in front of the cages and afterwards analyzed by a well-trained observer using event logging software. Tissue damage was evaluated by scoring pre-and postmortem hemorrhages and hematomas at the injection site and by measuring alanine transaminase, aspartate aminotransferase, and creatine kinase plasma levels. Submandibular bleeding appeared to be less time consuming ($P<0.05$) while providing higher sample volumes. However, this method led to increased acute stress responses in C57BL/6 mice ($P=0.03$). All animals of both strains undergoing this procedure expressed more pain-related behavior ($P<0.05$) and the extent of tissue damage at the injection site was significantly higher when compared to the saphenous venipuncture ($P<0.05$). Thus, in order to avoid the undesirable effects of stress on experimental outcomes and to reduce the extent of tissue damage, saphenous venipuncture should be preferred above submandibular bleeding whenever possible.

P283 Germ Cell Specific Apoptosis by Clusterin on Heat-Induced Canine Testis

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Clusterin (CLU) is a multifunctional heterodimeric glycoprotein that was first identified as a component of the fluid from the ram rete testes. It has been suggested that clusterin may be involved in a variety of functions like cell-cell interactions, sperm maturation, and cell survival and apoptosis. In the testis, CLU is strongly expressed especially in Sertoli cells and several studies have been reported the induction of clusterin expression following heat stress in rodent, but not in canines. The objectives of this study were to investigate the canine germ cell apoptosis by clusterin after heat exposure. Artificially unilateral cryptorchidism was induced in 3 mature male dogs. One testis was performed by surgically returning the testis and epididymis to the abdominal cavity and the other testis remains in scrotum as a control. Thirty days after the induction of cryptorchidism, the diameters of the seminiferous tubules of the in the heat-exposed testis significantly decreased compared to those of the scrotal testis by 45% in the histological appearance. Immunohistochemistry results showed that PGP9.5 positive undifferentiated spermatogonia were significantly reduced after heat exposure. In contrast, real-time PCR showed the expression of clusterin mRNA were significantly up-regulated in the heat-exposed testis as compared the control. Western blot analysis indicated a marked increase in the expression of clusterin and apoptotic markers in cryptorchid testis. These results suggest that clusterin expression of canine testis might be used as a potential biomarker of testicular heat stress and abnormal spermatogenesis.

P284 Cytotoxic Escherichia coli Strains Encoding Colibactin and Cytotoxic Necrotizing Factor (CNF) Colonize Laboratory Macaques

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Escherichia coli is normal flora found in the human and animal gut. Most *E.coli* strains are commensal but some strains carry virulence factors that enable them to cause intestinal and extraintestinal infection. Colibactin, encoded by a genomic island (pks island), and cytotoxic necrotizing factor (CNF), encoded by the cnf gene, are genotoxic and can modulate cellular differentiation, apoptosis, and proliferation. Some commensal and pathogenic pks+ and cnf+ *E.coli*strains have been associated with inflammation and cancer in humans and animals. In this study, *E. coli* strains encoding colibactin and CNF were identified in macaque samples. We performed bacterial cultures using rectal swabs and extraintestinal samples from clinically normal macaques. A total of 239 *E. coli* strains were isolated from 269 macaques. The strains were identified biochemically and select isolates were serotyped. Specific PCR for pks and cnf1 gene amplification, and phylogenetic group identification were performed on all *E. coli* strains. Among the 239 isolates, 41 (17.2%) were positive for pks only, 19 (7.9%) were positive for cnf1 only, and 31 (13%) were positive for both pks and cnf. One hundred and forty-eight (61.9%) *E. coli* isolates were negative for both genes. In total, 72 (30%) were positive for pks genes and 50 (20.9%) were cnf1 positive. Both pks+ and cnf1+ *E.coli* strains mainly belonged to phylogenetic group B2 including $B2_1$. Colibactin and CNF cytotoxic activities were confirmed using a HeLa cell cytotoxicity assay in select isolates. No cnf 2-positive isolates were detected. Macaque *E. coli* serotypes included O88:H4, O25:H4, O7:H7, and OM: H14. The genomic data supports the presence of virulence factor and antibiotic resistance genes in rhesus macaque *E. coli* isolates. Our findings indicate that colibactin and CNF-encoding *E. coli*colonize laboratory macaques and can potentially cause clinical and subclinical diseases that impact macaque models.

P285 Determining the Optimal Tumor Fragment Size for Cryopreservation

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Cryopreservation is the process of using low temperature to preserve cellular integrity of various tissues. This technique is used for developing patient-derived xenograft (PDX) models as platforms to investigate cancer therapies. PDX models are created by subcutaneously injecting a patient tumor into an animal model, such as mice. In order to store viable tissue samples at different passages or generations for future propagation, cryopreservation is required. Samples can be cryopreserved at varying sizes, but according to current methodology, smaller fragments are more optimal because their increased surface area maximizes exposure to the media. This would theoretically allow for better cryopreservation and more successful tissue recovery. The goal of this optimization study was to determine if cryopreserving at different sizes would lead to significant differences in tumor engraftment rate and tumor growth after tissue recovery and reimplantation. Method 1 required 5 small fragments (enough to trocar 5 mice) be cryopreserved and method 2 required 1 larger fragment (also enough to trocar 5 mice) be cryopreserved. Two tumor models were cryopreserved using these 2 methods at 2 time intervals resulting in 40 mice being injected. After 3 mo and 6 mo of the samples being cryopreserved, each model was thawed, minced, and then engrafted into 40 NOD.Cg-Prkdcscid Il2rgtm1Wjl/SzJ (NSG) mice using a 13 gauge trocar needle. Tumors were monitored weekly and the measurements were recorded once their volume reached 50mm^3. Data collected comparing the 3-mo and 6-mo time points suggested that there were no significant differences in tumor engraftment rate or tumor growth rate between method 1 and 2. It is possible that cryopreserving 1 larger fragment to engraft 5 mice would be the more efficient method due to it requiring less preparation.

P286 Can Lavender Essential Oils Reduce Distress in Mice During CO_2 Euthanasia?

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CO_2 euthanasia has been shown to induce varying levels of distress in mice. We sought to refine the euthanasia process to minimize this distress. Lavender essential oils have been shown to reduce anxiety in mice. Therefore, we hypothesized that introduction of lavender essential oil prior to CO_2 euthanasia could potentially reduce distress. Forty C57BL/6 background mice, otherwise slated for euthanasia due to lack of utility, were categorized by age and sex and randomly assigned to 1 of 3 experimental olfactory conditions: an unmanipulated cotton square (control); a cotton square treated with 5% lavender essential oil; or a neutral olfactory stimulus, 5% almond extract. Each mouse was placed in a clean cage with their randomly assigned cotton square located out of reach. Video recording began with the placement of the cotton square, included 2 min before CO_2 was added at a 20% displacement rate, and continued until respiration stopped. The videos were randomized and scored by a blinded observer using a behavioral ethogram. The ethogram evaluated 3 categories of behavior: active (walking, running, rearing, grooming); exploratory (sniff, climb, dig, touching the CO_2 entrance point); and stress (jump, kick, ataxia, freeze, avoid, sprint.) Analysis demonstrated that active and exploratory behaviors significantly decreased and stress behaviors significantly increased after CO_2 was introduced across all conditions, ages, and sexes. Before CO_2 was started, there were no significant differences between lavender and control condition for any of the behaviors. Similarly, after CO_2 started there were no significant differences between the 3 conditions for active or exploratory behaviors. However, there was a significant increase in stress behaviors in the lavender condition as compared to the control condition; though the lavender condition did not differ significantly from the neutral odor condition. Through this study, we validated a novel ethogram for recognition and assessment of stress behaviors associated with CO_2 euthanasia. Additionally, inhaled lavender essential oil introduced just prior to CO_2 euthanasia does not appear to be beneficial.

P287 Optimization of the Intratracheal and Intranasal Instillation Procedure in Ferrets

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The route of delivery is important to the efficacy of certain drugs and vaccines. Intratracheal (IT) and intranasal (IN) instillation may be effective routes to deliver test agents to the airway (lungs) or the mucosal immune system (sinuses) in ferrets, respectively, which could be useful in vaccine development. To determine to effectiveness of each route, 2 groups of 3 ferrets were sedated with atropine sulfate (0.1 mg/kg, IM), dexdomitor (0.06 mg/kg, IM), and Isoflurane (0.5-5%, INH), and placed in a supine position on an incline rack for IT, and laterally recumbent position (nares up) for IN administration. Group 1 received 0.3 mL of test agent IN (administered slowly during animal inspiration) and was kept

laterally recumbent to ensure the dose reached the mucosal immune system. Group 2 was dosed (0.3-0.5 mg/kg) with test material using a 15 GA x 78 mm cannula placed into the upper airway, followed by a bolus of air (1-2 mL). To ensure the test agent reached the airway, animals were kept at an incline for up to 15 m. This was also used to aid in respiration and recovery. Computerized tomography (CT) and positron emission tomography (PET) imaging, along with digital photography demonstrated the resulting localization of test material following IN and IT administration. It was determined that IN administration is effective at targeting the mucosal immune system located in the sinuses and IT is an effective method to deliver material to the airways of the lungs.

P288 Oral Gavage with Klebsiella pneumoniae to Establish Colonization in Neonatal Mice

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Antibiotic-resistant bacteria are on the rise in the United States. The superbug *Klebsiella pneumoniae* carbapenemase (KPC)-producing bacteria is a gram-negative, rod-shaped bacterium that can be found in the normal flora of the mouth, skin, and intestines of humans. As an opportunistic pathogen, it is a leading contender for bacterial pneumonia and other bacterial infections such as blood, kidney, and urinary tract in the clinical setting. Studying *K. pneumoniae*intestinal colonization in a mouse model is challenging because the mouse's normal gut microbiota can prevent this bacterium from colonizing this site. Developing a robust method to colonize the mouse intestine with *K. pneumoniae*is therefore a key first step towards studying *K. pneumoniae* pathogenesis in murine model. Here we have shown that dosing between 6-8 litters, each litter between 6 and 9 pups, of Foxp3-K1 neonatal mice between days 0-3 after birth while using a 22G gavage needle at a dose concentration of 10^6 colony-forming units of *K. pneumoniae* yielded the best conditions to allow for successful colonization of the neonate. This early postpartum gavage method yielded a greater response within the gut than compared to those neonates who were dosed between 5-7 dof age as we suspected that the innate immunity from the mother passing to the pup was playing a key role in preventing colonization. Gavaging neonatal mice is itself a challenge as it differs than the regular feeding method in which materials are administered directly into the stomach. Successful placement of the feeding needle into the pharynx of the animal is vital for dosing but also presents the opportunity for mortality due to inhalation. Furthermore, we found that using a 22G straight gavage feeding needle was the best when performing the procedure as we felt it fit the pharynx of the pup more naturally. We then followed this colonization on each mouse pup by performing qPCR on the feces at least once a week to show that *K. pneumoniae* can persist in the neonatal intestine for up to 3 wk after birth with no indication of this bacterium being suppressed by the mouse's normal gut microbiota. With this animal model, we can now begin a broader range of intestinal infection research on *K. pneumoniae* to gain a deeper understanding of this bacterium and explore novel ways for clinical treatment.

P289 Comparison of the Effect of Flutamide and Letrozole Therapies on Tumor Progression and Steroid Hormone Secretion in Human and Canine Inflammatory Breast Cancer Cell Lines

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Flutamide is an antiandrogen therapeutic strategy proposed for the treatment of inflammatory breast carcinoma (IBC) and canine inflammatory mammary cancer (IMC) which carry a poor prognosis. Letrozole is an antiaromatase therapy used for the treatment of estrogen receptor (ER) positive breast cancers. We determined the effects of 2 therapies in IBC and IMC cell lines on tumor progression and estrogen and androgen secretion. IPC-366 (canine inflammatory mammary cancer cell line) and SUM149 (inflammatory breast cancer cell line) were tested. Female BALB/cJHan Hsd-Prkdcscid (SCID) mice were divided into 7 groups (n=5) for each cell line: (1 control; 3 flutamide treated (5 mg/kg, 10 mg/kg, and 15 mg/kg of flutamide) and 3 letrozole treated (1 mg/kg, 5 mg/kg, and 10 mg/kg of letrozole). When tumors developed, mice received SQ flutamide or letrozole 3 times a week for 2 wk. At the end of treatment (or at $1.5cm^3$ endpoint), mice were euthanized and metastasis was evaluated by gross necropsy. Steroid hormones determination in tumor homogenates (testosterone (T), dihydritestosterone (DHT), 17β-estradiol (E2), and estrone sulphate (SO4E1)) were measured by EIA. In vivo flutamide showed a reduction of around 55-65% (IPC-366) and 50-60% (SUM149). In mice treated with letrozole a reduction of tumor was not found, but tumor growth was slowed. Percentage of metastasis decreased in flutamide treatment compared with control group and on letrozole treatment no metastasis were found. In tumor homogenates of flutamide treated mice, a dose dependent elevation of T and SO4E1 levels and a dose dependent decrease on DHT and E2 levels were found. Letrozole treated mice saw a dose dependent significant reduction in estrogen levels (SO4E1 and E2). T levels were similar in all doses and control group, although DHT levels increased in a dose dependent manner. We conclude that IPC-366 and SUM149 treated with flutamide significantly reduced tumors but letrozole does not, probably because estrogen levels are consumed by the tumor for maintenance. However, letrozole treatment prevents metastasis, possibly due to the increased DHT levels in tumor homogenates. Antiandrogen drugs could be an effective treatment for canine and human IBC and triple negative breast cancer and antiaromatase drugs could be useful for metastasis prevention.

P290 Effects of Some Environmental Factors on Reproductive Performance of Laboratory Mice

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With an increasing need to constantly produce more transgenic strains, it has become essential to focus even more on production and breeding efficiency with the aim of producing more strains with fewer mice. This is both a practical and an ethical issue. Mice are commonly housed at 22 °C, which is significantly lower than their thermoneutral zone. But, when given a choice, mice often seem to prefer higher ambient temperatures. Furthermore, novel bedding and nesting materials are constantly sought with the aim of enriching the cage environment. Additionally, phytoestrogens present in diet disrupt and affect reproduction in different ways. We studied whether the impact of higher ambient temperature, novel bedding material (cotton cloth), and different dietary phytoestrogen levels would improve reproductional response in embryo donors, as well as in foster females used for producing transgenic mice. The results are combined from 3 separate studies, out of which 2 are already published. The main hypotheses were that high temperature, cotton cloth as bedding, and low phytoestrogen would improve reproductional endpoints. We focused on pregnancy rates, as well as embryo and sperm yield and quality as indicators of successful reproduction. Thereby we could demonstrate that ambient temperatures of up to 28 °C can be tolerated by mice without an adverse effect on their early reproductive fitness. Also, our data show that the shift from 28 °C to 30 °C results in a significant drop in both male and female reproductive fitness (n=84 experimental days / n=440 mice). Significantly more pregnancies were observed in the wooden chip bedding compared to cotton cloth bedding (n=116 mice). High phytoestrogen content in mouse diet seem to benefit early embryology (high yield and good quality embryos), but conversely yielded the lowest number of pups born (n=57 experimental days / n=358 mice). We conclude that higher ambient temperatures than currently recommended could be used when producing transgenic mice. In addition, we demonstrated that a cotton cloth cannot be recommended as a sole replacement for bedding and nesting material. Additionally, high levels of phytoestrogens in mouse diet may not be that universally disadvantageous than often stated, and in certain cases may even support good reproduction.

P291 Evaluation of Zinc Gluconate as a Nonsurgical Sterilization Method in Rhesus Macaques (Macaca mulatta)

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It is widely known that rhesus macaques (*Macaca mulatta*) are prolific breeders in captivity and in the wild. Therefore, overpopulation can be problematic in research and feral populations. Currently, the most common contraceptive methods are hormonal control in female macaques and vasectomies in males. These methods each come with their own innate challenges, foremost being the alteration of necessary hormonal maintenance. In this study, we assessed the use of zinc gluconate as a novel, nonsurgical alternative to male contraception in 12 rhesus macaques. Zinc gluconate is an FDA-approved product for dogs that is given as a one-time, testicular width dependent, intratesticular injection to cause permanent infertility while theoretically sparing the testosterone producing Leydig cells of the testis. Complete blood counts, serum biochemistry analyses, testosterone levels, and testicular widths were evaluated at the time of injection and either 1 wk, 1 mo, 2 mo, or 3 mo post injection, and showed no notable changes. Daily post injection observations noted transient scrotal enlargement in 8 out of 12 animals but no indications of pain. Full necropsies, including testicular histopathology, were performed at study endpoints. Although some portion of every testis had evidence of seminiferous tubule loss, normal spermatogenesis was present in 22 of the 24 testes. Therefore, chemical castration with zinc gluconate is not an effective sterilization method in rhesus macaques.

P292 Evaluating Diastolic Dysfunction in the Sheep Model (Ovis aries) following Smoke Inhalation Injury with Echocardiography

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Diastolic dysfunction contributes to approximately 44% of total heart failures in the United States. Diastolic function is the ability of the heart to relax and fill, accepting blood into the heart chambers. Once there is a disturbance in ventricular relaxation, diastolic dysfunction occurs. Four different progressive patterns present during the abnormal relaxation of the heart: normal, impaired relaxation, pseudonormal, and restrictive cardiomyopathy. After smoke inhalation injury to the thermally injured patient, a cascade of pulmonary pathologies develop. These changes can cause serious sequelas including left ventricle diastolic dysfunction. To characterize the pathophysiology of diastolic dysfunction following smoke inhalation injury, we used the ultrasound modality by performing serial echocardiograms. After the smoke inhalation injury, an echocardiographic study was performed on 6 sheep (*Ovis aries*) at baseline preinjury, then at 24 and 48 h postinjury. The left lateral transthoracic parasternal and apical views were performed using a 3.0 MHz transducer. The LV diastolic and systolic diameters and volumes were recorded. Bi-dimensional left ventricle long and short axis views were consistently attained. Serial Doppler evaluation of the mitral valve inflow and tissue Doppler of the lateral mitral annulus were assessed. Velocities of Mitral E, A, and Tricuspid annular plane systolic excursion (TAPSE) were affected. In summary, the smoke inhalation injury can contribute to left heart diastolic dysfunction. Historically, the use of echocardiograms can accurately support a diagnosis of diastolic dysfunction. We used serial echocardiograms to evaluate mitral valve and tricuspid changes that developed over 48 h postinjury. Various profound cardiac changes were observed and documented including diastolic dysfunction.

P293 Maternal Infection with Bovine Viral Diarrhea Virus Impairs Thymic Gene Expression in the Bovine Fetus

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Bovine viral diarrhea virus (BVDV) infection of bovine fetuses in the first 125 d of pregnancy results in a persistently infected (PI) animal which is the main source of infections in cattle populations causing significant economic losses to the cattle industry worldwide. The immune mechanisms that lead to this immunotolerant state are not well defined. Fetuses respond to BVDV infection on day 75 of gestation with an innate immune response by day 82, and then express IFN-g mRNA in the thymus by day 89, with an upregulation of IFN-g protein in fetal blood by day 97. This indicates activation of a fetal adaptive immune response. Subsequently, BVDV titers in fetal blood decreased at least 10-fold; however, BVDV was not completely eliminated and persisted at low, but significant levels. We hypothesized that there is a defect in the development of the adaptive immune response in BVDV-infected fetuses. To clarify the steps of the adaptive immune response that might be activated by fetal PI, total RNA was extracted from fetal thymuses at day 89, 97, and 190 of gestation. Genes important in T cell differentiation and development including LMP2, CD4, and CD8 were quantified by qRT-PCR. CD8 and CD4 mRNA concentrations were significantly downregulated ($P \leq 0.05$) in BVDV-infected fetal thymus at 14 and 115 d (89- and 190-day gestation, respectively). Gene expression of LMP2, a subunit in the 20S proteasome core implicated in processing foreign proteins to peptides, significantly decreased ($P \leq 0.05$) at days 97 and 190 in PI compared to control fetal thymus. Persistent BVDV infection may initiate fetal adaptive immune responses, which are not fully activated over time because of immunotolerance caused by inhibition of antigen processing through inhibition of the proteasome and antigen presentation/recognition through downregulation of CD8 and CD4 T cells. Longer-term consequences of fetal PI remain to be determined in context of postnatal impaired immune responses to secondary infections.

P294 In Vitro Analysis of Psoriasis Linked CARMA2sh Gene in Keratinocytes and Mouse Embryonic Stem Cells towards Developing a Murine Transgenic Model

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Psoriasis is a debilitating skin disease affecting approximately 23% of human population. Genome-wide association studies have identified more than 40 susceptibility loci for psoriasis. CARMA2 proteins play a major role in regulating activation of NF-kB, which controls inflammatory response, cell survival, and proliferation. Missense mutations in the CARMA2 gene have been shown to dominantly transmit the psoriatic trait with high penetrance. It has been identified that CARMA2sh induces activation of NF-κB and this requires the function of another CARD-containing protein, BCL10 and TRAF2. Recently a novel CARMA Inhibitory Kinase (CIK) which inhibits the ability to induce NF-kB was identified; however these molecules are not tested in Human Primary Keratinocytes (HEK). We attempted to study the function of CIK and its associated molecules by in vitro and in vivo models. Our objective is to investigate the activity of CIK on HEK cells expressing wild and mutant CARMA2sh forms. Standard culturing method was used to maintain the normal human epidermal keratinocytes (HEK) cells. Active or inactive forms of CIK and CARMA2sh expression vectors were constructed. Transformation in DH5α *E.coli* cells was followed by transfection of respective constructs in HEK cells. Expression levels of target gene were analyzed by real-time PCR, immune blot, and immunohistochemistry. To confirm the target gene in mouse embryonic stem (ES) cells, generation of CARMA2sh mutant associated with psoriasis (Gly117Ser and Glu 138Ala) were created by site-directed mutagenesis and generating transgene via homologous recombination method. The gene targeting constructs electroporated into ES cells. Targeted ES cell clones confirmed by PCR and southern blot. Positive clones were microinjected into blastocysts and implanted into 10-15 pseudo-pregnant females. It has been observed that with in vitro analysis, active form of CIK significantly reduced the expression of mutant CARMA2sh gene in HEK cells. The gene targeted positive clones were successfully electroporated and expressed in mouse ES cells. Future studies will investigate the effect of CARMAsh RNA mediated knockdown CIK on the activation of NF-kB targeted genes and signal transduction pathways that control cell death and proliferation.

P295 Determination of Optimal Administration and Efficacy of Glucose Supplementation in Mice following Roux-en-Y Gastric Bypass Surgery

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An effective treatment for obesity in humans is Roux-En-Y Gastric Bypass (RYGB) surgery. Mouse models of RYGB surgeries aid in ongoing investigations of energy homeostasis and the mechanisms behind obesity. Hypoglycemia following RYGB surgery in mice is a significant adverse event, which is one factor to mortality rates up to 70%. Although glucose homeostasis has been studied in diabetic mouse models, the effect of postoperative hypoglycemia and glucose supplementation is not well documented. We characterized blood glucose kinetic profiles for SQ and

oral/transmucosal (OTM) dextrose administration and evaluate efficacy of dextrose supplementation in hypoglycemic mice following RYGB surgery. We hypothesized that morbidity and mortality would decrease with glucose supplementation. For phase 1, 16 C57BL/6 mice were administered SQ and OTM dextrose with a 5-d washout period between treatments. Blood glucose concentrations were measured over 3 h postdextrose administration. For phase 2, 16 different mice underwent RYGB surgery. A single dose meloxicam was administered prior to surgery and isoflurane was used for induction and maintenance of anesthesia. Once ambulatory mice received SC buprenorphine and sustained-release meloxicam for postoperative analgesics. Blood glucose measurements were collected prior to surgery and 3x/d for 3 d following surgery. Hypoglycemic (<60 mg/dL) animals were randomly allocated to either group 1) dosed (D) with 1 mL 5% dextrose SQ when hypoglycemic or 2) not dosed (ND). Pain assessment via grimace scale was recorded for 3 d following surgery. Mice were monitored for 3 wk to assess activity levels, fecal output, and mortality. Phase 1 results determined SQ and OTM routes had similar kinetics with no significant adverse effects. The SQ was more reliable, and OTM variability is suspected to be from inefficiency in transmucosal administration. During phase 2, 14 of 16 mice became hypoglycemic following surgery. During postoperative monitoring, 7 mice died (44% mortality), 2 from hypoglycemia (1 D group, 1 ND group). No difference related to mortality could be determined due to a small samples size; however, grimace scale assessment indicated greater discomfort in the ND group. The increased score may be related to the lethargy and the poor hypoglycemic state, rather than solely related to pain. Glycemic control in the RYGB protocol is recommended to minimize the occurrence of hypoglycemic events and decrease morbidity and mortality.

P296 Characterization of Reproductive Tract Infections following Experimental Infection with Chlamydia muridarum in Female C57BL/6J Mice

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Chlamydia trachomatis infections are an important sexually transmitted infection in women that can cause oviduct inflammation and subsequent infertility. We investigated whether intravaginal infection of *Chlamydia muridarum*, a related mouse adapted pathogen, would induce upper genital tract infection and lesions in C57Bl/6J mice that could serve as a model of chlamydial pathobiology. Sixty 4-wk-old female mice were infected intravaginally with 20uL of $3.0x10^5$ IFU of *C. muridarum*. Vaginal wash and swabs were taken at various time points postinfection to determine cytokine/chemokine profiles (multiplex assay) and bacterial load (qPCR and IFU). Mice (n=10) were necropsied at weekly intervals for 6 wk and reproductive tracts were prepared for histopathology. Changes in cytokine levels correlated with reproductive tract inflammation and infection levels. Twenty-three of the detectable 28 cytokines in the vaginal lavage were significantly elevated 2 days postinfection, including the pro-inflammatory cytokines IL-6, IFNa and IFNb. By 4 d postinfection the significantly elevated number of cytokines was 19/28 with IFNg, IL-1a and b, MIP1a and b, TNFa, GMC-SF, IL-10, IL-15, IL-17, IL-6, KC, LIX, MCP1, and IP10 peaking at this time point. By 10 d postinfection the inflammation in the vagina is resolving with only 4/28 significantly elevated cytokines (IP-10, MIP1a, MIC, and IFNg). Marked inflammation of the tract occurred within 48 h of bacterial instillation, resulting in pyosalpingitis at 1 wk postinfection. Hydrosalpinx and other tissue changes consistent with chronic inflammatory changes ensued over the time course of the experiment (6 wk). Intravaginal infection of C57Bl/6J mice rapidly results in acute inflammatory changes throughout the entire reproductive tract and results in a high incidence of hydrosalpinx following oviduct inflammation. Vaginal washes and swabs are an effective means to quantitatively monitor bacterial load inflammation and severity of infection in this model.

P297 Establishment and Utility of Antithrombotic Efficacy and Bleeding Risk Assessment Models in Cynomolgus Macaques (Macaca fascicularis)

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Antithrombotic drugs play a critical role in the management of thrombotic pathologies. Comprehensive evaluation of novel drugs is essential for preclinical efficacy and risk characterization. The need for nonhuman primate (NHP) models is high as NHP coagulation expression patterns lend greater translational potential clinically. The models of mixed arterial and venous thrombosis (arteriovenous shunt (AV-shunt)) and arterial specific thrombosis (ferric chloride (FeCl3)) and a template bleeding time (T-BT) model were selected for development in NHPs for evaluating antithrombotic efficacy and bleeding liability. Cynomolgus macaques were anesthetized and instrumented with femoral artery and vein catheters for AV-shunt studies or, the carotid arteries with flow probes for FeCl3 studies. AV-shunt NHPs underwent sequential dose administration using a 4 shunt paradigm/NHP to evaluate shunt thrombus stability and sensitivity wherein resulting shunt thrombus weights measured test agent efficacy. FeCl3 study NHPs underwent dose administration followed by arterial injury with topical 50% FeCl3 under continuous Doppler ultrasound monitoring of blood flow wherein time to occlusion measured test agent efficacy. Template BT tests were conducted in buccal mucosa, finger pad, and tail to characterize bleeding risk. The AV-shunt model showed consistent shunt thrombus generation in vehicle treated NHPs (n=3) and, was validated based on sensitivity to apixaban, a direct factor Xa inhibitor, wherein thrombus weight reduction was observed following apixaban treatment (n=3) compared to vehicle including apixaban titration (0.0015-0.015 mg/kg/hr,IV) which resulted in a dose dependent reduction in thrombus weight. The FeCL3 model, showed that arterial exposure to 50% FeCl3 for 5 min consistently resulted in sustained thrombus occlusion within 20 min (vehicle,PO:n=3) and, was validated using the P2Y12 antagonist, clopidogrel (1.0mg/kg,PO:n=3) and protease-activated receptor antagonists PAR4 (1.0mg/kg,PO:n=6) and PAR1 (1.0mg/kg,PO:n=6). The T-BT model was successfully used concurrently with the FeCL3 model. Approaches characterizing antithrombotic efficacy using AV-shunt and FeCl3 models of thrombosis were effectively used and a dual capability platform combining FeCl3 and T-BT models enabled simultaneous antithrombotic efficacy and derisking in the same NHP promoting a reduction in animal use while increasing efficiency and observation of differential responses.

P298 Assessment and Characterization of Hub Loss: Impact of Syringe Type, Repeated Withdrawals, and Interpersonal Variability on Substance Loss

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The need to monitor exact drug balances is critical for proper documentation of controlled drugs. Conventional syringes include a dead space within the hub, which retains a volume of liquid that cannot be expelled. Repeated draws from a drug vial often result in unaccounted net loss that is typically recorded as hub loss. When handling highly valued drugs or controlled substances, it is important to minimize and account for hub loss. Determining an acceptable range of loss is critical to identify incorrect recording or misuse. Information determining acceptable hub loss ranges and identifying sources of variation is scarce. We hypothesize the number and volume of repeated draws from the vial, initial drug volume, and interpersonal variability all contribute to differences in net hub loss. Three participants of different skill levels withdrew set volumes (0.1, 0.2, 0.5, or 1.0mL in 1 mL syringes) totaling 5, 10, or 20 mL from 10 and 20 mL vials. Each trial was repeated 5 times per user. The remaining volume in each vial was measured and subtracted from the initial volume to determine hub loss. Standard 1 mL syringes had a measured hub volume of 0.066-0.068 mL, which can be used to calculate the anticipated hub loss. However, this estimation may not be accurate due to other variables. There was a significant difference in interpersonal variability. Total hub loss increased proportionally with increasing numbers of draws from the vial. Additional sources of variability such as the precision and accuracy of starting volume, impact of air bubbles, and internal vial pressure also

seemed to contribute to fluctuations in volume loss. Acceptable hub loss ranges were established based on mean +/- 2 standard deviations from our trials. This acceptable range can be used to perform quality assurance checks on controlled drug records and be used as personnel skills validation criteria. Based on the results, we determined that hub-less syringes are optimal for minimizing drug loss. In situations where hub-less syringes are not used, we recommend having an acceptable range of hub loss given interpersonal variability and, most importantly, recording the number of syringes used per withdrawal for accurate accounting of hub loss in clinical and laboratory settings.

P299 Marble Burying for Assessing Postoperative Pain in Rats Treated with Meloxicam or Sustained-Release Meloxicam

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Laboratory rats are commonly used in studies involving pain that must be ameliorated. While multiple options for appropriate analgesa have been established as effective for this species, current assessments for pain, such as the rat grimace scale, are less established and continue to vary in execution based on assessor experience. The marble burying test is a unique pain assessment tool that utilizes natural behaviors of the rat, such as neophobia and digging-burying, where normal rats will be motivated to bury novel objects such as marbles. Our hypothesis is that painful rats will be less likely to perform these normal behaviors, and that meloxicam will ameliorate pain appropriately so that normal behaviors can be expressed. Male Sprague Dawley rats were randomly assigned to 6 different treatment groups (n=6/group): castration or anesthesia only and treatment with saline, regular meloxicam, or sustained-release (SR) meloxicam. Behavioral assessments, including marble burying, grooming, wound licking, orbital tightening, and rearing were performed at 0,1,6,12,24 and 48 h postcastration. Compared to saline treated, nonsurgerized rats, all 3 treatment groups had an increase orbital tightening, increase wound licking, decrease rearing, and decrease marble burying 1 h postop. Wound licking was the only parameter that demonstrated a significant increase over time ($P = 0.04$), being the greatest at 1 h. Marble burying activity was decreased the first 12 h postop in all treatment groups. Castrated saline treated rats had the lowest marble burying, while meloxicam and SR-meloxicam treated rats buried more marbles; however, it was not significantly different ($P = 0.31$), and by 24 h marble burying activity was similar to baseline. These results show a trend in increase marble burying activity in rats that receive postoperative analgesics. A similar trend is seen with grooming, orbital tightening, and rearing, while wound licking is the most sensitive indicator of pain over time. Although we were unable to determine a significant difference, the trends demonstrate marble burying may be a useful adjunct with other behavioral assessment to evaluate pain in rats.

P300 Environmental Enrichment Does Not Impact Immune Response to Ovalbumin Immunization

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The incorporation of environmental enrichment (EE) has been increasingly common in laboratory medicine, because of its well-documented ability to improve animal welfare. In addition to exploring EE welfare benefits, researchers have become interested in investigating the potential for EE to improve animal health and increase the biological model efficacy. To assess the influence of EE on the immune systems of laboratory rodents, 18 adult female Swiss-Webster mice were immunized with ovalbumin, and their responses were measured over a 12-wk period. Mice were divided into 6 groups, including positive and negative controls, and 4 EE treatment groups. The negative control did not receive EE, and the positive control received only nesting material. The 4 treatments either had access to manipulada, vertical climbing rings, food foraging tubes, or all 3 forms on a rotating schedule. Blood serum was extracted once at the beginning of the study in order to obtain baseline ova-specific immunoglobin values, and again at the end of the 12-wk period. Immediately following baseline blood serum extraction, mice received their first booster of ovalbumin; a second booster was administered during week 6 of the study. At the end of the study, ELISAs were conducted to determine the change in antibody titers over the course of the experiment. An independent t-test was conducted to test the hypothesis that enriched mice would exhibit stronger immunoresponses to ovalbumin immunization; however, the evidence did not support this hypothesis ($P = 0.87$). Using a 1-way ANOVA, significant differences between the immune responses of the 4 treatment groups were unable to be identified ($P = 0.616$). While other variables explored in this study suggest EE has a positive influence on mouse welfare, we were unable to demonstrate a statistically significant difference in immunoresponses as a result of access to EE.

P301 Female Wistar Han Outbred Rats as a Model of Obesity When Fed High-Fat Diets

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This study describes the development of a new rodent model of diet induced obesity utilizing non-ovariectomized, virgin female Wistar Han® rats. At 3 wk of age, rats were allowed *ad libitum* access to either high fat (HF-1; 60% kcal from fat) or control (CON; 13% kcal from fat) diet for a period of 30 wk. A third cohort was started on the same high fat diet (HF-2) at 8 wk of age for a period of 22 wk. Body weight was measured weekly. At 33 wk of age, food intake was recorded and body composition and energy expenditure were measured. Clinical chemistry parameters were assessed and plasma hormone levels were analyzed. At 33 wk of age body weight was significantly greater ($P < 0.05$) in HF compared to CON but not between HF groups (HF-1, 335 g, HF-2, 315 g, CON, 260 g). Percent body fat was also significantly greater in rats maintained on high fat compared to control diets but did not differ due to age at high fat diet initiation (HF-1, 27%, HF-2, 25%, CON, 13%). Leptin levels were significantly elevated in obese rats consistent with the increased proportion of body fat; all other clinical chemistry and hormone levels measured were within normal range for rats. Total energy intake during the monitoring period was approximately 11% higher in HF rats compared to CON (HF-1, 47.4 ± 3.1, HF-2, 45.1 ± 2.6, CON, 41.6 ± 2.8 total kcal). During the dark cycle, HF rats were approximately 20% less active than CON (HF-1, 15,667 ± 607, HF-2, 15,662 ± 1095, CON, 18,535 ± 1023 total beam breaks). In conclusion, these female rats became obese with increased energy intake and reduced activity in the absence of surgical or genetic modification, suggesting a potential model for further evaluation of the interplay between leptin and estrogen on energy regulating pathways. Additionally, the rats are nondiabetic despite a significant degree of obesity. This diet-induced obesity model may have utility for testing novel anti-obesity compounds to assess how gender specific hormonal differences may influence weight loss/gain outcomes.

P302 An Alternative Method for Urine Collection in Group-Housed Mice in Toxicology Studies Using Hydrophobic Sand Techniques

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There are many limitations associated with urine collection in mice, including urine volume obtained, extended fasting period, and separation of group-housed males that increases the incidence of fighting when returned to co-housing. A hydrpophobic sand urine collection technique addresses these issues and offers a refined procedure in line with the 3Rs, providing valuable advantages to toxicology studies. This commercially available sand keeps the urine afloat. The bottom of the bin is covered with the sand and urine drops can be collected using a pipette. This study compared the standard urinalysis and urine chemistry parameters values from single-housed mice in metabolic cages to values obtained from group-housed mice using sand. The urinary volume obtained was dependant on the time of collection. Twenty-seven CD-1 females (3/cage) were placed in solid bottom cages with the sand or placed singly in metabolic cages. The animals were food deprived with access to water for the duration of the urine collection. The urine from each cage was collected over a target period of 4-5h hours and analyzed for standard urinalysis parameters (using multistick 10SG) and chemistry parameters (using modular p-800 analyzer). A 5-h collection period provided a higher volume of urine. In addition, collecting the urine later in the day from 3 to 8 pm, when mice are more active, provided a higher urinary volume. The volume of urine obtained with sand when using the optimal conditions varied between 500 to 1200 µL. Results obtained with the 2 collection techniques provided similar urinalysis results (specific gravity, pH, blood,

glucose, proteins, bilirubin, ketones) or urine phosphorus, sodium, chloride; however urine calcium and potassium were elevated. Additionally, mice urine collected via metabolic cages was analyzed for urine chemistry parameters before and after contact with the sand, to mimic the in vivo conditions. Pre and postresults were comparable for urinalysis and showed differences within 11% for urine chemistry. The sand technique is considered an acceptable alternative for urine collection on toxicology studies for urinalysis, particularly with group-housed males where the singly housed metabolic cages are not optimal.

P303 Characterization of a New Spontaneous Mutation Affecting Myelination in Sprague Dawley Rats

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We introduce a new mutant line of Sprague Dawley (SD) rats called tembleque (SDt). The phenotype of the homozygous rats is mainly characterized by tremors and abnormal locomotion involving the hind limbs. In order to study the inheritance of the trait, male and female mutant SDt rats were crossed with wild-type (WT) SD rats and 100% of the F1 pups obtained showed no tremors. When F1 were intercrossed, around 25% of F2 pups showed the characteristic tremors, confirming the presence of an autosomal recessive mutation. Finally, crosses between mutant SDt rats produced 100% offspring with phenotype, which allowed us to develop a homozygous mutant line. In the mutant rats, the tremors appear by week 3 and decrease gradually after week 16. Histological analysis showed that the spinal cord of young (5-wk-old) and adult (20-wk-old) SDt rats had severe hypomyelination. These results were confirmed by electron microscopy studies, which also showed alterations in the compaction of myelin sheaths. We did not find differences in peripheral nerves myelination when comparing WT with mutant rats. We report here preliminary genetic and histopathology results from a new mutant rat affecting myelination process. This new spontaneous mutation in the rat is a potential new model for studying the myelination process, as well as the evaluation of new treatments directed towards diseases associated with myelin alterations.

P304 Preoperative Buprenorphine Administration Has Potential to Decrease Efficacy of Electrical Stimulation Therapy after Peripheral Nerve Injury

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Electrical stimulation (ES) is a continually evolving treatment modality for peripheral nerve injuries. ES has been beneficial in promoting functional recovery in rodent models of sciatic, femoral, facial, and recurrent laryngeal nerve (RLN) injuries, and in humans with carpal tunnel syndrome. Though ES has demonstrated success, efficacy is controversial. Anesthesia and analgesia are variables that may contribute to the effectiveness of ES. Buprenorphine has been shown to have a local effect on peripheral nerves to extend the duration of peripheral nerve blocks. Thus, we hypothesized that administration of buprenorphine-SR (b-SR) prior to surgery would reduce the efficacy of intraoperative ES in a mouse model of RLN injury. Sixteen C57BL/6J mice of either sex, divided into 3 groups, underwent a surgical compression injury of the right RLN. Group 1 (n=6) did not receive ES treatment and was given b-SR postoperatively. The other 2 groups both received ES treatment immediately after RLN injury. However, b-SR was given postoperatively to group 2 (n=5) and preoperatively to group 3 (n=5). Transoral laryngoscopy with a miniaturized endoscope was used to monitor vocal fold (VF) movement prior to RLN injury and following injury to confirm VF paralysis. After surgery, laryngoscopy was performed weekly for 12 wk to monitor VF recovery. Videos were analyzed using a subjective Likert scale and with our prototype VF tracking software. Though not statistically significant, mice that received preoperative b-SR (group 3) had decreased VF mobility recovery based on our Likert scale, while groups 1 and 2 had improved, but similar, recovery patterns. Our tracking algorithm was applied to all videos. Nine out of 224 videos could not be analyzed due to low quality. Graphs of VF motion for each mouse were generated at each time point. Preliminary results examining the ratio between right and left vocal fold movement indicate a drastic decrease of right VF mobility immediately postcrush that incompletely recovers by 12 wk in all groups. Choice of preoperative and perioperative anesthesia and analgesia may have an effect on functional outcomes after ES treatment and should be considered during experimental design. Once validated, tracking software will give further insight to VF recovery after injury as it has the potential to detect the synchrony and percentage of displacement between the right (impaired) and left (normal) VFs, as well as to identify the median and maximum effort of the impaired VF.

P305 Pregnancy-Associated Alterations in Pulmonary Function in the Mouse: Implications for Influenza Pathogenesis and Disease

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Human pregnancy is associated with alterations in normal physiology, including anatomic and functional changes of the cardiopulmonary system. Mice are a common animal model for studying respiratory infection and disease during pregnancy, but pregnancy-associated changes in pulmonary physiology have not been described in mice. Using a pregnant C57BL/6 mouse model, we sought to characterize changes in pulmonary structure and function during pregnancy in both healthy animals and following infection with an important public health respiratory pathogen, influenza A virus (IAV). We hypothesized that pregnancy-associated alterations in pulmonary physiology may contribute to the more severe outcome of IAV infection observed in this population. Pregnant (E10) (n=10) and nonpregnant (n=10) mice were intranasally inoculated with a sublethal dose of mouse-adapted 2009 H1N1 IAV or vehicle. Eight d postinoculation (dpi)–corresponding with E18 in pregnant females–pulmonary function testing (PFT), including diffusing capacity, quiet tidal breathing, forced-oscillation mechanics, and generation of pressure-volume curves, was performed to measure dynamic and static mechanical and functional properties of the lungs. Following PFT, animals were euthanized and lungs were collected for either immunophenotyping by flow cytometry or for histopathological analyses. Pregnant, uninfected animals had greater fixed lung volume, total lung capacity, residual volume, and lung compliance compared with nonpregnant, uninfected females (t-test, $P < 0.05$). No differences were observed in respiratory rate, tidal volume, minute ventilation, resistance, or oxygen diffusion capacity between pregnant and nonpregnant, uninfected mice. Following IAV infection, while nonpregnant females showed a significant increase in pulmonary resistance (t-test, $P < 0.05$), and a significant decrease in compliance and oxygen diffusion capacity (t-test, $P < 0.05$), these measures were preserved following infection of pregnant females, and they did not differ from uninfected, pregnant controls. Eight dpi, pregnant, and nonpregnant animals had similar numbers and proportions of pulmonary immune cells, but pregnant animals demonstrated reduced histological evidence of pulmonary inflammation and damage. Together, these data show that pregnancy in C57BL/6 mice is associated with alterations in pulmonary structure and function. Moreover, these measures are preserved following a respiratory insult compared with nonpregnant females, which suggests that pregnancy-associated alterations in pulmonary physiology may protect against IAV-associated disease in female C57BL/6 mice.

P306 Using Targeted Locus Amplification Analysis to Determine Transgene Integration Site for Commonly Used Molecules to Improve Genotyping and Breeding Efficiency

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Transgenic animal models are necessary resources for the study of gene function and disease. Pronuclear microinjection is the preferred method for generating these lines and results in the random integration of foreign

transgenic DNA into the genome. This can lead to the disruption or alteration of gene function, potentially causing an unrelated phenotype. Knowing the chromosomal location of the transgene is also useful when planning crosses, especially in cases when the alleles are on the same chromosome. Unfortunately, many transgenic lines remain uncharacterized. We have used targeted locus amplification (TLA) to efficiently map the transgene location in 40 colonies (transgenic lines). The TLA technology is based on the crosslinking, fragmentation, relegation, and selective amplification of DNA and results in the amplification of tens to hundreds of kilobases of surrounding DNA. TLA works without detailed prior locus information, and one or a few primer pairs are sufficient for sequencing. Even with limited knowledge of the transgene sequences, it is possible to generate a complete or near-complete picture of the transgene integration event and genomic/transgenic borders, including all single nucleotide variations (SNV) and structural variants. Detailed knowledge of the transgene integration event not only allows for improved genotyping assays, it also helps inform the interpretation of phenotypes obtained when using mice carrying transgenic alleles. Thus, identification of the integration site can prevent unnecessary losses of time and resources. With the power of the TLA method, we have found the integration sites for 40 commonly used transgenic mice models. Here we provide the sequence and integration sites for a number of models that are publicly available. Our methods, data, and genotyping assays should serve as a useful resource for the research community and our results illustrate the power of the TLA method in contributing to genetic research and diagnostics.

P307 Improved Site Selection and Sampling of Göttingen Minipig Skin for Dermal Studies

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Miniature swine are increasingly used in biomedical research and are the preferred non-rodent species in preclinical dermal safety studies, as minipig skin has been reported to be similar to human skin. However, there is a wide variance in skin morphology reported for domestic pigs, depending on the region of the body sampled. Thus, selection and sampling of dosing and comparator control sites may introduce unwanted variance and thus skew histopathological assessment of test article-related findings. We worked to develop a map for skin site sampling as well as improved sampling methods to allow improved standardized assessment and comparison of sites across animals. A site map for prospective sample collection was created with 17 separate sites identified for sampling, including routinely used dosing and control sites. Seven adult Göttingen minipigs were euthanized and 3x3 cm skin samples taken from sites on each animal. Various methods of fixation, including standard floatation, cloth bagging, and waxed card with filter paper, were assessed to determine the most consistent method. Samples were sectioned, stained, and assessed for basic histoanatomical comparisons. Fixation on a waxed card overlaid with filter paper was most effective in minimizing tissue distortion. Among sectioned samples, there were notable differences in skin thickness between the commonly dosed dorsal sites, along the back, and the ventral sites, commonly used for routine skin sampling, with the dorsal sites having thicker dermis and subcutis. Careful site selection for dosing and sample handling of minipig skin should be a consideration and may allow for more scientifically accurate assessment of compound-related effects in the research setting.

P308 The Effects of Intracage Ammonia on Markers of Pulmonary Endothelial Integrity in Mice Housed in Static Microisolator Cages

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Time-weighted exposure limits to ammonia are established for humans (NIOSH 25 ppm and OSHA 50 ppm); however, no defined guidelines exist for laboratory rodents. The *Guide* recommends air pollutants be maintained at concentrations below levels irritating to mucous membranes, but does not provide definitive threshold values. Numerous studies have examined intracage ammonia and its effects on animal health and wellbeing by evaluating damage to the upper respiratory tract, yet none have evaluated lower pulmonary epithelial and endothelial integrity. This study used a battery of assays commonly used in the mouse acute lung injury model to assess the effect exposure to cyclical, naturally occurring ammonia has on pulmonary integrity and inflammation. Assays included bronchoalveolar lavage fluid (BAL) cell counts and protein concentration, excess lung water content (ELW), Evans blue permeability (EBA), myeloperoxidase (MPO), and lung histology. For this study, B6 mice (n=60) were maintained in either static microisolator or open-top control cages. Cages were changed weekly and ammonia levels were measured on days 0, 3, 5, and 7 of each cage change cycle for 6 wk prior to assessing pulmonary endothelial integrity and inflammation. Ammonia levels in static microisolator cages began to increase on day 3 and peaked at a maximum average of 141.3 ppm on day 7. Ammonia levels in open-top cages never exceeded 5 ppm. No significant differences between groups were observed in BAL cell counts (P = 0.8602) or protein concentration (P = 0.2614), ELW (P = 0.1266), EBA (P = 0.3064), or MPO (P = 0.5879). Lung histopathology showed minimal, incidental changes in all mice. Results suggest that exposure to naturally occurring ammonia in static microisolator caging does not adversely affect the integrity of the lower pulmonary tract in the mouse nor does it negatively impact murine models of acute lung injury.

P309 Developing Combination Gene Therapies for the Treatment of Corneal Fibrosis

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Corneal fibrosis (scarring) is a leading cause of blindness worldwide. Safe and effective treatments for full resolution of corneal fibrosis are lacking. The persistence of myofibroblasts in the stroma heavily contributes to corneal fibrosis. Hepatocyte growth factor (HGF) selectively eliminates myofibroblasts via apoptosis in nonocular tissues. We have shown that bone morphogenetic protein-7 (BMP7) modulates Smad signaling pathway to limit excessive wound healing and fibrosis in the cornea. We postulated that targeted combination gene therapy (HGF+BMP7) delivered to the cornea with polyethylenimine nanoparticles (PEI-NP) following injury will safely and effectively attenuate corneal fibrosis through myofibroblast-specific apoptosis and by counter balancing pro-fibrotic factors in vivo. For in vitro studies, stromal fibroblasts (HSF) were isolated from donor human corneas and stromal myofibroblasts (HMF) were produced by growing HSF in serum-free media with 5ng/ml TGFβ1. Corneal fibrosis was created in vivo in 12 New Zealand White rabbits by a 30 sec topical application of 1 N NaOH. HGF+BMP7 gene transfer was performed with a single 5-min topical application of PEI-NP vector. Serial stereo- and slit-lamp microscopy evaluated response to treatment. Real-time quantitative PCR and immunoblotting quantified delivered gene copies and the mRNA and protein of fibrosis markers. Immunofluorescence and TUNEL assays evaluated levels of fibrosis and apoptosis. HSF and HMF treated with HGF showed many TUNEL+ apoptotic cells in HMF but not in HSF (> 15 TUNEL+ in HMF vs < 2 in HSF). PEI-NP treatment showed substantial HGF and BMP7 gene delivery into stroma in vivo (> 104 gene copies/ug DNA). HGF+BMP7 gene therapy significantly decreased corneal opacity in vivo (Fantes scale 3.3 control vs 0.6 treated; $P < 0.001$). Treated corneas contained significantly reduced amounts of α-smooth muscle actin, f-actin, and collagen-I mRNA (up to 15-fold; $P < 0.01$). Immunofluorescence revealed significantly fewer myofibroblasts in treated corneas (83%; $P < 0.001$). This novel HGF treatment caused myofibroblast-specific apoptosis in the cornea. Combination HGF+BMP7 gene therapy can be delivered through a single topical application of PEI-NP to safely and effectively attenuate corneal fibrosis in vivo.

P310 Drug Profile of Sustained Released Meloxicam in Sheep (Ovis aries)

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Postoperative analgesic regimens for sheep undergoing major surgery typically use a multimodal approach. This often requires multiple sessions of handling and restraint for injections which may subsequently induce stress. Sustained release (SR) formulations may, therefore, serve as a valuable refinement to current analgesic regimens. While a variety of

analgesic SR formulations are already used in lab animal medicine, there are currently no studies evaluating the pharmacokinetics of an SR NSAID in sheep. In this study, we evaluated the drug profile of 2 dosages of meloxicam given subcutaneously (SC): conventional meloxicam (CM) versus SR meloxicam (SRM). We hypothesized that single dose SRM would provide 72 h of stable plasma levels equivalent to CM, without the need for repeated injections over 3 consecutive days in sheep. Six naïve female Dorset sheep, roughly 1 y of age weighing 60-80 kgs were used to compare CM 0.5 mg/kg SC once daily for 3 consecutive days to SRM 1.5mg/kg SC given once. There was a 14 d washout period between drug administrations. Blood was collected at time points 1, 4, 12, 24, 36, 48, 60, 72, 84, 96, 120, 144, and 168 h post injection. Physical exams, urine, and hematologic analysis were performed at time points 0, 24, 48, and 120 h. Plasma levels of drug were evaluated using liquid chromatography-mass spectrometry and a clinical pathologist analyzed blood and urine results. CM and SRM had elimination half-lives of 12.1±4.2 and 15.2±2.4 h, respectively. The peak of CM and SRM were C_{max} =1057±433 and 3238±1480ng/mL corresponding to time points 4±0 and 6.7±4.1hrs, respectively. One animal experienced transient acute renal azotemia at 24 h after SRM administration that improved by 120 h. No other significant clinical findings were noted. In comparison to CM, plasma levels of SRM were significantly higher throughout the initial 24 h period postinjection. SRM did not sustain therapeutic levels the entire 72 h as expected. The high peak levels could pose a concern, considering that acute renal azotemia was observed in the sheep with the highest SRM plasma levels. This study found no apparent advantage for SRM usage, other than a higher plasma level for some animals up to 48 h. Additional clinical investigation is warranted.

P311 Genipin Conjugation of Gold Nanoparticles to Decellularized Porcine Tissue

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The application of nanoparticles in tissue engineering has grown expansively in the past decade. However, there is currently no method to rapidly apply nanoparticles to tissue without causing cytotoxicity. The current methods require days of washing to remove hazardous byproducts which makes their use in the operating room difficult. Recently, genipin has been proposed as solution to this previously unsolved problem. Genipin is a naturally derived crosslinking agent isolated from the fruits of *Gardenia jasminoides*. It reacts spontaneously with amino-group-containing compounds such as proteins, collagens, and gelatins. We hypothesized that genipin can be used to attach gold nanoparticles to tissue in a fast, cell safe manner. To test the hypothesis, porcine diaphragm and carotid artery were decellularized and then immersed in cystamine functionalized gold nanoparticles and genipin at concentrations of 3 mM, 5 mM, and 10 mM for numerous time points. The thermal stability of the tissue was measured using differential scanning calorimetry to evaluate the degree of crosslinking and potential damage to the tissue. Nuclear activation analysis was used to measure the amount of gold attached to each sample, and WST-1 assay was used to measure viability of the fibroblast cells cultured on the scaffold. The results show the genipin began crosslinking the tissue by the 5-min time point and gold nanoparticles successfully attached to the carotid tissue in 30 min and with no greater cytotoxic effects than the current methods used.

P312 Differential Vulnerability of Muscle Fiber Types in Spinal Muscular Atrophy with Respiratory Distress Type 1 (SMARD1) Mice

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Spinal muscular atrophy with respiratory distress type 1 (SMARD1) is an autosomal recessive disease caused by mutations in the immunoglobulin mu biding protein 2 (IGHBMP2) gene. The exact mechanisms of IGHMBP2 gene mutations leading to the disease are not well understood; however, it is speculated to be related to DNA/RNA processing. SMARD1 affects distal muscular groups initially and progresses to proximal muscular atrophy. While human patients show a wide spectrum of clinical signs, mortality associated with SMARD1 is due to diaphragmatic paralysis. Analyses of the SMARD 1 mouse model have shown different muscle groups have differential vulnerability to denervation. Based on this finding, we wanted to investigate whether SMARD1 selectively affects specific muscle fiber types leading to the selective vulnerability to denervation of different muscles. Type I fibers are slow oxidative fibers that produce low force and slow contraction. Type II fibers (IIa, IIb, and IIx) are fast glycolytic fibers that produce high force and fast contraction. Using antibodies specific to each muscle fiber type, we quantified the proportion of fiber types in several different muscles, which had shown differential vulnerability to denervation. Our results revealed no significant change in the proportion of type I muscle fibers in SMARD1 gastrocnemius (highly vulnerable) muscle compared to control. Interestingly, there was a dramatic reduction in the proportion of Type IIb and IIx fibers in SMARD1 gastrocnemius muscle compared to control. The proportion of Type IIa fibers was mildly affected in SMARD1 muscle fibers compared to control. In the EDL and TA (intermediately vulnerable) muscles, the proportion of Type I and IIa muscle fibers was not different. However, the proportion of all Type IIb and IIx muscle fibers was severely reduced in SMARD1 compared to control. These results indicate that there is a correlation between fiber type composition and vulnerability to denervation. Furthermore, this provides a basis for the selective vulnerability to denervation of specific muscles and identifies a new target for potential therapeutic intervention.

P313 Impact of Fenbendazole Treatment on the Canine Fecal Microbiota

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The gut microbiome (GM) consists of hundreds of distinct microbial species that equals or outnumbers host somatic cells. The GM influences a multitude of physiological and immunological processes in the host and changes in the GM have been shown to cause changes in animal model phenotypes. Previous studies using rodents have also shown that the composition of the GM is affected by many factors including diet, husbandry, housing types, and genetic backgrounds. However, limited information exists about factors that may modulate GM in other laboratory species such as dogs, which remain a critical model for the study of a variety of human and animal diseases. We set out to eliminate sporadic *Giardia* infections in dogs on our campus using fenbendazole (FBZ). FBZ is widely used to treat and prevent gastrointestinal parasites in dogs used in biomedical research. That said, the influence of FBZ on the resident GM is unknown. We speculated that this use of FBZ could have inadvertent effects on the canine GM and sought to document any changes that might occur over the course of treatment. We collected fresh fecal samples from dogs that were housed in 3 different facilities (n=19-25). FBZ treatment (50 mg/kg) was given orally to all dogs for 10 consecutive days. The fecal samples were obtained 2 d before the initiation of treatment, on the last day of treatment, and 2 wk after the completion of treatment and targeted 16S rRNA gene sequencing was utilized to analyze fecal microbiota. All dogs were clinically normal throughout the sample collection period. Statistical analyses of data showed significant differences between dogs housed in the 3 different facilities, further emphasizing effects of housing and husbandry factors on the GM. Furthermore, negligible differences were seen between time points, indicating that FBZ did not significantly alter the canine GM. These findings suggest that FBZ may be used therapeutically in dogs with minimal impact on the GM, including studies potentially modulated by changes in GM.

P314 A 3D Printed Apparatus for Small Animal Imaging: Minimizing Attenuation by Increasing kVp

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Computed tomography and positron emission tomography are vital imaging modalities necessary for proper cancer diagnosis, staging, and treatment. Computed tomography is most often used for anatomical purposes, while positron emission tomography allows functional assessment of tumors. When used together, they give researchers and clinicians unrivaled information for cancer identification, prognosis, and therapy. As small animal models are commonly used to gather data prior to human

clinical trials, these imaging modalities have become a crucial part of rodent research. However, in order to limit image distortion due to motion, animals often are anesthetized for their scans. Other important factors to consider with rodent imaging include biosecurity (inability to return animals to home environment after exposure to imaging suites), cost (large number of rats and mice necessary to complete experiments, which are commonly imaged solitarily), and feasibility (time needed to anesthetize and properly image individual animals). We created a 3D printed model, allowing us to simultaneously image 9 rodents anesthetized with flow by gas. Rats were anesthetized in series, and stacked in a 3x3 fashion. However, an increase in numbers results in an increase in mass, potentially causing excess attenuation and leading to suboptimal images and increased image noise. We hypothesized that by increasing kVp, or increasing x-ray energy, we would decrease attenuation associated with imaging multiple rats with our device. We performed 3 serial scans with our apparatus while increasing kVp. Attenuation, measured as Hounsfield units, was quantified using our computed tomography scanner software and compared between kVp groups. Hounsfield units decreased as kVp increased, with correlating decreased image noise. In summary, our apparatus allows 9 rats to be simultaneously anesthetized and scanned. Overall, our imaging model limits motion, decreases time needed to scan multiple animals, and makes positron emission tomography and computed tomography more cost effective in a small animal model.

P315 Seasonal, Yearly, and Life-Stage Comparisons of the Fecal Microbiota of Mink—A Representative Carnivore

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With the advent of next generation sequencing (NGS) technology, our understanding of the role that the microbiota has on host health and disease, as well as its influence on research outcomes, has increased significantly recently. With few exceptions, most studies have focused on herbivores and omnivores. We sought to characterize the fecal microbiota of commercial mink, a representative carnivore, and to determine how the microbial population changes between seasons and years and between adult females and weaned kits. Pooled fecal samples (n=366) were collected from weaned kits and adult females in the summers of 2014 and 2015, and from adult females during the winter of 2016. Bacterial DNA was extracted and the V4 region of the 16s rRNA gene was amplified using NGS. Following quality control filtering, approximately 22 million sequences were identified. Together, sequences from the phyla Firmicutes and Proteobacteria account for >95% of those identified. At each taxonomic level, differences were identified in the relative abundance of bacteria by year, life stage, and season. The greatest number of significantly different taxa were noted between 2014 and 2016, and the fewest between adult females and weaned kits from the same year. There were no differences in richness, evenness, or diversity when comparing season and life stage. Significantly more operational taxonomic units (OTUs) were seen in 2014 than 2015 or 2016 ($P < 0.05$), 2014 was richer than 2016 ($P = 0.013$), and was more even but less diverse than 2015 ($P < 0.01$). There were significant differences in community membership and structure by year and season for adult females (all P values <0.001). Together, these indicate that, once the microbiota reaches maturity, time is an important factor in the dynamics of the fecal microbial population. Additionally, at the farm level, the vast majority of the relative abundances of predominant phyla and genera identified were similar from year to year ($P > 0.05$), suggesting that the environment is an important factor in the stability of the microbiota over time. In addition to adding to what little is known about the microbiota of carnivores, this study will contribute to our understanding of the dynamics of the microbiota.

P316 Efficiency of Genome Editing in Mouse Embryos by Lipofection of CAS9/sgRNA Ribonucleoproteins

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Genome editing technologies in the mouse are often used in biomedical research. We examined the efficiency of genome editing in mouse embryos by lipofection, a lipid-based transfection technology, of Cas9/sgRNA ribonucleoproteins (RNPs) to establish a simpler method of the technique without any special equipment (for example, micromanipulators or electroporation apparatus). We designed 2 sgRNA bound to different sites on mouse genome to induce genomic deletion of ~1,500 base pairs between the sites. In vitro-produced zygotes of 4C30 mice (C57BL/6N) were cultured approximately 2 h with various concentrations of complexes with lipofection agents and Cas9/sgRNA RNPs. Then, the zygotes were transferred to fresh KSOM/aa media and cultured for 5 d. We confirmed the genomic deletion in mouse blastocysts by detecting smaller amplicon production than those from the wild type by PCR. We used 5 doses of lipofection RNP complexes (LRC): original concentration (= 1x) described in the instruction manual of the lipofection agent, and 2×, 3×, 4×, and 5× higher concentrations of the original. Blastocyst formation rates (# of blastocyst / # of zygotes cultured) were 30/34 (88%), 25/37 (68%), 31/40 (78%), 31/40 (78%), and 20/30 (67%) at 1×, 2×, 3×, 4×, 5×, respectively. The efficiency of genomic deletion in mouse embryos (# of blastocysts with genomic deletion / # of blastocysts tested) were 2/30 (7%), 1/25 (4%), 1/29 (3%), 1/30 (3%), and 3/20 (15%), respectively. These results indicate that high concentration of LRC had some adverse effect on preimplantation embryo development, but that there was a tendency of higher genome editing efficiency in higher concentrations of LRC. In addition, we obtained 28 pups after embryo transfer of 71 embryos treated with 2× concentration of LRC, but there were no pups with genomic deletion. Genome editing by lipofection is promising but still needed to be optimized for mouse embryos.

P317 Gut Microbiome Diversity in C57BL/6 Mice Is Associated with Age and Gender within the Same Barrier Facility

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There are hundreds of species-level microbiota permanently colonized in the human body. The gut microbiota composition plays a role in several diseases pathogenesis and drug discovery, including inflammatory bowel diseases, type 2 diabetes, and autism. Elucidation of the microbiota composition in lab animals, which are used for studying the disease of humans, is important because of its impact on the continuity of the research model performance. We hypothesized that the gut microbiome diversity in C57BL/6 mice acquired from within the same barrier may vary by age and gender. To test this, we used a 16S rRNA analysis by next generation sequencing (NGS) to investigate the fecal microbiome. Fecal samples were collected from 3 age groups (3-4 wk, 8-10 wk, and >20 wk) and each gender (n=8 per age-gender). DNA libraries were prepared for targeting the variable V3-V4 regions of 16S rRNA and sequenced to generate pair-ended 300×300 reads. Taxonomy was assigned to sequences via the GreenGenes database. The number of operational taxonomical units (OTUs) obtained for the 3-4 wk group was significantly lower compared with 8-10 wk and >20 wk groups. The number of OTUs is not statistically different between 8-10 wk and >20 wk groups. Beta diversity evaluation by principal coordinate analysis (PCoA) produced 3 overlapping distinct clustering locations. Within the 8-10 wk group, alpha diversity of OTUs among females was higher than males. We identified 10 dominant organisms at the genus and phylum level among all 3 age groups and compared average relative abundance of each by gender. Substantial variation was observed at the phylum level for *Bacteroidetes*, *Proteobacteria*, and *Firmicutes*. In summary, the microbiota composition among C57BL/6 mice in the same barrier room is different by age and gender. Thus, to maintain continuity among investigations, it is important to consider the age and gender of the mice.

P318 Diet Alters Fecal Microbiota Transplantation Efficiency in Germ-Free Mice

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Fecal microbiota transplantation (FMT) in germ-free mice is a common approach in microbiome research and drug testing. However, FMT with complex, human microbiotas in mice does not fully capture the microbial community of the donor, nor does it stimulate the murine immune system in the same way as a murine microbiota. It was hypothesized that diet affects the efficiency of a human microbiota to establish in recipient germ-free mice and subsequent immune responses. Therefore, it was tested whether custom diets with altered fat content or fat/protein sources (soybean oil versus milk fat or soy protein versus casein), compared to a standard rodent chow, would improve human microbiota establishment and immune system characteristics. Germ-free C57BL/6NTac mice were colonized with human or mouse microbiota and fed different test diets (n=8-10): (1) animal-sourced (with casein and 4.3% milk fat), (2) human profile (grain-based with 10.5% soybean oil), or (3) control rodent chow. Fecal microbiota were characterized by 16S rRNA amplicon sequencing of the V3 region. Lymphocyte and dendritic cell populations were measured in mesenteric lymph nodes and Peyer's patches by flow cytometry. Gene markers for lymphocytes, dendritic cells, and pro and antiinflammatory cytokines were measured in ileum and colon by qPCR. It was found that the animal-sourced diet increased the colonization efficiency of mouse microbiota-colonized mice significantly and differentially altered dendritic cell populations. In contrast, the human-profile diet improved the colonization efficiency of human microbiota-colonized mice, although only slightly. The immunological phenotype of the human microbiota-colonized mice was characterized by an increase in regulatory T cells in mesenteric lymph nodes and inflammatory cytokines in colon, and this effect was greater in recipient mice on the animal-sourced diet. Altering the diet of mice transplanted with complex human fecal microbiotas is a promising approach for optimizing FMT efficiency in mice and for modulating subsequent immune system function. Further studies to optimize fat content and diet constituents are warranted in order to successfully model the human microbiota in mice and its effects on the immune system.

P320 An Easy Microsampling Device for Routine Serosurveillance of Nonhuman Primate Colonies

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Traditional serology has been performed using serum and singleplex ELISAs. Multiplex ELISA techniques, including multiplexed fluorometric immunoassay, require much smaller volume of serum thus paving the way for use of microsampling techniques, including dry whole blood spot (DBS) requiring only a single drop of blood. In this study a new microsampler was optimized for routine serosurveillance of nonhuman primate (NHP) colonies. The absorptive tip of the device consists of inert, porous, hydrophilic material that wicks up a consistent volume (20µL) of whole blood. This sampling tool has several advantages versus serum and DBS as it is quantitative; has improved reproducibility; and reduces animal stress due to shorter restraint times, anesthesia, and technician handling. Qualification studies were conducted comparing analytical and diagnostic performance of paired sample eluates and standard immune serum from rhesus and cynomolgus macaques. Matching sera samples (8 per species) from naturally and experimentally infected macaques (known positives) as well as from SPF macaques (known negatives) were tested. Study samples were tested by MFIA for infectious agents including SIV, STLV, SRV, B-Virus, Measles, SVV, SCMV, MRV, LCV, and Chagas. Average scores (analytical performance), titration curves, and limit of detection using polyspecific and monospecific sera were similar for tip eluates and serum. Diagnostic reproducibility and ruggedness were tested by 2 analysts performing MFIA on 3 different days (6 runs total). Diagnostic sensitivity between tip eluate and serum was found to be 99.6% and 99.8% whereas diagnostic specificity was 98.6% and 99.6%, respectively. Average eluate scores and number of positives between the 2 analysts as well as tip elute versus serum were comparable with CVs around 10%. Qualification data demonstrate that the device is an alternative to submitting serum for routine NHP serology testing. It eliminates the steps, reagents, material, and equipment needed to prepare and ship serum samples, thus saving labor and time in the collection and shipping of samples. Also, it reduces overall stress in NHPs during the collection of blood for routine serosurveillance or from animals on study, promoting the animal welfare principle of the 3Rs.

P321 Decrease in Trimethylated H3k9 Level Was Effective in Reprogramming of Donor Nuclei by Interspecies Nuclear Transfer Embryos

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Our study group was successful in improving somatic cell nuclear transfer technology. This cloning method requires refinement in the establishment of nuclear transfer embryonic stem cell (ntES) and the production of interspecies somatic cell nuclear transfer (iSCNT). We investigated the embryonic development and histone modification of interspecies reconstructed embryos produced using our novel method. To create donor cells, fibroblast cells were created from tail tissues of the large Japanese field mouse (*Apodemus speciosus*). After collection of the donor cells, fibroblast cells were plated with deionized bovine serum in Hepes-buffered CZB medium. After the recipient oocytes (B6D2F1, *Mus musculus*) were enucleated, the fusions of cell-cytoplasm were performed using an envelope cell fusion kit. The reconstructed oocytes were activated by incubation for 6 h in 5mM $SrCl_2$ and 2mM EGTA-containing KSOM supplemented with 5µg/mL cytochalasin B. Then, the reconstructed oocytes were treated with 50nM trichostatin A (TSA) for 8 h followed by treatment of 10µg/mL vitamin C (VC) for 7 h. The cloned embryos were immunostained with trimethylated histone H3 lysine 9. The rate of cell fusion in iSCNT oocytes was 96% (218/226). After activation, pronuclear forming rates of reconstructed iSCNT oocytes were 78% (170/218). This treatment revealed that 2% of the reconstructed embryos had developed into the blastocyst stage. Significant reduction of H3K9me3 fluorescent signal intensity in 2 cell stages embryos compared to nontreatment ($P < 0.05$) was observed. We demonstrated the successful development of iSCNT embryos and somatic nuclear reprogramming in the reconstructed oocytes and embryos via this technique.

P322 Evaluating Consumption of Oral NSAID Products as Part of Multimodal Pain Management in a Mouse Thoracotomy Model

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At our institution, Ibuprofen added to the drinking water is commonly used to provide nonsteroidal antiinflammatory drug (NSAID) for rodent surgical manipulations and clinical concerns. More selective NSAIDs are available as oral formulations but consumption is variable. The purpose of this study was to quantify voluntary consumption of carprofen gel (C) and meloxicam bacon-flavored chewable tablets (M) relative to ibuprofen water (I) in a mouse model. Ten-week-old C57BL/6Crl male mice housed 3/cage were assigned to 3 NSAID groups; C(5 mg/kg), M(5 mg/kg), or I(40 mg/kg). NSAIDs were made available 24 h prior to intubation and anesthesia (60 min of isoflurane) and remained for 72 h. A thoracotomy was performed, with subsequent myocardial infarction, in the surgical group (Sx) and twice daily subcutaneous buprenorphine (0.1 mg/kg) was provided for 72 h (n=27) in addition to the assigned NSAID. Nonsurgical groups (NS) had no surgical manipulation, only intubation/anesthesia, and received buprenorphine plus NSAIDs (n=27) or NSAIDs alone (n=27). Body weight was tracked individually, while behavioral assessments, food, and NSAID consumption were tracked by cage. Differences were not identified between NS groups so data were combined for final analysis. Transient weight loss (6% average) occurred following anesthesia regardless of surgery. Postoperative mortality in the Sx group (44%) limited consumption comparison, as animal numbers were variable. The NS group consumed ibuprofen at significantly greater rates than C and M groups at 48, 72, and 96 h and specifically reached the target dose in mg/kg for C at 24 h and for I at 48, 72, and 96 hours. In conclusion, our data supports that intubation/anesthesia has minimal impact on voluntarily consumption of C and M, and that therapeutic doses can be reached voluntarily; 3.4 and 2.6 mg/kg respectively. When using these products in practice, close oversight is needed to ensure consumption and to confirm analgesic efficacy.

P323 Development of a Skin-Bleeding, Time-Test Procedure in an Anesthetized Cynomolgus Monkey (Macaca fascicularis)

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A bleed time test is a useful tool to evaluate the anticoagulant drug efficacy. The Ivy method is a quantitative coagulation assay based on a standardized skin wound, which measures platelet and vascular responses to injury. It is traditionally used in clinical settings. In the procedure, the blood pressure cuff is wrapped on the upper arm and inflated to 40 mm Hg. A standard-sized cut wound is created on the forearm skin and the bleeding time is recorded. However, the test used for new drug preclinical evaluation is not well established in the monkey. We modified the Ivy method in male cynomolgus macaca (n=7) to develop a reliable bleeding test method. The animals with platelets in the normal range were selected and anesthetized by Zoletil 50 at 4-6 mg/kg. The blood pressure cuff was wrapped on the upper arm and inflated to 70 mm Hg and laceration was created through standardized equipment (depth: 0.1cm, length: 0.5 cm) in both forearms that had no visible vein. And then blood was wicked from the cut with filter paper in a 30-sec interval until active bleeding had totally stopped, at which point the bleeding times were recorded. The incisions of all animals were healed within 7 d after the procedure. The bleeding times in both forearms in the same animal were not significantly different (P>0.2). The mean bleeding time was 155 ± 34.4 sec. The correlation between collected blood weight and bleeding time was significantly different (r = 0.67, n = 7). In conclusion, the modified Ivy method is a reliable method to assess drug efficacy in monkey.

P324 Development and Characterization of the Ultra Immunodeficient B6;129-Rag2^{tm1Fwa}IL2rg^{tm1Rsky}/DwlHsd (R2G2) Mouse Model

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The R2G2 (B6;129-*Rag2^{tm1Fwa}IL2rg^{tm1Rsky}*/DwlHsd) knockout mouse is the latest advancement to provide an alternative option in the highly immunodeficient mouse model category for oncology, immunology, and other biomedical research communities. This model was generated by backcrossing an IL2rg (common gamma) knockout model to an RAG2 (recombinase activating gene) knockout model. The resulting mouse lacks various cytokines, including IL-2, IL-4, IL-7, IL-9, and IL-15. In addition, this model lacks B cells, T cells, NK cells, and has a deficiency in lymphocyte development. The R2G2 mouse model is not only ultra immunodeficient but provides a model that is less leaky and more tolerant to gamma radiation than traditional *SCID* models. Herein we describe the development and characterization of the R2G2 mouse model, which includes breeding history, growth curve, complete blood count and serum chemistry, flow cytometry, tumor growth, and radiosensitivity.

P325 Comparative Study of Clinical Blood Examinations in the Cynomolgus Monkey (Macaca fascicularis) as a Heart Disease Model

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Heart disease greatly affects human quality of life. It is advantageous to use experimental nonhuman primate for human medical research because their circulation system is complicated and show many similarities to the human mechanism of circulation. Considering the intracellular ions and heart structure, research should be conducted using animals that are closely related to humans. However, reports of blood values in nonhuman primates are not sufficient, and there is no report on analysis of arterial blood and heart disease, in particular. Thus, we developed a spontaneous heart disease model in the cynomolgus monkey (*Macaca fascicularis*) that closely resembles humans. We used monkeys bred in our facility. The control group included 27 healthy macaques showing nonabnormal findings in screening tests, whereas the heart disease group included 20 macaques with valvular heart disease and cardiomyopathy confirmed by X-ray and echocardiography. The test monkeys showed no symptoms with no treatment except for imperceptible exercise intolerance. The studies were conducted according to the *Guide for Care and Use of Laboratory Animals*. Blood samples were collected from the femoral artery under ketamine sedation. In addition, X-ray imaging and echocardiography findings at the time of examination in the heart disease group showed pathological conditions such as turbulence and hyposystolic failure, which are similar to human heart disease conditions. Therefore, humanlike heart disease was observed in the heart disease group. An increased red blood cell distribution width was observed in the complete blood count because of chronic inflammatory related to heart disease, CO_2 excretion failure, and increased HCO_3 following the correction of acidosis in arterial blood gas. Furthermore, the development of a heart disease model was supported by significant increases in natriuretic peptides. In conclusion, the cynomolgus monkey can be used as a model of human heart disease. This is the first report that demonstrates the analysis of arterial blood and heart disease in nonhuman primate. This data may facilitate human research and help in the management of nonhuman primates.

P326 Seroprevalence of Common Murine Pathogens and Radiation Mortality in Pet Store Vendor-Acquired and Cohoused Mice

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While most rodent research colonies are specific-pathogen free (SPF) for murine pathogens, there is an increased demand by some investigators to perform studies using wild-caught or non-SPF vendor-acquired mice due to their varied microbiota and therefore varied immunity compared to SPF mice. A total of 80 pet store vendor mice were purchased and then continuously cohoused with SPF C57BL/6J mice under ABSL-3 conditions in individually ventilated cages (1 pet store mouse and 4 C57BL/6J housed per cage). Roughly 10% (n=10) of these pet store vendor mice were tested for a variety of murine pathogens at the time of arrival. Eight wk later, the C57BL/6J mice (n=10) housed with the original 10 pet store mice were tested for the same murine pathogens. Only one C57BL/6J mouse per cage was tested. The pet store vendor mice were all seropositive for at least 1 of the following murine pathogens: epizootic diarrhea of infant mice (60%), mouse adenovirus-2 (10%), mouse hepatitis virus (90%), murine norovirus (20%), mouse parvovirus (90%), minute virus of mice (20%), pneumonia virus of mice (80%), and Theiler's murine encephalomyelitis virus (80%). Similarly, the cohoused C57BL/6J were seropositive for mouse adenovirus-2 (40%), mouse hepatitis Virus (80%), murine norovirus (70%), pneumonia virus of mice (30%), and Theiler's murine encephalomyelitis virus (100%) after exposure to the pet store vendor mice. Cohoused mice were also seropositive for *Encephalitozoon cuniculi* (10%) and *Mycoplasma pulmonis* (20%), for which the pet store vendor mice were seronegative at the time of arrival. Additionally, cellophane tape tests of the original pet store vendor mice as well as exposed C57BL/6J were positive for fur mites and fur mite eggs, presumptively *Myocoptes musculinus*. Necropsy findings of 3 cohoused mice after 10 wk of exposure to pet store vendor mice revealed enteric infections of pinworms, *Cryptosporidium muris*, and *Hymenolepis (Rodentolepis) nana*. The cohoused mice had significantly increased survival times after total body irradiation when compared to SPF mice of the same strain. These findings are important as they identify some of the infectious, as well as potentially zoonotic, diseases present in pet store mice and also demonstrate that these agents can be transmitted to colony animals. This information may also provide better data for decisionmaking when housing pet store vendor acquired mice within research colonies.

P327 Inhibitory Effects for Rheumatoid Arthritis of Celecoxib in Collagen-Induced Arthritis Using Fluorescent Probes

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Celecoxib, a selective cyclooxygenase-2 inhibitor, is well-known to treat the symptoms of rheumatoid arthritis, osteoarthritis, and ankylosing spondylitis, and is widely used for positive control drug in preclinical studies of rheumatoid arthritis. To date, a number of studies show that visualization techniques by fluorescent probes can identify correctly, quickly, and efficiently. Especially, molecular expression level of target

protein can be identified by optical imagers. We induced rheumatoid arthritis in DBA/1 mice using type II collagen-induced arthritis (CIA) method, and then confirmed destruction of cartilage and bone of CIA model using commercially purchased 2 near-infrared (NIR) fluorescent probes. We induced arthritis at 9 DBA/1 mice, and then separated 3 mice per 1 group (normal, vehicle treated, and celecoxib treated groups). The effect of celecoxib on arthritis was assessed by clinical scoring system. Fluorescence signal intensity was compared to clinical scores and microCT evaluation. We identified that clinical score of vehicle-treated mice is 2-fold higher than celecoxib-treated mice. In the result of fluorescent probes intensity, moreover, we confirmed that celecoxib-treated mice showed 2-fold and 1.5-fold lower than vehicle-treated mice, respectively. In addition, celecoxib-treated mice showed that bone erosion of paws was decreased on 3D microCT images. These results demonstrate cathepsins and MMPs probes support the translational utility of molecular imaging for preclinical studies of antirheumatic drug such as celecoxib in the CIA model during disease progression.

P328 Use of Ultra-High Resolution X-Ray to Measure Tibia Length in Rodents

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Measurement of tibia length in mice and rats is commonly done as part of cardiovascular studies and is used to normalize heart weight in studies where cardiac hypertrophy may be observed. In comparison to body weight, which can continue to increase as mice age or decrease due to other conditions, tibia length remains stable once animals reach maturity and have been found to be a more accurate normalization standard. Collection of tibias at animal necropsy and bone preparation to obtain accurate measurements with calipers is both time consuming and difficult. In order to get an accurate caliper measurement of tibia length, technicians must disarticulate the lower leg and completely strip the soft tissues from the tibia, essentially creating a bone ready for histological preparation when we are not interested in a histological endpoint. We proposed to replace caliper measurements with ultra-high resolution x-ray imaging, which would allow for removal of the leg, followed by x-ray. This allows us to collect tibia length without having to undergo any further tissue trimming. This saves time, is more efficient, and we get an image with a scaled measurement. The caliper method creates a paper record that is prone to more typographical errors. Twenty animals from 2 studies (n=10 mice and n=10 rats) were x-rayed while under isoflurane/oxygen anesthesia and tibia length was measured using digital calipers. Following necropsy, those 20 tibias were measured by a single technician using traditional caliper methods and logged on a paper record. The difference between the 2 types of measurements in this validation pilot was less than 4%. Use of x-ray to measure tibia length in rodents represents a replacement of the caliper method and ultimately is more efficient. Additionally, x-ray has the added benefit of allowing for in vivo multiple measurements to be collected throughout the duration of a study, particularly if rodents are in a growth phase. X-ray measures for tibia length can be done in vivo or ex vivo depending on study needs.

P329 Effects of Water Decontamination Methods and Bedding Material on the Gut Microbiota

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Rodent models are invaluable to understanding health and disease in many areas of biomedical research. Unfortunately, many models suffer from lack of phenotype reproducibility. Our laboratory has shown that differences in gut microbiota (GM) can modulate phenotypes of models of colon cancer and inflammatory bowel disease. We and others have also shown that a number of factors associated with rodent research, including vendor, cage system, and bedding can alter GM. The objective of this study was to expand these studies to examine the effect of additional bedding materials and methods of water decontamination on GM diversity and composition. To this end, Crl:CD1 (ICR) mice were housed on corn cob or compressed paper chip bedding and provided water that was decontaminated by 4 commonly used procedures: reverse osmosis, autoclaving, sulfuric acid treatment, or hydrochloric acid treatment. This resulted in 8 different bedding and water combinations with 12 mice in each group. Feces was collected at day 0, and at day 28 (endpoint), fecal and cecal samples were collected. DNA was extracted from samples, amplified by PCR using conserved bacterial primer sets and subjected to next generation sequencing. Raw sequence data was analyzed and resulting metagenomics data compared among groups using principal coordinates analysis (PCoA) and permutational multivariate analysis of variance (PERMANOVA). Two factor PERMANOVA of cecal GM data revealed significant changes when comparing bedding and water decontamination methods, while no significant effects were noted in the fecal GM data. Subsequent PERMANOVA and PCA of cecal data revealed that several combinations of bedding and water decontamination methods resulted in differing GM, with corncob bedding most often associated in changes. These findings highlight the complexity by which environmental factors interact to modulate GM.

P330 Establishing Enhanced Gut Microbial Richness in Laboratory Mice (Mus musculus)

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There is speculation that the richness of the gut microbiota (GM) of laboratory mice has decreased over several decades through practices designed to render research mice free of pathogens. Unfortunately, this lowered GM richness may not replicate the GM richness seen in humans. As differences in microbial richness have been shown to influence disease phenotypes of rodent models, it is conceivable that mice with greater microbial richness resembling that found in humans would result in more translatable model phenotypes. We sought to establish mice with a higher GM richness than is currently available in contemporary rodent colonies. Forty mice from pet stores were obtained and 9 wild mice were caught. DNA was extracted from fecal samples, amplified by PCR using conserved bacterial primer sets and subjected to next generation sequencing. Taxonomy was assigned to selected operational taxonomic units (OTUs). GM richness, defined as the number of OTUs detected via sequencing, was then compared among groups. Their GM was characterized and found to be of significantly higher richness than that of contemporary laboratory mice. While the GM of pet store mice had the highest richness, they were also contaminated with the highest number of pathogens, including but not limited to mouse hepatitis virus, mouse parvovirus, fur mites, pinworms, and *Cryptosporidium* sp. Using broad spectrum antibiotics and antiparasitics, stop breeding strategies, and selective culling, these pathogens were eliminated from 5 pet store mice. Unfortunately, analysis revealed a significant decrease in the richness of their GM resulting in a profile similar to that of a laboratory mouse. Five wild mice were found to be *Cryptosporidium*-free and thus did not require the use of broad spectrum antibiotics. An antibiotic-free regimen consisting of strategic cessation of breeding and selective culling resulted in clearance of other pathogens with maintenance of GM richness, which was intermediate between that of lab mice and pet store mice. These mice will serve as invaluable sources of an alternative GM for future studies on the role of microbiota in model reproducibility and translatability.