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Animal Welfare, Training, and the 3Rs Posters

P100 3D Printed Caps to Protect Chronic Cranial Implants in Rhesus Macaques (*Macaca mulatta)*

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Chronic neurobiological studies in Rhesus macaques (*Macaca mulatta*) often involve the implantation of cranial hardware, including headposts, chambers, and screws. However, these animals often exhibit picking behavior around the cranial devices, leading to inflammation, dehiscence, and compromised tissue integrity, which can necessitate early study termination. Current literature on chronic cranial care primarily advocates for topical treatments, which may inadvertently exacerbate picking behavior. To address these challenges and enhance animal welfare, we propose the use of 3D-printed caps designed to protect cranial implants. These caps aim to mitigate the adverse effects of picking behavior by providing a physical barrier without impeding research protocols. By safeguarding the integrity of cranial implants, our approach seeks to prolong study durations and ultimately improve the quality of life for research animals. We designed and developed a 3D-printed head cap that securely attaches to a head post, covering cranial implants and surrounding tissue margins. Initially, we created the caps by soldering together multiple 3D printed grids made from PLA (*Polylactic Acid*) filament, which proved to be cumbersome and prone to breaking due to the strength of the macaques' manipulation. With the acquisition of our own carbon fiber 3D printer, we can now create a solid cap and connector piece that is more durable and autoclavable. The caps are secured to the head post using two screws and can be easily removed. One of our animals developed an insistent habit of picking at his implant site, which led to our design solution. By creating the initial PLA cap, we were able to successfully discourage this behavior and minimize further tissue damage. We then upgraded this animal to a carbon fiber cap, which has further reduced its ability to pick at the area, thanks to its increased coverage. The carbon fiber cap has proven durable and resistant to damage, requiring no modifications or repairs. We've since applied this same design to a second animal post-implantation, and it has been unable to access the surgical site, allowing for uncompromised healing. The development of these 3D-printed caps has significantly prolonged the research duration of one animal, extending its participation by 27 months to date. This has enabled us to collect valuable data for two separate investigators while also enhancing the animal's welfare and overall health. With a second animal now equipped with a 3D carbon fiber cap, we anticipate a reduction in the frequency of future implant and tissue revision surgeries. Going forward, we will continue to design and implement custom caps for other animals as they undergo implantation, further advancing our research goals and prioritizing animal well-being.

P101 3R Benefits of Using Star-Oddi Micro-HRT for Monitoring Temperature and Heart Rate in Group Housed Laboratory Mice

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The 3Rs (Replacement, Reduction, Refinement) are crucial for ethical and scientific progress in animal research. This study explores the benefits and practicality of implanting Star-Oddi micro-HRT loggers, designed for rats, into mice to monitor temperature and heart rate (HR), refining data collection and improving animal welfare. In a series of three experiments, ten (n=10) laboratory mice were implanted with Star-Oddi micro-HRT data loggers. Each experiment involved chronic stress (CS) exposure over seven days, with an injection of Isoproterenol on the last day to elicit a maximal HR response. Two variations of ECG amplification and bandwidth were tested, with two surgical approaches: tethering the logger to the skin and subcutaneous implantation without tethering. The quality of the recordings was assessed using raw ECG data and manual annotation, compared to on-board HR calculations and their associated quality index (QI) ranging from 0 (Best) to 3 (Worst). Amplification and bandwidth of the ECG signal were also assessed using power spectral density (PSD) analysis. Both ECG bandwidth variations provided high-quality data, but the higher bandwidth setting yielded more consistent HR readings with a lower QI due to the higher frequency content of the QRS waveform in mice. The onboard HR calculations correlated strongly with the raw ECG data, especially during rest, with a maximum HR of 804bpm, minimum HR of 329bpm, and average HR of 548±105bpm. The average HR after the Isoproterenol injection was 693±10.3bpm. The subcutaneous body temperature had a maximum of 36.93°C, a minimum of 34.03°C and an average of 35.41±0.69°C. Movement artifacts during peak activity were reduced by anchoring the logger. This study demonstrates significant 3R benefits by refining the data collection process with subcutaneous implantation, reducing the need for additional surgical procedures, and replacing more invasive methods for ECG and HR collection in mice. The ability to group-house mice with these data loggers further enhances animal welfare. Implementing Star-Oddi micro-HRT data loggers in laboratory mice provides reliable physiological data with a minimally invasive surgical approach, promoting a more humane and efficient approach to animal research.

P102 Dotting the Difference: Pre-Weaning Rodent Identification

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In laboratory animal research, reliable and humane identification methods are crucial for maintaining animal welfare and data integrity. We present a simple method to uniquely identify rodent pups in the pre-weaning phase while they are housed in the dam prior to performing traditional identification methods post-weaning. The method allows for accurate individual pup data collection with a litter, such as body weight, ano-genital distance, clinical observations, etc. The method utilizes a 27-gauge sterile needle and green paste animal tattoo ink to create unique identification numbers (1 through 20) based on a dot system. A single dot tattoo is applied to the plantar surface of 1 or more paws for numbers 1-10, and an additional single dot is added to the dorsal surface of the tail for numbers 11-20. Training for this new technique incorporates the 3 R's principle (Replacement, Reduction, and Refinement) using non-animal models for initial practice. Gummy bears, comparable in size to PND1 mouse pups, serve as an effective and engaging training tool. Trainees practice needle insertion into gummy bear paws to achieve the correct depth, ensuring that the tattoo ink does not penetrate through

to the opposite surface and using gentle handling to ensure the gummy bear maintains its original shape. This hands-on approach allows trainees to master the numbering scheme and accurately read the tattoos on previously marked gummy bears prior to handling animals. The technique's reliability has been confirmed, as the tattoos remain easily readable from neonatal stages through weaning age. Applicable to both mice and rats, this method proves versatile across different rodent models. This simple, easy-to-train procedure minimizes handling stress, ensuring the welfare of the pups, and does not interfere with traditional post-weaning identification methods. By integrating non-animal training tools and ensuring accurate identification, this method supports the 3Rs and robust data collection across rodent research models.

P103 A Comparative Study of Identification Methods in Non-Human Primates: Evaluating Tiny Mouse Ear Tags, Long Clamp Ear Tags, and Paint Identification for Optimal Animal Welfare

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In the field of non-human primate research, identification methods are crucial for tracking individual animals and collecting accurate data. This study compares three common identification techniques: tiny mouse ear tags, long clamp ear tags, and paint identification. Tiny mouse ear tags, despite their initial design for rodents, are increasingly favored by non-human primates due to their minimal size and enhanced comfort, reducing stress and behavioral disruptions. Long clamp ear tags, while durable, are larger and can cause more discomfort and complications such as ear tearing or infection. Paint identification, though non-invasive, is temporary and can fade or be removed by the animals, necessitating frequent reapplication and increasing handling stress. In this study, there were three different groups (six animals in each group, three pairs of DAMs, and infants). This study was conducted over a three-month period. The paint identification was prominent for the first 24 hours after application, but after that point, it was 75% non-visible. The long clamp ear tags stayed in place. However, the weight of the clamp caused the ear to droop down. Long ear clamps were also too distracting for other DAMs and caused them to be ripped out. The tiny mouse ear tags are painless, small, and bright enough in color to be visible from a distance. Our findings indicate that tiny mouse ear tags offer a superior balance of permanence, animal welfare, and ease of use, making them the optimal choice for researchers prioritizing the well-being and accurate identification of non-human primates.

P104 Leveraging Remote Behavioral Guidance for Successful Pair Housing of Non-Human Primates

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Pair housing of non-human primates (NHPs) poses many challenges, particularly due to the necessity for adequately trained staff. Despite the recognized benefits of social housing and regulatory requirements, resources offering practical guidance on pair housing techniques are scarce. To address this gap, we sought expertise from a collaborating institution and implemented remote behavioral coaching using Zoom video technology. Through Zoom sessions, personnel were guided in the process of pair housing NHPs, which included bar-pulling techniques, behavior observation, and management of antagonistic and affiliative behaviors. Coaching sessions followed a structured institutional pair housing process comprising of six stages of increasing physical contact, with advancing timeframes contingent upon observed behaviors. Typically, institutions may halt pair housing due to antagonistic interactions. However, this approach enabled behavioral specialists to validate antagonistic behavior in real-time, allowing the continuation of the pair housing process. This supported the successful introduction of twenty pairs of both male and female rhesus macaques with ages ranging from 4 to 14 years. It also facilitated the re-establishment of pair housing for NHPs who previously engaged in conflict resulting in wounding. By sharing our experiences, we aim to encourage similar collaborations to enhance social housing opportunities for NHPs and provide support for institutions lacking personnel with expertise in pair housing techniques. The innovative use of remote behavioral guidance via video conferencing presents a viable solution for institutions seeking to improve NHP welfare through successful social housing initiatives.

P105 A Cross-Species Comparison of Abnormal Behavior in Two Species of Macaque Monkeys (*Macaca mulatta* and *Macaca fascicularis*)

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Abnormal behavior has been studied in many species of captive nonhuman primates and may be an indicator of well-being. However, the display of abnormal behavior often varies across species, making comparisons difficult. The purpose of this study was to further assess similarities and differences in abnormal behavior and associated risk factors in two closely related species, rhesus (*Macaca mulatta*) and cynomolgus (*Macaca fascicularis*) macaques. Subjects were 194 cynomolgus macaques (105 male, 89 female) aged 2.4 to 20.3 years (M = 5.3 years) and 734 rhesus macaques (402 male, 332 female) aged 2.7 to 23.1 years (M = 7.8 years) housed at a laboratory facility. Trained observers conducted four 1-minute quarterly observations using a one-zero sampling method to record the presence or absence of abnormal behavior for each animal. Assessments were conducted in 2022 (September and December) and in 2023 (March and June). Animals were included in this study if they were observed during all four assessments and if their housing situation (i.e., singly- vs. socially housed) did not change during the four assessments. Results showed that significantly more rhesus macaques exhibited abnormal behavior in comparison to cynomolgus macaques (13.76% vs. 7.73%; $p < 0.05$). The most common abnormal behavior in both species was pace. Neither species showed significant age or sex differences in the display of abnormal behavior. However, there was a significant species difference in the impact of housing. Rhesus macaques were significantly more likely to exhibit abnormal behavior when singly housed (15.78% vs. 5.96%; $p < 0.005$). In contrast, there was no difference in abnormal behavior between singly- and socially-housed cynomolgus macaques. These results suggest that cynomolgus macaques may have different responses to the effects of a captive environment.

P106 Assessing for the Body Condition Score (BCS) of the Common Marmoset (*Callithrix jacchus*): A Standardized Method

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Body condition scoring (BCS) serves as a valuable tool in monitoring the health and condition of animals under human care, such as nonhuman primates like the common marmoset (*Callithrix jacchus*). As the common marmoset continues to grow in popularity as a New World primate research model, there is an increasing need for adapting standardized clinical measures, such as BCS, specifically to marmosets. Wasting and obesity are important health concerns

in common marmosets, yet a validated scoring system to track body condition has not been published. We propose a validated and standardized BCS system for marmosets based on observable and palpable anatomical features upon examination. During routine examination of the marmosets during semi-annual TB testing, we implemented a scoring system comprised of visual and tactile assessments of body fat disposition, muscle mass, and overall body conformation. This has allowed for the development of an illustrated guide for the numeric BCS system, specifically of the marmosets. Interobserver reliability testing was performed between members of the veterinary and research staff, and BCS scores were correlated with established health parameters to demonstrate effectiveness and reproducibility. Implementation of a standardized BCS protocol for common marmosets and all nonhuman primate species holds substantial implications for enhancing veterinary care, research management, and overall welfare. This systemic and objective means of evaluating body condition in the marmoset will allow for the early detection of health issues and will foster appropriate husbandry and dietary interventions, thereby promoting overall colony health and ensuring high-quality scientific research.

P107 ARRIVE Study Plan: Including Rigour Assessment Before the Conduct of Each Experiment to Maximize Output

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Reviewing individual experiments in the animal unit is a critical quality-control step to increase experimental rigour. Beyond details of the procedural and animal care and monitoring plans, it is a missed opportunity not to incorporate an assessment of the reliability of the prospective study. The ARRIVE Study Plan was developed based on the ARRIVE guidelines (an internationally recognised standard to describe animal experiments), current study plan documentation, and stakeholder engagement. An accompanying user guide and reviewer checklist allow assessment of the rigour and reliability of each proposed experiment. The benefits of animal research are realised through the production of high-quality, rigorous science that is ultimately reported in the literature. As such, each study's experimental design strategy needs to be reviewed to ensure best practices in animal research are followed and animals are not wasted due to the production of unreliable results. The ARRIVE Study Plan and reviewer checklist ensures that each study plan assessment is conducted in a robust and accurate manner and encourages the justification and collaboration of the experimental design strategy presented. For researchers, the ARRIVE Study Plan provides guidance and direction to conduct animal studies based on best practices. It promotes early adoption of rigour strategies such as randomisation and blinding and provides the researcher with a record of the experimental design plan that can be directly translated into a manuscript. Providing explicit detail on the experimental design upfront will further enable the animal units and IACUCs to identify opportunities to support researchers in implementing best practices, e.g., help with blinding and randomising cage location. The ARRIVE Study Plan provides the infrastructure for all those involved in animal research, including animal technicians, veterinarians, animal facility managers, IACUCs, and researchers, to achieve high-quality studies, maximizing animal welfare and experimental outputs. With high variation in the amount of information reviewed before the start of a study, the ARRIVE Study Plan, user guide, and reviewer checklist offer a standardized approach to maximize the reliability of a well-designed study.

P108 Artificial Intelligence: A New Tool to Enhance Animal Welfare and Research Outcomes?

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The timely identification and provision of veterinary care, achieved through proactive animal monitoring by knowledgeable, skilled, and dedicated research professionals, is an essential element of all high-quality animal care and use programs. This presentation outlines how a global research organization has successfully developed and validated a new animal welfare monitoring tool that incorporates innovative artificial Intelligence (AI) technology to predict when an animal may need veterinary care based on historical and current data. It will explain how partnerships were developed between veterinarians, data scientists, computer programmers, and end users across the organization to leverage existing knowledge of AI and apply it to the complex world of animal research. During the presentation, we will explain our journey so far, what we have achieved, the tools we've developed, and what we've learned about the use and value of AI in research animal welfare. The timely identification and provision of veterinary care, achieved through proactive animal monitoring by knowledgeable, skilled, and dedicated research professionals, is an essential element of all high-quality animal care and use programs. This presentation outlines how a global research organization has successfully developed and validated a new animal welfare monitoring tool that incorporates innovative artificial Intelligence (AI) technology to predict when an animal may need veterinary care based on historical and current data. It will explain how partnerships were developed between veterinarians, data scientists, computer programmers, and end users across the organization to leverage existing knowledge of AI and apply it to the complex world of animal research. This poster explains our journey so far, what we have achieved, the tools we've developed, and what we've learned about the use and value of AI in research animal welfare.

P109 Technical Refinement: A Gatekeeping Mechanism

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National Laboratory Animal Center (NLAC) has opened our AAALAC-accredited facility for biotech and academic scientists in animal studies. Users will complete an online orientation, qualification assessment, animal protocol application, and facilities tour before starting work at NLAC. In the past, we trusted their experience and technological capability as described in the protocol application. However, during the post-approval monitoring, we found instances of errors. These could significantly impact experiments and result in wastage of animals, for example, gavage errors resulting in animal deaths, head tilt after submandibular blood sampling, tail swelling due to leakage of intravenous injection, and abdominal metastasis due to inappropriate subcutaneous tumor transplantation. These skills can be improved by user technical assessment prior to any lab work and by providing training opportunities, if necessary. Therefore, we established an on-site skill assessment system. Users will first give an on-site demonstration of animal handling and techniques. For those needing more assistance, we provide correct or refined technical methods. In addition, we teach how to recognize abnormalities in animals caused by common technical errors. Since we started this system one year ago, human-caused abnormality, or mortality rates have been significantly reduced. Our work has successfully reduced unnecessary animal wastage while ensuring more stable experimental quality.

P110 I Wink You Got It - Dosing Caustic Substances by Retroorbital Injections

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Retroorbital (RO) injection is a common route for intravenous (IV) delivery of therapeutics and experimental substances in rodents. Chemotherapeutics rely on optimal IV administration, as they are often caustic, and extravasation is a known adverse sequela in many species. Animal models of oncologic disease experience frequent dosing of these agents and caustic substances. At our institution, trabectedin is used as a chemotherapeutic in glioma and osteosarcoma models. The original route for trabectedin injections was via the tail vein; however, a high incidence of tail necrosis was observed. RO injections were also trialed and demonstrated lower adverse effects compared to previous studies, but the incidence of clinical ocular cases remained high. We sought to establish a refined technique for the administration of trabectedin. Using our technique, mice received a protective ophthalmic ointment and proparacaine for local anesthesia around the globe. Injections resulted in the lowest volume possible for injection, the absence of air bubbles, and the cleaning of the needle hub with alcohol before injection into the animal. Trabectedin was injected slowly into the RO sinus of the mice, and the globe was wiped clean afterward. In total, 132 mice received trabectedin at least once, and 54 mice received two doses of trabectedin in a 2-month period, and no ocular issues were reported. We observed an effective reduction of ocular clinical cases with this refined technique, improved animal welfare, and animals were kept in the study longer. At our institution, when IV administration of a chemotherapeutic or similar caustic substance is required, we recommend following this refined approach to mitigate potential adverse reactions.

P111 The Impact of Experience on Clinical and Histologic Outcomes Following Retro-Orbital Injections

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The retro-orbital sinus in mice is a common site for blood collection and intravenous administration. While best practices for retro-orbital bleeding are established, the impact of user training and experience on post-procedural health following retro-orbital injection (ROI) has not been studied. We hypothesized that standardized training would enable novice injectors to achieve outcomes comparable to experienced injectors and that clinical and histological scores would worsen immediately post-injection but improve quickly. To test this, two novice injectors underwent a single hour-long training session, and their injection outcomes were compared to those of a highly experienced injector. Histopathological evaluations were conducted on three mice per injector at four-time points: immediately after, one day, two days, and seven days post-injection. Histologic assessments included evaluation of blepharitis, corneal abnormalities, hemorrhage, inflammation, Harderian gland inflammation, and Harderian gland atrophy, each graded on a scale from 0 (not present) to 4 (highly present). Clinically, minimal findings were noted across injectors and time points, with only occasional blepharospasm and blepharitis that was rarely persistent for greater than 24 hours. Less than three of 36 mice exhibited corneal lesions or Harderian gland atrophy. The most common histological findings were minimal inflammation, Harderian gland inflammation, and blepharitis, observed in over 50% of the mice within seven days post-injection. Novice injectors' histologic scores were similar to those of the experienced injectors, although the latter had lower scores on days one and seven. When all injectors were grouped, the average total histologic score decreased from 3.3 immediately post-procedure to 1.6 over time. These results suggest that ROI is well-tolerated in mice, rarely resulting in clinical complications or significant inflammation or trauma, even when only a minimal amount of training is provided prior to the researcher's implementation of the technique.

P112 Creating Institutional Change Using Multiprong Training and Communication Strategies

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Have you ever heard, "I wasn't aware of this initiative" or "When did this change in process happen"? Do you ever get frustrated when key messaging doesn't seem to reach the right people or resonate with them? Large or small, initiatives impacting people need effective change management strategies and continual reinforcement. This requires planning, multiple communication avenues, and building relationships to garner buy-in. The role of training in supporting the implementation of key initiatives is not a new concept. Uptake of training, its connection to the scientific purpose, why it is important to those providing the training, and doing the work can be more challenging. Implementing Low-Stress Handling (LSH) in mice and rats is a paradigm shift in how we do our work across regions and types of businesses. We will share how our organization is using training and multiple communication modalities to change fundamental business practices. By taking advantage of social learning, technology, gamification, and other engagement tools to reach audiences large and small, we have increased the uptake of messaging around LSH with >10,000 employees globally. Providing training resources and support in multiple languages, as well as continual reinforcement of the benefits to animal welfare, has had a positive impact on shifting our handling practices across our organization, with 24% of sites fully implementing LSH as of May 2024 and the majority of sites committing to fully implementing in 2024-2025. Open and transparent communication at all levels of the organization increases awareness and buy-in for initiatives like this from the top down and bottom up, ensuring we will reach our goal of >85% of all rooms, all study types, and all departments working with mice and rats of all ages transitioning to LSH. These strategies can help ensure your messages are reaching the right people from top to bottom.

P113 Give the Learners What They Want: Expanding Beyond Required Animal Welfare and Behavior Management Training

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Animal welfare and behavior management training for In-Life and Post-Life personnel is an essential part of a Culture of Care. These trainings help introduce and reinforce concepts, principles, and standards related to working with animals in a research setting. However, development, implementation, and oversight of this type of training program can pose several challenges due to a variety of factors. At our company, our animal welfare and behavior management training program is managed by three trainers/instructional designers who serve over 70 facilities globally. To ensure our training is meaningful, relevant, and engaging to all learners, they must incorporate a diverse array of species and the various types of work conducted, represent cultural differences, be available in multiple languages, be accessible and available in different modalities, are budget-friendly to develop, and time conscious to complete. To navigate these challenges, we have started to offer recommended or optional training (e-learning and webinars), engagement activities/games that coincide with industry-wide events (i.e., International Laboratory Technician Week) and/or internal animal welfare and behavior management initiatives (i.e., Low-Stress Handling), and job aids/posters that respect and celebrate the achievements of our personnel. These initiatives champion key messaging and reinforce and expand upon required animal welfare and behavior management training

completed throughout the year. This model promotes a Culture of Care and helps build resiliency by offering employees additional opportunities for professional development throughout the year. Since implementation, data collected has shown increased engagement, feedback from learners indicates they see these as value-added, and we have seen facilities taking ownership of the internal animal welfare and behavior management initiatives. These improvements all positively affect our animals' and personnel's well-being.

P114 Implementation of UV-Fluorescent Food Dye in Oral Gavage Training Procedures to Highlight the Importance of Control Contamination Procedures

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The prevention of test article exposure of control animals and biological samples is important for maintaining the integrity of a safety assessment study. Following several updates to site procedures, SOPs, and training sessions, instances of control samples with reportable concentrations of test articles were still occurring. A detailed review of study-specific information for the samples with contamination was completed, and it was noted that the use of non-aqueous vehicles was a common factor. An internal study using a common non-aqueous vehicle containing UV-fluorescent food dye and standard oral gavage dosing procedures was conducted in rats, rabbits, and dogs. Following the completion of dosing procedures, the condition of the technician's PPE, dosing supplies and surrounding area, animal caging, animals, and cleaning procedures were evaluated for traces of the food dye using a black light. The information gained from the evaluation was used to improve site practices for PPE, dosing procedures, animal caging concerns, and general cleaning procedures. One of the major components of a successful training environment is to provide information in several manners that will allow for it to resonate with individual trainees and learning styles. The implementation of the use of UV-fluorescent food dye when training oral gavage procedures has assisted with technician self-awareness during dosing procedures and has provided a memorable visual experience that is unique to an individual trainee's success. This addition to our training program has aided in highlighting the importance of controlling contamination procedures and how easily substances can spread.

P115 The Effect of Positive Reinforcement Training on Housing and Transport Stress in Laboratory Pigs

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The health and well-being of pigs used as translational models can affect the outcomes of studies. Increased levels of stress secondary to housing and handling have detrimental effects on pigs, including decreased immune function, inhibition of reproduction, and delayed wound healing. This stress can cause inherent study errors that may result in increased animal numbers, repetition of experiments, inaccurate results, and ultimately decreased animal welfare. Positive reinforcement training has been shown to decrease stress in a variety of animals, but this has not been published in pigs. We hypothesized that pigs undergoing positive reinforcement training would display fewer stress-linked behaviors in both housing and transportation. A total of 18 research pigs were evaluated. The experimental group consisted of 6 pigs from a wound study in which pigs underwent daily positive reinforcement training where both commands and human touch were reinforced to achieve study-specific goals, such as salivary cortisol collection, bandage changes, daily weights, and sling training. The control group consisted of 12 pigs from another study who did not receive training. All pigs were individually but socially housed in normal housing conditions. Ten-minute videos were taken of each pig with a handler present, both in normal housing conditions and post-van transport. Videos were randomized and scored by blinded reviewers for behaviors linked to stress or welfare using location-based ethograms. The results were analyzed via a t-test for statistical significance. Results showed a significant decrease in stress-linked behaviors in pigs who undergo training versus the control pigs ($p<0.05$). In addition, the use of training also enabled data collection for the study with minimal need for sedation and, subjectively, a better environment for the pigs and handlers. Based on this pilot study, we conclude that positive reinforcement training can decrease the occurrence of stress-linked behaviors following transport and decrease vocalization and stereotypic behavior in housing. Therefore, this type of training for laboratory pigs should be considered in translational pig models for better animal welfare and potentially more accurate outcomes.

P116 Training New Zealand White Rabbits (*Oryctolagus cuniculus*) to Facilitate Cooperative Participation for Research Procedures

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Non-aversive training methods, including positive reinforcement and the classical conditioning methods of desensitization and counterconditioning, can be utilized for animals in the laboratory setting to facilitate voluntary participation in veterinary exams, routine husbandry, and study procedures. Voluntary participation mitigates stress and improves animal welfare and the quality of research. When stressed, research rabbits exhibit behaviors such as avoidance of handling, spontaneous urination, thumping, boxing, certain vocalizations, scratching, and biting. In our facility, a plan of desensitization and counterconditioning was implemented for rabbits prior to research use to foster a positive association with common handling and research procedures. A food preference test found that pumpkin seeds were consistently a high-value reward. Technicians were provided a written step-by-step plan, which began by creating a positive association with human approaches towards rabbits in their home cage. Over time, staff systematically worked through creating positive associations to cage side staff, handling, restraint, blood collection, hair shaving, and physical exams. Rabbits were exposed to each aspect in small increments, increasing cumulatively across training sessions. Progression from one step to the next could only occur when rabbits met behavioral criteria indicating a lack of stress, including continued consumption of treats and relaxed body postures. Adjustments were made to the training plans based on each individual rabbit's behavior, and cumulatively, the time to study started did not significantly delay. These adjustments included remaining at a particular step for multiple sessions or additional sessions. Overall success was defined by cooperative participation in procedures as indicated by relaxed body postures and the absence of stress behaviors, including continued consumption of treats and decrease or elimination of required restraint. Implementation of non-aversive training methods demonstrates how a robust behavior and training plan can aid in low-stress completion of husbandry, veterinary, and study procedures and should be considered as standard practice for improving research outcomes and animal welfare.

P117 ApRATments: Unique Housing Option for Training Colony Animals

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In our animal facility, we have colonies of training animals, including rats and hamsters. These animals are donated from labs after study completion and are usually in our training colony long-term, resulting in weight gain for some. We previously implemented ventilated caging with platforms for our training rats, but staff raised concerns that the space was too small for the large males, even though it met floor spacing guidelines. Our facility had recently purchased new multi-level ferret caging, which inspired us to try with our teaching colonies. We have staff who use these cages for their pet rodents at home and gave us advice about large group housing. We purchased four cages: one cage for each sex of rats to combine 11 males from five cages and five females from two cages; one cage for our hamsters to combine four males from two cages; and one cage for change-outs. We were concerned about introducing the animals in these cages since they were previously housed in pairs or trios, so we provided food and water stations and many types of enrichment on each level of the cages to prevent resource guarding and fighting. We also included litter/forage boxes with contact bedding. We were pleasantly surprised that none of the animals fought in these new cages, including the hamsters that were previously fighting in our standard rodent caging. After a week in their new caging, there were some cases of rat pododermatitis that we were not expecting but were resolved by placing fleece lining on the cage pans and ramps. We chose fleece because we already use it for other species, and it does not fray or make strings. We have observed that the animals have had increased activity – especially bar climbing- and have been more curious and interactive with their caretakers and have been choosing to sleep in groups together. This trial has been successful in many ways, such as providing more space, enrichment, and opportunities for social interaction for these training animals. One very unexpected result was the induction of estrus for the females, who were housed in the same room as the males, and they have since been moved to a new room. This was a great learning opportunity for our staff to see rat estrus behaviors. Overall, this has been a popular change for staff because they are able to watch the rats and hamsters display more species-specific behaviors and address space concerns.

P118 Tunnel Trouble? A Cost-Effective Way to Introduce Mouse Tunneling to your Facility

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Tunneling is a method to move mice in a less stressful way than the widely used tail-handling method. It reduces stress in mice by allowing them to choose to walk into the tunnels as opposed to being forcibly picked up by the tail, mimicking predator/prey behavior. Although mouse tunneling is not a new technique in rodent husbandry, we have found a cost-effective and environmentally friendly way to implement this practice. Instead of purchasing tunnels, we evaluated two no-cost options: 1. utilizing the enrichment huts already in the mouse cages and 2. modifying broken/cloudy water bottles by cutting both ends off to create a tunnel. The broken water bottles were screened before modification to assess the safety of the broken bottles. If they were broken in a way that was unsafe to modify for the animals, they were not used. Our facility has a supply of broken water bottles collected for recycling that we can repurpose for use as tunnels. An advantage of this cost-effective option is that the tunnels can be easily replaced if needed since they are repurposed water bottles. All options were tested for ease of use and mouse preference. Mice showed a preference for their own hut that was already in the cage, followed by a new, clean hut and then the repurposed bottles. We tried commercially available samples, but the mice did not show a preference for them over our no-cost options. Once we determined mouse tunnel preference and best practices, we introduced this to our animal husbandry staff and held multiple training events to learn this new technique. Staff were initially worried about this, making cage changes take longer and resistant, but after timing cage changes, we found it takes the same amount of time or less than moving the mice with the tail handling. Previously, staff averaged changing 40 cages/hour using tail handling and tunnels. We were able to increase this average to 50 cages/hour. Tunneling is now a standard practice for our rodent husbandry program. Utilizing existing huts and repurposing mouse water bottles are great no-cost options to improve mouse welfare and introduce tunneling to your rodent facilities.

P119 To Have or to Hold? Refined Methods in Mouse Handling

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The standard approach of grasping laboratory mice by their tails has been demonstrated to significantly impact their anxiety levels & physiological stress responses. Consequently, there is a pressing need for more refined methods of mouse handling to gain deeper insights into their biological & affective states. Techniques such as tunnel or open handheld methods offer mice a lower level of anxiety and are therefore deemed preferable for physical restraint. Our aim was to investigate if implementing tunnels for routine husbandry handling could lead to decreased anxiety during behavioral tests compared to mice handled by their tails. 24 male & 24 female C57BL/6J mice were divided into 3 groups. Each group underwent routine husbandry with three differing animal handling groups over three months. Group one (n = 16) animals were handled by the tail every two weeks at cage change. Group two animals (n = 16) animals were transferred via a tunnel that resided in the cage from the start of the study. Group three (n = 16) animals were transferred via tunnel every two weeks at cage change, but the tunnel did not remain in the cage. Afterward, the mice were behaviorally evaluated through spontaneous alternation, novel object recognition, elevated maze test, & fear conditioning. Tunnel handling, in general, resulted in reduced anxiety, improved spatial learning, & enhanced exploration. Mice housed with tunnels in their home cages displayed the most significant improvements, while those transferred via tunnels showed some benefits but encountered practical challenges as they were less inclined to enter the tunnels voluntarily. The results between the groups were sex-dependent in the majority of tests. Neither of the two tunnel-handled mouse groups exhibited the anticipated increase in locomotion among females; instead, tunnel-handled males displayed elevated locomotion, indicating that tail handling may suppress exploratory behavior in males. Tunnel handling, in both groups, led to the most significant improvements in spatial learning among females, an effect driven by their increased exploration of objects during the familiarization phase. In summary, our results endorse the adoption of tunnel handling as it enhances behavioral performance and, in turn, conveys an improvement in animal welfare.

P120 Development and Refinement of Intratracheal Inoculation of Syrian Gold Hamsters in ABSL-4 Containment

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Before the pandemic, intratracheal inoculation of Syrian hamsters was not a commonly requested procedure in our biocontainment facility, but recently, this method has been used for inoculation with SARS-CoV-2 and ABSL-4 viruses. In ABSL-4 containment, we work in a biosafety cabinet while wearing a positive pressure suit, so visibility, range of motion, and dexterity are all impeded. Additionally, some traditional methods of intratracheal inoculation in rodents involve percutaneous access and the use of a sharp, which poses a safety risk. While there is commercially available

positioning equipment for mice and rats, species-specific equipment is not available for hamsters. So, we have modified equipment to satisfy these constraints and improve workflow while maintaining technician safety and animal welfare. Our first attempt used umbilical tape to suspend the hamster from its incisors, similar to published intubation platforms for mice. Because hamsters have shorter incisors, they often became disengaged before inoculation, requiring repositioning and prolonged sedation. To mitigate this, we created a "hammock" to suspend the animals and adjusted the lighting for better visualization. This resulted in a 6-8-minute gap between each administration with high variability. This was unsatisfactory because increased manipulation of the animals increases the risk of human exposure to the virus. Our second attempt utilized an intubation stand for mice, suture, the same "hammock," and a fiber optic light source from a rodent intubation kit, permitting visualization of the opening of the trachea well enough to inoculate. The interval between administrations dropped to 3 minutes. A final version will elevate the platform unilaterally and attach the light source for improved visibility with less manipulation. If notably better, it will also be used to train other technicians. Although all attempts resulted in successful inoculation, our changes have increased accuracy and efficiency and reduced anesthetic time and staff time in maximum containment.

P121 Development, Refinement, and Implementation of Home Cage Tail Vein Microsampling in the Nonhuman Primate – A 3R's Advancement

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Nonhuman primates (NHPs) remain a critical model in drug discovery and development. The refinement of microsampling (collecting no more than 25µL blood) has been applied to other species in our vivarium, and we investigated implementation in primates. Typical blood sampling procedures frequently involve removing conscious animals (cynomolgus macaques) from the home cage and transporting them to a nearby procedure room, where the minimal amount of restraint needed is used to acquire an approximately 250µL blood sample from a femoral vessel, noting that typical analysis only requires a 20µL aliquot. After background research and benchmarking were conducted, with institutional and research support, we developed a method for tail vein collection of 20µL from conscious monkeys remaining in their home cage. This multidisciplinary effort involved members of scientific as well as animal welfare/veterinary teams and utilized positive reinforcement methods to acclimate animals to novel procedures. Compared to typical procedures, this method resulted in an approximately 75% reduction in total blood sample volume collected for NHP DMPK studies and an approximately 50% reduction in chair restraint instances and time, as well as improved sample collection efficiency and safety. This 3Rs advancement refines and improves our use of NHPs while maintaining sample quality and integrity. This work was conducted according to GSK's Policy on the Care, Welfare, and Treatment of Lab Animals and reviewed by the Institutional Animal Care and Use Committee at GSK.

P122 Early Sex Determination in Axolotls (*Ambystoma mexicanum*) Using Non-Invasive Genotyping

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The axolotl (Ambystoma mexicanum) serves as a valuable vertebrate model for regeneration research due to its remarkable limb and central nervous system regenerative capabilities. Additionally, axolotls have gained significant popularity in the pet trade. However, reliable sex determination in these amphibians remains a challenge. As with other animals, overbred female axolotls are subject to stress, illness, disease, and even death. Traditional methods rely on the presence of a cloacal bulge in mature males, which can take up to 18 months to develop, hindering breeding programs and research efforts. This study aimed to develop a non-invasive genotyping method for early and accurate sex identification in axolotls. Skin/slime swabs were collected from a cohort of n=20 axolotls with known sexes (obtained from a reputable pet breeder colony) to minimize sampling bias. Leveraging established PCR protocols, we developed a species-specific PCR assay targeting a female sex-linked gene. The implemented assay achieved 100% specificity and sensitivity in sex determination. This novel genotyping method offers significant advantages for both research and pet management applications. Early and reliable sex determination facilitates the establishment of appropriate social housing configurations, preventing unplanned breeding in research colonies and pet environments. Moreover, it aligns with the 3Rs (Replacement, Reduction, and Refinement) principle in animal research by potentially reducing the number of animals required for breeding purposes and providing a more positive life experience.

P123 Getting to the Root of the Problem: How to Maximize Swine Environmental Enrichment Opportunities in the Laboratory Animal Setting

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Swine rooting refers to the natural behavior of pigs to root or dig in soil with their snouts. Given the significance of rooting, providing opportunities for swine to engage in this and other naturalistic behaviors is an important aspect of promoting their overall welfare and ensuring regulatory compliance. We describe the environmental enrichment methodologies and materials developed at the author's institution to promote natural swine behaviors such as rooting. Methods described in a "DIY" format include rooting/foraging tubs, puzzle feeders, electrolyte ice blocks, "Piggy Picasso" artwork, and swine raking. Designating specific areas of the vivarium for swine exercise and storage of edible enrichment items are also outlined for readers. Notable benefits of incorporating these enrichment concepts have been reduced stress during caretaker interactions, reduced handler acclimation times, and allowing swine a sense of control over their environment by choosing their desired enrichment activity and item. Implementing these measures as part of an animal welfare enrichment program not only impacts the sensory and mental stimulation of the swine but builds a work culture of top-notch animal care, engaging staff to directly interact with their patients and work as a team to create new ideas to enhance overall well-being for our animal heroes.

P124 Sustainable Enrichment

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Enrichment is an essential part of any animal's life. The purpose of enrichment is to stimulate the animal's minds and encourage them to perform species-specific behaviors. Our company implemented a donation program to collect empty glove boxes, toilet paper tubes, and paper towel tubes from our employees site-wide to be repurposed to make enrichment items for many different species. Originally, 100 training rats and 20 training rabbits received the tubes with approval from the IACUC, and after permission from the study directors, all animals were eligible for enrichment tubes and glove boxes. This program has been running for over a year, and there have been no animal welfare issues or observed adverse behaviors related to the enrichment items. These cardboard items are autoclaved before use to ensure that no outside pathogens are introduced into the animal's home cage. Glove boxes can be used as foraging boxes for animals such as rats and non-human primates by filling them with species-specific treats and foraging materials. Cardboard tubes

can also be stuffed with various species-specific treats for rabbits, rodents, and non-human primates. Rodents will often shred the cardboard to use as nesting material. They can also use the tubes and boxes as shelter. These enrichment devices can last for days or be shredded in a matter of hours. These items are safe for use and can be left in an animal's home cage. By creating a donation program, institutions can save money on enrichment devices for their animals. Disposable tunnels or shelters for mice can be bought for about $0.50 each, and plastic tunnels for up to $5.00 each. Alternatively, donated toilet paper tubes can serve the same purpose, which could save companies money on materials and cagewash costs. Providing animals with extra sustainable enrichment can reduce the wear and tear on the reusable enrichment devices, decreasing the need to purchase replacements. This reduces our carbon footprint and the cost of supplies. Additionally, this can provide companies with an opportunity to form a sustainable enrichment program while educating employees on the responsibilities of housing laboratory animals in a research setting.

P125 Maximizing Space While Enriching It: A Novel Enrichment Device

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Providing enrichment to laboratory animals enhances their quality of life and promotes a healthy physiological state of being. Rotation of enrichment items provides the opportunity for the animal to be stimulated in various ways. Currently, there is no device available to use for efficient rotations of enrichment items. A major factor that inhibits the use of some enrichment devices is floor space in an enclosure. The enrichment mobile device we created has the capacity to hang four different enrichment items, allowing you to have components that stimulate a variety of species-specific behaviors. It is made from a PVC pipe crossbar with stainless steel chains and attaches to the sidebars of the enclosure via a sturdy metal pipe frame. All materials can be sourced at hardware stores, making it cost-effective and accessible to build at any facility. The design also allows for easy rotation of the mobile so staff can move it out of the way to clean the enclosure or to switch enrichment items out. In addition, all the parts can be easily cleaned and decontaminated for reuse. Our device was designed with smaller pigs in mind but could be scaled or modified for other research animals. When used, it encourages animals to engage in species' typical behaviors, such as foraging, when used in conjunction with edible items. The creation of this enrichment mobile allowed for enrichment items to be held off the floor, maximizing floor space, and the chains allowed for easy change of enrichment during rotations.

P126 Evaluation of Rodent Enrichment Preference in Common Laboratory Stocks and Strains

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Providing enrichment is a key facet of good animal care and welfare. The specific enrichment items provided can vary at each facility. While some literature exists on possible rodent enrichment preferences, there have been few recent studies. Additionally, there has been little examination of possible preference differences among rodent stocks/strains. For this study, six different common rodent enrichment items were tested using both sexes of four stock/strains of mice and two stock/strains of rats. Animals were divided into groups based on the enrichment item they received. The purpose of the study design was to observe any preference differences between individual stocks/strains or sexes within a species. The study comprised 72 distinct groups, each group representing a set of cages of socially housed animals of a single-sex of one stock/strain and given one of the six enrichment items. To evaluate preference, an ethogram was utilized for recording observed animal behavior and interaction with the enrichment. Additionally, enrichment items were observed daily for evidence of use as well as scored on the remaining quantity of the item to evaluate their durability over the course of a typical cage change cycle. Preference results were assigned based on the number of observed interactions with the enrichment, as well as observable evidence of use. The durability results were assigned based on the scores given for the amount of enrichment remaining in the cage. Results showed that there were significant differences in rates of interaction and evidence of use of the different enrichment types. Overall, across all strains and sexes, for both mice and rats, enrichment items that allowed the animals the ability to shelter in addition to chew on (cardboard tunnels, paper half huts) appear to be strongly preferred over the other enrichment types tested. There were also some differences in the preference results between individual stocks/strains within the same species. Many of the inbred strains tested (such as C57BL/6 and Balb/c mice) seemed to show a stronger preference for certain enrichment items, such as paper twists, as compared to the outbred stock (ICR/CD-1), which showed a stronger preference for other items tested instead. This may indicate that different strains may prefer different enrichment types, and animal welfare may be enhanced by tailoring enrichment to the specific stock/strain of animal being housed. In summary, our results indicate there is a preference for certain enrichment in mice and rats. Animals are more likely to utilize these preferred enrichment items, and these preferences may be worth considering when choosing enrichment options. The study described in this abstract was approved by Inotiv's IACUC.

P127 Biologically Appropriate Enrichment and its Role in Barbering NSG Mice

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Barbering in laboratory mice raises welfare concerns and affects research quality. NOD.Cg-Prkdcscidll2rgtm1Wjl/SzJ (NSG) are a prominent model for translational studies of human disease. Despite their prominence, strain-specific barbering research is lacking. While enrichment is recommended to reduce barbering, its effectiveness remains unclear, leading to arbitrary selection based on availability or cost. In our NSG production colony, barbering significantly impacts welfare and timely mouse supply to researchers. From January 2024 to the present, we provided different housing conditions with biologically appropriate enrichment (one that fulfills a biological drive for the mouse) to NSG mice on separate ventilated racks (n=5). Cages in our production room housed up to five mice, either of one sex or paired for mating, with enrichment items, including sunflower seeds for foraging, red plastic hides for shelter, wooden blocks for chewing, or new paper-based bedding. We had four different housing conditions and one control with no added enrichment beyond a nestlet. We collected six weeks of baseline data from 796 cages in our production room. During this period, barbering was observed in 24.7% of cages (197/796). Significant variations in barbering were observed among sex groups (Fisher's exact test, $p < 0.001$), with 13.8% (28/203) occurrences in female-only cages, 30.3% (69/228) in male-only cages, and 27.2% (100/367) in mixed-sex cages. Barbering was notably associated with longer observation periods (Fisher's exact test, $p < 0.001$). Specifically, barbering was observed in 12.9% (39/302) of cages monitored for 1-2 weeks, 22.5% (65/289) for 3-4 weeks, and 44.9% (93/207) for 5-6 weeks. No statistically significant variances were found in barbering among different ventilated racks (Fisher's exact test, $p = 0.088$). An ongoing analysis will assess the effectiveness of enrichment on barbering at the cage level. These results contribute to our understanding of strain-specific barbering, underscore the importance of enrichment, and identify priorities for future research, including the assessment of enrichment effectiveness.

P128 Enhancing The Enrichment Experience: Incorporating Cultural Foods into a Nonhuman Primate Enrichment Program

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When nonhuman primates are enrolled in Good Laboratory Practice Studies, they become limited to specific types of enrichment and food. Traditionally, GLP guidelines restrict the use of non-edible destructible enrichment items such as paper and cardboard. Exploring underutilized food items can bring novelty to the implementation of animal enrichment. The idea of introducing banana leaves and rice paper as destructible items stems from their use in Filipino cooking. Drawing inspiration from elements in my own culture has continued introducing novelty into the environmental enrichment program. Rice paper and banana leaves are commercially available food-grade items that are safe for consumption in most nonhuman primates. This allows them to be the perfect substitute for paper and cardboard. Like paper and cardboard, they can hold a smear and tear fairly easily. These foods are readily available at a reasonable cost through wholesale and established food vendors. By adding these items to our enrichment rotation, we have been able to increase the novelty of common-use toys and manipulanda. Banana leaves can be wrapped around or weaved into Holee Molee's. They also work great as standalone items; banana leaves are great for shredding, as browse, or made into woven packets. Rice paper can be dry, wet, or frozen, adding additional tactile stimulation through the different preparations. It can be layered onto or wrapped around hanging tires. Additionally, rice paper is easily dyed with food coloring. Both items add an olfactory element of engagement and serve as colorful additions to enrichment. Their dynamic textures enhance the animals' experience. One benefit to Rice paper is that it requires no after-use cleanup, as it is consumed or dissolved completely. This may be especially beneficial for facilities with concerns about paper-based products entering drain systems. Leftover banana leaves may need to be removed prior to washdown. These additions are simple solutions to add novelty to an animal's enrichment plan, even in a GLP setting. The exploration of underutilized food items by considering food items from multiple cultures and traditions has allowed us to continue finding innovative ways to modulate NHP enrichment.

P129 What is the Perfect Primate Enrichment? Using A Multi-Beneficial Enrichment System for Non-Human Primates Used in Biomedical Research

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Environmental enrichment is critical to promoting animal welfare and species-specific behaviors for non-human primates used in research. There are multiple types of environmental enrichment, one being occupational enrichment, which requires the animal to use species-specific behaviors to manipulate a device or puzzle to get the food/reward. There are many prefabricated occupational enrichment devices available on the market for animals housed in laboratory settings. Many of these devices are well received by primates; however, over time, the animals may become bored/uninterested. Additionally, these enrichment options are often cumbersome to get on/off the cage without potential injury from the animal, as these devices are clipped directly to the cage. When placing and removing these items, our staff were required to use a metal "shield" to keep the animal from being able to access/injure the staff. Our institution attempted to address this issue by using an enrichment system that locks into a frame that is bolted to the front of the cage. There are over fifteen different device options that all lock into this frame with the use of a screwdriver. We decided to start with two of these options to see how they were received by our animals and found that most of our macaques preferred the new devices; animals spent more time performing foraging behaviors when compared to the previous enrichment device options. In addition to being preferred by our animals, the new devices were found to be a safer option for our staff to attach/remove from the cage as the devices are taken on/off with a screwdriver, putting the staff's hands at a safe distance from the cage. The behavioral benefits for our animals and the safety benefits for our staff made the new devices an optimal occupational enrichment option.

P130 Novel Approaches to Social Housing and Enrichment to Improve Welfare for Rabbits, Guinea Pigs, and Mice

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Male mice and rabbits traditionally have not been social housed at our facility due to conspecific aggression. Guinea pigs, although successfully social housed, tended to outgrow the standard caging that was readily available, which would preclude social housing due to floor space (~105 in.^{2}/animal, The Guide minimum). The Animal Care and Training teams worked collaboratively to come up with various combinations of ideas to improve the welfare of these three cohorts of animals. For male mice, we used larger rat caging, more enrichment, and dual access to water lixits and feeders. For rabbits, males were paired with females (once they were neutered), and additional enrichment was added, including human interaction outside their cage environment. For guinea pigs, we utilized older-style rabbit cages with tunnels for improved floor space (~784 in.2/animal) and modified the flooring using a recycled polycarbonate sheet to mimic a solid bottom caging with bedding. Improvements were incremental, but overall, we found a balanced approach (i.e., more space, two more pieces of enrichment/animal (rather than four), and solid flooring with bedding). For male mice, we observed far less flooding and wet cages (zero wet bedding events, compared to the handful of cages every morning), animals were less aggressive (subjective assessment based on fewer observations of fighting, vocalizations, and vet requests), and we used far fewer cages due to social housing (housing 3/cage, we used 66% fewer cages). Male mice commingling worked better with litter mates or before sexual maturity, but it still worked as long as there was group housing (3-5/cage), not pair housing, and multiple points of access to both water and food. For male and female cohabitating rabbits, they were far less skittish and more of a "willing partner" as they were more likely to come to the front of the cage when interacting with animal handlers, which, although subjective, was a comment repeated multiple times in this context and was a great win for compassion satisfaction. For guinea pigs, the recycled rabbit caging with tunnel allowed us to keep the animals social housed since space limitations would have precluded social housing as the animals gained weight, and we also saw an increase in activity levels in the form of guinea pig "popcorning" that we subjectively feel can be ascribed to the additional floor space and enrichment that was provided. In conclusion, the interactions of the two teams with the animals and the continuous idea-sharing from all staff members allowed the program to truly blossom and benefit the welfare of these three species. The combinations of non-standard housing, novel enrichment, and human interaction made these efforts successful.

P131 Implementation of a Novel Mouse Plethysmography Model for Measuring Acute Changes in Respiratory Parameters During Inhaled Dosing

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The potential for inhaled compounds to induce adverse effects in the airways is a challenging hurdle in inhaled drug development. Respiratory irritation following acute inhalation in in vivo studies is one issue that should be mitigated early in the drug discovery process before progressing to studies using large numbers of animals. At AstraZeneca we have adopted a novel approach when it comes to performing early in vivo assessment of new chemical entities for pulmonary drug delivery. Our approach ensures we have calm, well-trained animals in statistically powered experiments using modern, innovative technology. During lead optimisation it is uncommon to have synthesised the amount of test material needed to perform rodent inhalation studies. It is more common to administer the test material by intratracheal administration (IT). This dose route comes with inherent limitations: it is more invasive for the animal, the distribution in the lung is less homogenous when compared to inhalation, and it is not possible to measure the acute effect of the test material on a conscious animal as IT dosing is performed under anesthesia. To mitigate these issues, AstraZeneca has designed and built a new rodent nebulisation system that utilises 15 times less test material when compared to commercially available in vivo inhalation systems. Along with this new inhalation system, we have tested and validated the Buxco® Head-out Plethysmographs and Alley restraint system to measure respiratory parameters during inhaled dosing. We have also adapted the training of our mice to the Alley restraint with an ethical and 3R mindset using a refined training procedure and positive reinforcement to ensure our animals are handled and trained so they are calm and breath well in the plethysmograph tube. With the new inhalation method combined with the sensitivity of the Buxco system and calm well trained animals we can reduce our animal numbers and can still detect changes in multiple respiratory parameters during inhaled studies earlier in drug discovery. We can provide an early robust plethysmography assessment across our diverse inhaled portfolio to de-risk and rank compounds prior to committing to inhaled MTD/DRF studies.

P132 Incorporating a Token Economy System as a Reinforcement Schedule for Rhesus Macaques

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In the laboratory environment, high levels of motivation and engagement are crucial for the reliability and validity of behavioral data used to demonstrate functionality in the neuroscience field. Most importantly, the animal's experience should be prioritized from an ethical perspective. However, traditional motivation and teaching strategies for Rhesus macaques (Macaca mulatta), such as water restriction and trial and error, have been shown to cause frustration in humans with overnight water restriction (Zhang J et al., 2020; Zhang J et al., 2021). In the science of applied behavior analysis, token economies are used with children to maintain behavior over extended time periods due to increased motivation while providing the subject with the agency to diversify their own rewards (Hackenberg TD, 2009.) A token economy system involves the use of secondary reinforcers (tokens) that can be exchanged for larger primary reinforcers (such as an animal's top food preference for that day). This study investigates the incorporation of a token economy system as an alternative motivation and teaching strategy to increase trials per minute in a behavioral computer screen task. To measure animal experience, we compared animal-led breaks and session duration in a control condition (no token system provided on screen) with a study group (token system provided on screen) in a troop of male rhesus macaques acting as their own controls. The macaques were trained to earn visual tokens (stars) by completing specific gameplay tasks, and once all were earned, they were able to be exchanged for preferred reinforcement that the animal previously selected in a daily preference test. Preliminary findings show animals engaged for longer periods (mean = 78 min vs. 61 min, 24% increase) and performed fewer frustration-related behaviors (mean frequency = 0.1 vs. 56, 199% decrease). However, the number of trials per minute was not significantly different between conditions (mean = 49 tpm for both), suggesting token systems improve animal experience and maintain data quality. This study contributes to the development of more effective and humane research practices in the study of primate behavior and cognition.

P133 Innovative Cage-Based Technique for Sampling High-Quality Mouse Urine

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Accurate and humane collection of mouse urine samples is essential for various research studies. Conventional methods often cause stress to the animals and compromise sample quality. A natural approach to urine collection for mice was developed to ensure high-quality samples while prioritizing animal welfare. The technique involved a specially designed urine collection device with individual compartments and urine collection plates. Each compartment contained a single-use 96-well collection plate, and mice were allowed to urinate freely for up to 2 hours. The design of the compartments, along with a spacious, transparent housing environment, effectively minimized stress and improved animal welfare. The optimal collection period was found to be from 7 AM to 9 AM, during which mice produced urine quantities ranging from 80-810 µl. The use of 96-well plates mitigated sample evaporation and contamination from fecal material. Overall, this innovative cage-based technique provides a user-friendly solution for obtaining high-quality mouse urine samples, benefiting animal welfare and facilitating research studies.

P134 Lighting the Way: Illuminating Animal Welfare with Advanced Lighting Controls

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Proper light cycles are imperative for the welfare and health of animals cared for in indoor research facilities. Inconsistent lighting controls can cause stress, which can affect an animal's psychological well-being. For this reason, in traditional research animal housing rooms, non-human primates have lighting controls that are on a 12:12 cycle set to turn on in the morning and off in the evening. Non-human primates are primarily diurnal, and it is natural for them to have 24-hour light-dark cycles. Prediction strategies in non-human primates are essential for allowing adaptability in future events and navigating complex environments and social structures. Therefore, we were interested in enhancing the predictive strategies of NHPs housed indoors by introducing environmental enhancements that enable a slower transition between light and dark periods. We hypothesized that implementing simulated dusk and dawn that more closely resembled the natural circadian rhythms would improve sleep efficacy and research task performance. We predicted that animals would display resting behaviors earlier at the start of their dark cycle and that sleep quality would increase. Additionally, research task performance should improve or be maintained at a

mastery level. In this study, we observed 9 Rhesus macaques (Macaca mulatta) in three different lighting conditions. We collected behavior observations on seven days of simple automatic on-and-off lighting, seven days in which lights were dimmed at descending increments of 10% over the course of 15 minutes, and seven days in which lights were dimmed continuously over the course of 15 minutes until full darkness was reached. In between these recording periods, we provided the animals with a two-week acclimation period to the new light system before collecting a full week of behavior scans on each animal in the room. Data on the quality of sleep was also collected through the use of an accelerometer. Preliminary results suggest that animals had decreased latency to begin rest at the beginning of the dark cycle and increased sleep quality overall. This study suggests that allowing animals access to more prediction strategies and more closely mimicking their environment to that of their wild counterparts leads to improved health and research outcomes.

P135 Towards the 3Rs in Marine Toxicity Testing: Comparison of Tests Employing Larval Fish, Fish Embryos, and Mysid Shrimp

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Current regulatory guidelines require that effluents be assessed for potential toxicity using standardized toxicity testing methods, often featuring fish. Though these methods have proven effective in the screening of potentially toxic effluents, there are growing ethical concerns surrounding the current use of vertebrate animals as part of routine effluent testing. These ethical concerns coincide with legislative demands for the implementation of alternatives that promote animal welfare whenever possible. This has catalyzed efforts for the development of alternative methods for freshwater effluent assessments, but there is a lack of analogous development for marine effluent testing. The goal of this work was to improve animal welfare in marine effluent testing by performing a comparison between standardized larval fish tests and fish embryo toxicity tests using the sheepshead minnow and inland silverside as well as a standardized invertebrate test employing mysid shrimp. To achieve this, each toxicity testing method was used to evaluate the toxicity of 3,4-dichloroaniline (DCA), phenanthrene, Ni, and a crude oil standard. Toxicity values, including median lethal concentration (i.e., LC50 values), were determined and compared between each testing strategy. In this comparison, it was found that mysid tests possessed comparable LC50 values to the larval fish tests for DCA and significantly lower LC50 values for all other chemicals. The larval fish tests possessed comparable LC50 values across the chemicals tested, and the fish embryo tests possessed greater LC50 values than the other test types. Based on these results, the mysid tests appear to represent a promising alternative to the larval fish tests for acute toxicity testing. Though marine fish embryos tests were less sensitive than larval fish tests, a predictive relationship has been previously shown between freshwater species; a greater comparison across a broader range of chemicals would be needed to see if this holds true for marine species. This work represents a step forward in the development of alternative methods that could improve the welfare of animals in marine effluent testing.

P136 Marine Fish Embryo Toxicity Test: Investigation of Sublethal Endpoints to Enhance Test Sensitivity

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The fish embryo toxicity (FET) test was first developed as a substitute for the acute fish toxicity test. The FET test has since undergone a series of refinements and extensive validation processes to be adopted as a standardized toxicity testing guideline. The FET test method has been applied to multiple test species, including two marine fish species: the sheepshead minnow (*Cyprinidon variegatus*) and inland silverside (*Menidia beryllina*). Currently, the standardized FET test is an acute-only test and lacks sublethal endpoints. This work sought to investigate the utility of sublethal endpoints, specifically hatchability and pericardial edema, for increasing the sensitivity of marine FET tests. To achieve this, sheepshead minnow FET and inland silverside FET tests were performed using an array of chemicals, including 3,4-dichloroaniline, phenanthrene, Ni, and a crude oil standard. For each chemical, exposures were performed with five concentrations over a 7- or 6-d exposure period for the sheepshead minnow FET and inland silverside FET tests, respectively. The inclusion of pericardial edema and hatchability was found to increase the sensitivity of the tests in both test species; only hatchability was observed to be altered in all exposures. Though hatchability was found to increase test sensitivity, lack of hatch is not traditionally considered a negative outcome; further experiments were conducted to assess the fate of unhatched embryos. Both sheepshead minnow and inland silverside embryos were exposed to a crude oil standard up to 24-h prior to the anticipated hatch of 6- to 5- days, respectively. The embryos were then transferred to clean seawater for a 7-d monitoring period to assess hatch status and mortality. The results of this study found that unhatched embryos were less likely to survive than their hatched counterparts following chemical exposure, with ~56% and ~98% of unhatched sheepshead minnow and inland silverside embryos perishing by the end of the monitoring period, respectively. As such, delayed hatch may be indicative of delayed onset mortality, especially in the inland silversides. Further testing is needed to determine the consistency of this response following chemical exposure and to strengthen its applicability as a test endpoint.

P137 Motorization and Automation of Micromanipulation for Microinsemination and Blastomere Biopsy

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Reproductive engineering in laboratory animals involves the collection of embryos and gametes, cryopreservation, and production of offspring. Intracytoplasmic sperm injection (ICSI) is used to fertilize sperm that has become immobile due to damage caused by cryopreservation or disease and is useful for the production of offspring. Blastomere biopsy (BB) is used for preimplantation diagnosis of background genes or transgenes. Both techniques are performed using a micromanipulator, but long-term training is required to ensure the reproducibility of micromanipulation. We aimed to motorize and automate micromanipulators so that these techniques could be accurately performed with little training. An automatic micromanipulator (AM) was constructed by integrating motorization of the pipette movement, sample stage movement, pump suction, and ejection, and automation of the pipette 3-dimensional movement, sample 2-dimensional movement, and microscope field focusing. Gametes and embryos for ICSI or BB were collected from sexually mature BDF1 mice. A novice performed the ICSI and BB micromanipulation using the newly designed AM. In ICSI, after perforating the zona pellucida, sperm were aspirated into an injection pipette. The pipette was automatically moved to the perforation site, and sperm were injected into the cells. More than 80% of ICSI-treated oocytes were fertilized, and fetal development rates were similar to those of in vitro fertilization-derived embryos. In BB, automation allowed the pipette and microscope field to move seamlessly between media to treat five 8-cell stage embryos in succession. All manipulated embryos survived. Fetal development rates were similar between untreated (control) and treated embryos, suggesting minimal invasiveness. These results suggest that even novices can achieve high and stable results when performing ICSI and BB using the AM. This AM developed for laboratory animals may also be applicable to assisted reproductive technology and the production of domestic animals.

P138 Navigating Surgical Parabiosis Challenges and Pioneering Solutions

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Parabiosis is a well-established model that has been used across many fields to study aspects of blood circulation. Despite its wide application, it is a non-standardized, technically challenging procedure, and there are gaps in the understating of procedure-related complications and their solutions. We published an optimized protocol that describes commonly observed complications, including variable responses to anesthesia, external and internal dehiscence, dehydration, and weight loss, along with successful management strategies for these adverse outcomes[1]. We have further refined our protocol by incorporating additional suture modifications and other simple interventions to prevent dehiscence and skin lesions. Through the incorporation of parabiosis procedural solutions, we observe qualitative improvements in outcomes across experiments, including skin healing duration and severity of external elbow dehiscence. Ultimately, these technical advances foster our ability to improve animal welfare and experimental success by reducing the need for surgical dehiscence repair and the incidence of clinical decline after surgery.

P139 Overcoming Challenges to Implement Studies in Nonhuman Primate Pens that Meet Good Laboratory Practice (GLP) Regulations

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Many institutions have made the transition from cages to large pens for nonhuman primates in academia, industry, and contract research organizations. Our institution joined the list of those facilities installing European Union (EU)-style and sized pens as part of a large-scale toxicology facility renovation. At our facility, >50% of our work is conducted under Good Laboratory Practice regulations. Although literature reviews, tours at peer institutions, and networking aided in the design and the initial occupancy of our ~80 sq ft. pens, less information is readily available on how institutions have maintained GLP compliance in this housing. We utilized a team-based approach consisting of technicians, managers, veterinarians, and behaviorists in Comparative Medicine and our toxicology researchers, Study Directors, and pathologists to test and address questions and concerns related to the GLP study process and data. Components of animal randomization were addressed with pre-study mapping since the movement of animals to new locations after pen occupancy was suboptimal. Quantitative food consumption for studies requiring closer assessment of appetite was monitored and not found to be negatively impacted by larger areas for animals to hide or throw biscuits. Sanitation protocols were created to alleviate any concerns about any contamination of Control Animals with test articles. Observation of chronic animals was followed to understand if historical control values of body weight would be impacted by a larger cage size and more opportunity for movement. Progressive housing size and complexity is an important component of the 3Rs in all settings, including terminal toxicology studies. This poster describes how our institution overcame both real challenges and perceived obstacles of utilizing pens in a GLP setting with the goal of promoting refinement in NHP housing and aiding in breaking down barriers to get there.

P140 Rearing C57BL/6J Mice in Partially Divided Caging Demonstrates Reduction in Anxiety-Like Behavior and Improved Welfare

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Choosing environmental enrichment for mouse cages often prioritizes cost over welfare benefits like reducing aggression and anxiety. While various devices aim to promote natural behaviors and curb aggression, their effectiveness varies. In previous research, we found that partially divided caging led to significant reductions in aggression among group-housed male mice. To further investigate, we raised male C57BL/6J mice from weaning to 180 days of age in either partially divided caging or standard caging without dividers. Behavioral test analyses included 25 mice from each condition for a total of 50 mice. Animal behavior was tested on rotarod, open field, novel object recognition, elevated plus maze, and Y maze. Body weights were taken weekly beginning at weaning and bite wounds were counted weekly beginning at 133 days old. Aggressive behavior was recorded weekly, beginning at 133 days old. Our findings revealed less anxiety in the elevated plus maze, fewer bite wounds, and reduced aggressive behavior in mice housed in partially divided cages compared to standard cages. Mice from divided cages had more open arm entries (t_{47}=2.28, p=0.03), spent more time in the open arms (t_{47}=2.57, p=0.01), and spent more time in the center of the maze (t_{47}=2.67, p=0.01), indicating less anxiety and more exploratory behavior. Total bite wounds correlated with the total number of aggressive events. A Pearson's correlation between aggressive events (video recording) and bite wounds (visual inspection) showed a significant positive correlation (r=0.82, p<0.05). Mice in standard caging showed 2-fold increases in aggression rates and 4-fold increases in bite wounds compared to mice in divided caging. These results suggest that raising mice in partially divided caging may enhance the overall welfare of non-sibling, group-housed male mice by decreasing anxiety, aggression, and bite wounds.

P141 Refinements to Monitoring Requirements in Subcutaneous Air Pouch Studies in Mice

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The subcutaneous air pouch model is commonly used to study acute and chronic inflammation. In this study, historical study data was analyzed to determine if the frequency of mice being weighed during air pouch studies could be reduced from daily to twice per study. The air pouch model is used to evaluate the effects of positive and negative regulators of pro-inflammatory antibodies, biomarkers, and potential mechanisms of action of relevant molecules. The air pouch is formed by injecting sterile air into the subcutaneous area of mice, which can later be injected with irritants to induce inflammation. Endpoints such as inflammation levels, resolution of inflammation, and the efficacy of anti-inflammatory drugs can be quantified using this model. Based on the analysis of over 30 studies for changes in animal body weight over the course of the study, it was found that there were no significant changes. As a result of this finding, the principal investigator, with IACUC approval, reduced the required frequency of weighing from daily to twice per study (once on the day the study is initiated and again halfway through the study). Cage-side clinical observations on all study animals will be performed twice a day by trained husbandry staff. This change will save staff approximately 150 hours per year (based on a 7-day study duration, including weekend coverage) and reduce stress on the animals, as handling is a known stressor. Overall, reducing the frequency of animal body weights in air pouch studies is justified, based on historical data showing no changes in body weights over the course of 30+ studies, and will be beneficial for both researchers and the welfare of the animals.

P142 Refining Standard Weight and Age Requirements for Mouse Weaning to Reduce Cannibalization, Technician Time, and Breeding Cage Handling in a Transgenic Mouse Breeding Colony

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The gestational period of a laboratory mouse lasts between 19 to 22 days, and they can become re-impregnated almost immediately after giving birth. Our team traditionally weaned pups at postnatal day 21 and had a minimum wean weight requirement of 7g. Failure to meet this weight requirement resulted in pups being left in the parent cage for up to two additional weeks, delayed one week at a time until the weanlings weighed more than 7g. Anecdotally, we noticed a high incidence of cannibalized new litter when an older litter was present in the parent cage at the time of birth. To reduce the risk of cannibalization, we decided to lower the weaning age to postnatal day 20 to provide a less crowded cage for new litters. However, contrary to our goal, this change resulted in more pups falling below the 7g weight requirement, increasing the chance of these older animals being present in the cage when the next litter was born. We chose to re-evaluate our 7g weaning weight requirement to counteract the overcrowding caused by the younger wean age. We hypothesized that there would not be a negative impact on pups weaned out of the parent cage at postnatal day 20 that weighed as little as 6g. To test this, we designed a study to measure the average daily weight gained by weanlings weighing between 6g and 7g on postnatal day 20, which are left in the parent cage compared to those weaned into a new cage. We found that mice weaned from their parent cage at 6g or greater gained weight similarly to those that were left in the parent cage (0.65g/day vs. 0.64g/day, respectively) and noticed no negative impact on the health or survival of the smaller weanlings. By adjusting both the required weaning date and weight, we were able to clear out space and resources in the parent cage for new litters, and possibly increase new litter survival. This refinement also reduced the time that technicians spent weaning animals by reducing the number of delayed weaning tasks, as well as the number of times the breeding cages had to be disturbed.

P143 Utilization of Broth-Infused Gauze Wipes for Canine Oral Gavage Dosing

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Dose administration via oral gavage, while common in laboratory animal research, requires well-trained and careful technicians to maintain animal health, welfare, and data integrity. An animal struggling during dose administration can cause many complications, including increased stress for animals and technical staff, increased indices of behavior-based observations, and the risk of a dosing-related error. This can compromise research data, as it can be difficult to distinguish a transient adverse reaction or observation versus the stress stemming from repetitive restraint and procedures. Typically, during oral gavage-dosing, a gauze square that has been submerged in water and wrung out is used to wipe the gavage tube prior to placement. We propose a slight alteration to this process by utilizing broth-infused gauze wipes (one/animal) during dose administration to help alleviate the stress caused by the procedure while also rewarding the animals with a sapid treat. We noted in a 40-dog oral gavage study and a BID 32-dog oral gavage study, both over the course of 28 days, that the introduction of broth wipes caused a moderate decrease in clinical observations relating to dosing. Utilizing data from these two internal studies, we can conclude that the introduction of broth wipes during oral gavage procedures can moderately reduce clinical observations, including vocalizations during gavage tube placement and the number of animals struggling during dose administration. This stand-alone study will support this theory by using a behavioral scoring system to determine the positive and/or adverse effects of using broth wipes for canine gavage, in addition to any significant differences in the flavors of broth used.

P144 Replenishment of Nutrients via Iron-Rich Treats After Blood Collection in Canines

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In the realm of animal research, the process of blood collection is a crucial step for obtaining the necessary data required in the drug development process. Although each individual collection may seem small, often ranging from 0.5 to 1.0mL for large animals, the cumulative impact becomes significant when multiple time points or multiple blood samples are required. This rapid accumulation results in a continuous loss of red blood cells without adequate replenishment, ultimately leading to acute anemia in animals and could potentially influence study data. The consequences of anemia and/or iron deficiency, stemming from continuous blood loss, manifest in various clinical observations, such as lethargy, fatigue, pale mucous membranes, and more. It is noteworthy that the stress induced by repeated collections alone can contribute to these symptoms. Most importantly, these clinical signs may erroneously be attributed to the test article rather than recognizing them as outcomes of the routine demands for data collection. To address this issue and uphold the optimal health and safety of laboratory animals, we propose the implementation of an evidence-based approach, with data collection still ongoing. Specifically, we advocate for supplementing animals with an iron-rich treat immediately after blood collection. To keep our data consistent in this study, our animals are given a flavored canine iron supplement tablet in place of an iron-rich food after each blood collection. The replenishment of RBCs and their physical characteristics will be evaluated by a Clinical Pathologist via a complete blood count (CBC) completed at regular intervals. The evaluation of RBC indices in the animals, along with any notable observations, will determine the effects of iron supplementation. This implementation serves a dual purpose – rewarding the animals for positive behavior during the collection process and efficiently replenishing the lost red blood cells. By doing so, we aim to mitigate the adverse effects of repeated blood collections, ensuring accurate research outcomes while prioritizing the well-being of the laboratory animals.

P145 Smell Ewe Later: Examining The Use of Wool Clippings to Reduce Stress Behaviors in Recovering Sheep

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As a highly social prey species, sheep are susceptible to panic and stress when separated from their flock *(Kalyan De et al., 2018)*. In research settings, projects may require sheep to be intermittently or continuously singly housed, leading to increased stress and reduced welfare *(Kalyan De et al., 2018)*. Previously published studies indicate that sheep can recognize odor cues from conspecifics *(Baldwin BA, Meese GB 1977)*. Additionally, it has been identified across animal models that "odors influence autonomic responses…[and] pleasant, novel odors can decrease heart rate" *(Fletcher and Wilson, 2001; Wilson, 2009)*. We hypothesized that providing solitary sheep access to their freshly clipped flockmates' wool would lead to a lower frequency and duration of stress behaviors observed during necessary periods of isolation, such as post-sedation recovery. To evaluate the effects of olfactory cues from conspecifics on sheep recovery from sedation, three cohorts (each n=6, three males and three females) were provided with fresh flockmate wool in a hanging device, synthetic wool in a hanging device, or an empty hanging device, respectively. All sheep were video-recorded for 30 minutes following sedation, and the frequency of stress behaviors was collected. Preliminary analyses suggest that sheep provided with fresh flockmate wool

spend a greater amount of time in proximity to the device compared to sheep provided with an empty device or synthetic wool. Sheep in the flock mate wool group also demonstrated decreased vocalizations and lip-licking stress behaviors. To further explore these findings, we then introduced the following conditions: one cohort in which a large wool sack was present, one with a mirror present, and one with a wool sack and mirror present simultaneously in order to compare behavioral responses following sedation and further measure the impact of visual and olfactory components on stress behaviors. Preliminary findings suggest that providing solitary animals access to their flockmates' wool as a source of olfactory enrichment may aid in lowering stress during separation and, in turn, increase welfare.

P146 Standard Peri-Surgical Procedures to Improve Survival Rates after Thoracic Surgery in Mice (*Mus musculus*)

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Thoracic surgical procedures used in cardiopulmonary research (e.g., transaortic constriction) have the potential to produce high mortality rates; refining surgical and peri-surgical procedures results in decreased mortality, effectively reducing the number of animals needed per study. In addition, more refined techniques also reduce post-surgical stress, which can obfuscate experimental outcomes. Using a series of novel, enhanced peri-surgical procedures, our lab has successfully reduced surgical and post-surgical mortality in male C57BL/6NCrl mice (n=291) by as much as 63% when compared to standard surgical and peri-surgical procedures. The techniques developed include provisioning animals with gel diet and pre-, post-, and peri-surgical medication according to a specific timeline; holding the animals in a pre-warmed high oxygen environment both pre-and post-surgery; maintaining body temperatures between 34-38°C during surgery; and eliminating the use of lidocaine (due to its tendency to cause respiratory dysfunction in the animals). Finally, animals were kept on a warmed surface for up to 24 hours during the post-surgical monitoring period; during this time, they were also given additional gel diet and subcutaneous fluids. By using these techniques, we have effectively reduced the number of animals needed in our research and increased our ability to provide reproducible results.

P147 Refinement of Jugular Access Venipuncture in Swine for Sequential Blood Collection

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Many research studies require sequential blood sampling for pharmacokinetics in swine. Unlike other large animal species, pigs are not easily restrained for venipuncture and generally require sedation or anesthesia. To refine blood collection techniques in swine requiring multiple collections over short or long time periods, the objective was to identify an improved technique of intravenous catheterization of the jugular vein using peripheral veins, such as the auricular and cephalic veins. These minimally invasive techniques eliminate the requirement for a surgical approach to the jugular vein and allow for conscious blood collection over consecutive days or weeks as required. The use of a short-term angiocath does not allow for consistent, larger blood volumes to be collected easily from peripheral veins. Intravenous catheter placement into the jugular vein using the auricular and cephalic veins allows for easy access while the animal is standing and awake. The use of the auricular vein technique has proven to be a successful method for long-term catheter placement. However, sometimes, this technique can be difficult given the challenges of repeatedly using the ears or if the vascular anatomy is not compatible. Intravenous catheter placement into the jugular vein using the cephalic vein is an excellent alternative to placement in the auricular vein for short-term procedures. The cephalic vein is superficial and large, allowing for easy advancement of the catheter. However, securing the catheter at this site is more difficult due to the position on the forelimb. For both sites, extension sets can be attached to the catheter and secured in place using bandage tape. We have used the MILACATH kit with a guidewire introduction that can remain inserted for up to 30 days before removal or replacement. Both procedures have been successfully accomplished with minimal complications, such as infection, removal by pig, or catheter failure due to patency or damage. Catheters were placed into 50 Yorkshire swine ranging between 35-75kg. Of the 50 catheters placed, 90% were inserted into the marginal ear veins, and 5% were inserted into the cephalic veins. We had an 86% success rate for placement for the allotted timeframe for the specific study. Of the 50 catheters placed, 62% of catheters were inserted for greater than 24 hours, and 38% were inserted for less than 24 hours. There was a 12% failure due to displacement either during blood collection or by the animal. We only had a 2% failure due to localized infection. Despite the catheter remaining patent due to health concerns, the catheter was removed by the technician, and the animal was treated with antibiotics.

P148 Rat Fashion: DIY Vest to Practice Vascular Access Handling and Restraint

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Standard introductory rat handling sessions involve instructing the learner on how to manipulate a normal rat appropriately and adequately. Current handling and restraint techniques require the handler to cover the upper dorsum of the rat with their hands. However, some researchers must restrain rats that have vascular catheterization devices implanted in this area. Purchasing catheterized rats from a vendor or performing surgeries in-house to produce a rat solely for training purposes increases animal numbers, is costly, causes delays in training, and requires potentially stressful surgical techniques. As a refinement, we made vests to emulate the devices found on catheterized rats using self-adhesive elastic bandage wrap and other supplies commonly found in the animal research facility. The vests were simple to make and apply onto the animals, easily customizable, reusable for multiple training sessions and caused little stress to the rats after acclimation. Having the rats wear the vests with the pseudo vascular catheterization devices helped the learners visualize and alter their holds for their specific needs (i.e., oral gavage, blood collection, or drug administration via catheter, etc.). The researchers who practiced using this training tool were able to start their study once their own catheterized rats arrived, and our training team was able to successfully implement the 3R's by refining an invasive procedure and reducing our needs to purchase and operate on additional animals. Here, we describe the materials needed and provide a step-by-step guide on creating this training tool.

P149 Skin Lesions Related to Implantation of Transcutaneous Button in Male Sprague Dawley (SD) and Wistar Han (WH) Rats

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Transcutaneous vascular access button (VAB) is routinely used in catheterized rats, allowing easier access to the catheter for sampling and dosing. VAB use also promotes group housing, improves patency, and minimizes stress to animals and staff. The surgical implantation of VAB is a more involved surgical procedure with potential sequela, including such as seroma and skin lesions. Our experience with VAB suggested the development of seromas and skin lesions depends on the technique and skill of the surgeon as well as the strain of the rat. In this study, we investigated the

incidence rate of skin lesions in the most requested rat strains, SD and WH rats, related to surgical implantation of VAB. Thirty male SD (Crl: CD® (SD)IGSBR) and thirty male WH (Crl: WI® (Han) IGSBR) rats, 175 to 200 grams were used. Trained and verified surgeons performed surgery on five rats per strain. Rats were surgically implanted with a femoral vein catheter (FVC) and carotid artery (CAC) and then connected to a two-channel transcutaneous VAB. Rats were housed in individual cages and maintained at 23 ± 3 °C with a relative humidity of 30%-70% and a 12:12 hour light: dark cycle. Feed and water were provided *ad libitum*. Body weights and detailed observations for skin lesions around the VAB were performed once a week for four weeks. An antibiotic ointment was applied if lesions were observed. Rats recovered smoothly from the surgery without complications. One WH rat developed blepharospasm related to surgery and was excluded from the study. One week after the surgery, skin lesions were observed in 10% of SD rats and 24% of WH rats. The skin lesion rates for week two, week three, and week four were 7%, 10%, and 0% in SD rats and 45%, 17%, and 3% in WH rats. The data shows that skin lesions resolve with time and minimal intervention. We did not find any relation between skin lesions and surgeon technique. The data indicates WH had more skin lesions and took longer to resolve compared to CD rats. Additional studies are required to understand the mechanisms for the difference in skin lesions in SD and WH rats related to VAB implantation.

P150 Optimizing Blood Sampling Techniques in Immuno-compromised Mice for Longitudinal Collections and Improved Animal Welfare

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Certain mice strains are genetically engineered to exhibit immunodeficiency that is characterized by the absence of functional T and B cells, which are critical components of the immune system. Blood collection from laboratory mice is widely used for scientific investigations. Conventional techniques often induce stress and discomfort in animals, potentially altering research results. Studies revealed notable disparities in blood compositions between C3HeB/FeJ (Kramnik) mice and other immune-compromised laboratory mice strains, potentially contributing to heightened clotting properties during blood collection. Elevated levels of analytes, such as chloride, phosphate, and sodium, have been linked to increased coagulation, highlighting the importance of strain-specific factors in blood collection methodologies. The serum contains fewer proteins than the plasma and is preferred for use in clinical assays. The addition of anticoagulants can potentially interfere with test analysis results. Immuno-compromised mice typically have smaller blood volumes compared to healthy counterparts, demanding precise techniques and specialized equipment to obtain sufficient samples without harm. Our hypothesis was that the correct choice of blood collection methods could mitigate the potential interference of anticoagulants, such as heparin, and obtain sufficient volumes for analyses. Investigation into an efficient technique to obtain high-quality blood samples from mice can be achieved without employing heparin to mitigate the rapid clotting tendencies while ensuring the integrity of collected samples for downstream analyses. The animal's tail must be heated, followed by the venipuncture procedure into the lateral tail vein using a 27G needle and a blood capillary tube to draw the blood in a temperature-controlled environment. This blood collection technique offers a safe and efficient approach for immuno-compromised mice to collect adequate blood volumes, eliminating the need for retro-orbital blood collection. Collecting blood samples from immunocompromised mice requires careful consideration of their unique physiological and immunological characteristics to ensure the integrity of the samples and to improve overall animal welfare.

P151 Successful Execution of Intravenous Vascular Access Button Dosing on BALB/c Mice with Submandibular Blood Collection

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AmplifyBio, like many preclinical CROs, increasingly faces the challenge of novel dosing routes and new blood collection methods. The route of dose administration, blood collection methodology, and safety precautions played a role in designing and preparing technician training for a client-funded project. The project was to administer test articles to BALB/c mice as an intravenous (IV) infusion via vascular access button (VAB) while concurrently collecting toxicokinetic (TK) blood samples from the submandibular vein. Infusion durations were 30, 60, or 90 minutes, with TK collections occurring while mice were continuously connected to the dosing system. AmplifyBio technicians performed all dosing and blood collections in a Class II Type A2 BSC (biological safety cabinet) in a BSL (biological safety level) 2 environment. Training started six months before the project and began with teaching submandibular bleeds in mice, a new blood collection technique for our company. Mice were surgically instrumented with VABs from an approved vendor for training purposes to develop the procedures outlined in job aids and SSMs (Study Specific Methods) for techniques specific to the study. AmplifyBio developed the training for a project that resulted in a record of zero mis-doses and 99.54% blood collection time points met on time. Blood collection success due to the training led to the team utilizing the submandibular vein as the standard for biological sampling in mice. Post-project meetings and surveys revealed positive attitudes towards training techniques. Technicians indicated that hands-on training and mock project practice boosted their confidence to perform dosing and blood collections on this and future projects. Survey results revealed that over 80% of technicians would volunteer to work on a similar project again due to the proper execution of training and how prepared they felt before the project started.

P152 Surgical Implantation of a Catheter System Used for Repeated Collection of Blood and Cerebrospinal Fluid in Rats over Time

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Historically, the collection of cerebrospinal fluid (CSF) in rodents required the use of repeated anesthetics or terminal collections, which increases the animal numbers of any given animal use protocol. With the use of animals in research, we are always looking to reduce the number of animals in order to be compliant with the 3Rs. The use of terminal collections makes it impossible to obtain samples over the course of a disease within a single animal, and repeated anesthetics can cause interference with biological samples and create confounding data. This study sought to develop a technique for repeated CSF sampling to 1) allow animals to serve as their own controls in time-course studies and 2) obtain samples over time without repeated anesthesia. Our technique involved surgical implantation of cannulas to allow repeated CSF (cistera magna) and venous blood sampling from the same animal without repeated anesthesia. Ten Sprague-Dawley rats were anesthetized, and cannulas were surgically placed and secured in the jugular vein and the cisterna magna. These cannulas were then connected to an access button, which was surgically implanted between the shoulder blades. Upon recovery, a maximum of 200uL of blood and 50uL of CSF was collected daily for up to 14 days, using a sterile technique. The samples provided sufficient blood and CSF for extracellular vesicle isolation. The animals were able to be group-housed without any interference with the implants or surgical sites. The use of this technique allows for a

large decrease in animal numbers required, as individual animals can serve as their own controls for experimentation, and samples may be drawn from the same animal over at least 14 days without needing terminal collections. As well, animal welfare is increased for the animals, including the ability to group house the rodents, reduced number of needle punctures, and reduced restraint.

P153 Use of Tear Film Measurement as an Antemortem Diagnostic Tool for Ocular Lesions in Mice

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Ocular lesions are highly prevalent in laboratory mice, yet there are limited antemortem diagnostic tools for ophthalmology cases beyond visual examinations and fluorescein staining in cases of corneal ulcers. In other species, tear film measurement, including methods such as Schirmer's tear test, tear film break-up time (TBUT), and tear film osmolarity, are commonly used. However, TBUT and tear film osmolarity are impractical for laboratory animals. However, the endodontic absorbent paper point test (EAPTT) offers an easy and objective method for measuring tear production rates in mice. An increased incidence of corneal lesions was noted in 5XFAD, a common mouse model of Alzheimer's Disease (AD), with a correlation between AD, blink reflex, and dry eye. To determine if corneal ulcers in these mice could be detected prior to clinical signs, baseline data were collected using EAPTT. The study compared tear production rates in 5XFAD mice alongside NSG and C57B6 strains. NSG and C57B6 mice had similar rates, averaging 2.01 mm and 2.14 mm, respectively, while 5XFAD mice had a higher baseline value, averaging 2.67 mm, a statistically significant difference. The use of EAPTT as an antemortem clinical tool proved to be more effective for diagnosing ocular problems, aligning with the principles of the 3Rs (Reduction, Refinement, Replacement) by reducing the number of animals needed and refining disease endpoint measurements. Implementing tear film measurement as a standard diagnostic tool in laboratory mice could enhance early detection and treatment of ocular lesions, improving animal welfare and research outcomes.

P154 What's All the Buzz - Easing Baa-d Reactions to Subcutaneous Injections in Sheep

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With the immense contributions that animals make to the field of science, it is paramount that we continue to refine medical procedures to optimize animal welfare. In the field of animal research, animals are often restrained to safely perform medically necessary procedures. Studies have shown that restraint can cause a great deal of stress, leading to regression in cooperative care and handling, as well as injuries to animals and staff. Although training medical behaviors using positive reinforcement may reduce this stress response, there may be non-invasive devices that will also aid in improving an animal's experience during medical procedures. The ShotBlocker is a small, inexpensive device that has been effective in reducing pain responses in human neonates and adults during injection delivery. According to the device maker, the plastic blunted tips minimize pain from injection by saturating sensory signals at the injection site. Additionally, studies showed that Buzzy, an FDA-cleared product utilizing vibrations and a cold pack, is also effective in reducing pain in children. We aimed to examine the efficacy of the ShotBlocker and Buzzy for subcutaneous injections in an animal research setting. We hypothesized that these would reduce animals' behavioral responses to pain during injections and would reduce the number of recovery sessions needed for animals to return to mastery of their voluntary injection behavior following injection. To test this hypothesis, 42 polypay sheep (*Ovis aries*) were randomized into a control group or one of two test groups. Animals underwent the same voluntary subcutaneous injection training plan. In the control group, trainers "tented" the skin with their hands, whereas, in the ShotBlocker group, trainers "tented" the skin with the device. In the final test group, trainers utilized the Buzzy device according to the manufacturer's instructions during injection. Behavior observation data was collected at the time of the saline injection. Preliminary results suggest that, while not significant, flight response was lower in the buzzy group (M=3.14) compared to the control group (M=6.14). Continued data collection across more animals in the next year will provide further support for lessening perceived pain and discomfort experienced during vet med practices.

P155 Comparison: Intrarenal (IR) and Intravenous (IV) Injections of Sodium Pentobarbital for Euthanasia in Rabbits (*Oryctolagus cuniculus*)

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Peripheral venous access in rabbits may be technically challenging. According to the 2020 AVMA Guidelines for Euthanasia, intrarenal injection is categorized as an acceptable with-conditions method that can only be performed on a sedated and unconscious animal. On average, the renal blood flow contains 20-30% of the resting cardiac output, and the intrarenal route utilizes this circulation to deliver drugs systemically. Sodium pentobarbital is a barbituric acid derivative and the most commonly used euthanasia solution used in a variety of species. Its mechanisms cause depression of the central nervous system (CNS), loss of consciousness, anesthetic overdose, and apnea followed by cardiac arrest. Minimal studies using intrarenal administration have been conducted in felines, but there are no current studies measuring the efficacy, efficiency, and validity in rabbits. In a pilot study using 26 rabbits (13 males and 13 females), we evaluated the time to cardio-pulmonary arrest (TCPA) following injection of sodium pentobarbital intrarenal (n=13) compared to intravenous (n=13). Intravenous CPA times averaged 6.81 seconds for cardiac and 9.7 seconds for respiratory. Intrarenal CPA times averaged 12.33 minutes (409 seconds) for cardiac arrest and 4.84 minutes (290.92 seconds) for respiratory arrest (p=0.001 and p=0.016). These average times are comparable to a retrospective study performed on cats and are suited for the euthanasia of anesthetized rabbits. Factors such as variable times to cardiopulmonary arrest and individual technique skills should be considered when performing this as a main route of euthanasia. The overall information from this pilot study can be used to guide both laboratory and practicing clinicians who are considering performing this technique.

P156 You Can Have Your Cone and Eat Too! Soft Cone Collars Lead to Improved Postoperative Rabbit Care

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Laboratory animal research focuses on animal welfare and care approaches to minimize pain and distress. Postoperative measures must be taken to prevent rabbits from self-traumatizing surgical sites. Traditionally, this has been in the form of rigid Elizabethan collars and/or tight-fitting bandages and jackets. These methods can lead to weight loss, stress, inappetence, and decreased fecal and urine output in rabbits due to their restrictions on normal behavior. Because rabbits are hindgut fermenters, they must continually eat high-quality fiber to establish healthy microbial populations within their ceca. Any disruption to feeding and water consumption by rigid collars, bandages, and jackets must be avoided. Additionally, they are prey species and rely on superb panoramic vision for survival in the wild. Any impedance to their peripheral vision may cause stress and anxiety. The goal of this study was to establish a method to prevent self-trauma at surgical sites in rabbits but not impede their normal, instinctive behaviors. This clinical refinement would result in less GI stasis, dysbiosis, stress, and weight loss in postoperative patient models. Thirty rabbits with surgical incisions located on the dorsum of the body and five rabbits with inguinal incisions of the hindlimbs were retrofitted with cotton cone collars designed for cats. Compared to rigid E-collars, jackets, and bandage wraps, the application of soft collars allowed rabbits to remain engaged in natural behaviors and resulted in less inappetence and weight loss. Soft collars adequately prevent self-trauma to surgical sites located on the dorsum of the body; however, rigid E-collars, jackets, and/or bandages may be necessary to properly protect incisions on the caudal aspect of the body. Acclimation to postsurgical collars is recommended to alleviate the stress of initial placement, and modifications to properly fit the soft collars to each rabbit may be necessary.

P157 Enabling Male Mouse Social Housing: Assessing Aggression in Male and Female CD1 Mice Under Standard Toxicology Study Conditions

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While international regulations recommend social housing, most institutions that perform GLP-regulated toxicology studies still singly house male mice due to concerns about aggression compromising study outcomes. This study evaluated the impact of aggression on socially housed male and female mice maintained under otherwise standard toxicology study conditions. To assess whether the age of pairing has an impact on aggression, we compared arrival at 5- and 7-wks (wk) of age; mice are typically seven weeks at arrival. Mice were housed in same-sex pairs (n=14/group/sex) upon arrival and acclimated for 7- or 21-days, administered drinking water (Oral Gavage) for 28 days (dosing phase), and were maintained without dosing for an additional 28 days (recovery phase). Aggression was assessed daily, and mice were weighed twice weekly. Food consumption was measured at least once weekly, and nest quality was scored (NQS) twice weekly. Wounding was assessed using the pelt aggression lesion scale (PALS). No aggression was observed in female mice. Aggression was observed in 5 males (5-wk) with 12 observations of aggression and four males (7-wk) with six observations of aggression. Only one male (7-wk) had visible signs of wounding, resulting in a single cage requiring separation. Most animals constructed well-formed nests, with females demonstrating higher NQS than males. 5-wk Males had increased NQS in the dosing and recovery phases ($p<0.05$) compared to the 7-wk males. There were no significant differences in body weight between groups for the dosing and recovery phases. 7-wk Males gained more weight in the baseline phase ($p<0.001$) than 5-wk males. There were no differences in food consumption. There was minimal evidence of wounding, with males in both groups demonstrating higher PALS scores than females and the 7-wk group having higher scores than the 5-week group. Posterior PALS scores between the male mice that arrived at 5- and 7-wks was statistically significant ($p<0.05$). These data suggest that in the current context, it is not necessary to order animals younger than the standard age of arrival (7-wk). Importantly, this evidence supports social housing in both male and female mice with minimal impact on study outcomes, enabling optimal animal welfare, 3Rs impact, and alignment with international recommendations and requirements.

P158 Social Housing of Male Mice in the Toxicological Setting

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Group or pair housing social animals in laboratory settings is beneficial to animal welfare. However, social housing of male mice can be difficult due to possible aggressive behaviors and can lead to injury that can result in animals not being able to continue on study. Techniques used to decrease male mice aggression and territorial behaviors have been described in recent literature, including pairing mice before sexual maturity, moving used nesting material over during cage changes, initially removing high-value items that can encourage territorial behaviors, and using low-stress handling techniques. Using these suggested tactics, we conducted a 28-day study determining the social compatibility of CD-1® male mice in a standard toxicological study design. Forty-eight mice, aged five weeks old, were equally divided into single-housing and socially housed pairs. To observe social compatibility when exposed to the test article, theophylline was administered daily on weekdays as it is known to cause significant neurological effects that may impact social compatibility. Animals were exposed to common toxicological procedures known to cause additional stress on the animal, including repeated blood collections, daily dosing, weekly clinical observations and body weights, and terminal urine collections. Behavioral assays, including nest scores and TINT (time to integrate nest material) testing, were performed weekly, and pelt scores were collected postmortem. Fecal samples were collected intermittently for fecal corticosterone analyses. No mouse pairs required separation throughout the dosing phase of the study. In preliminary data analyses, nest scores appeared to be affected by theophylline, while TINT testing showed no obvious trends in scores, and pelt scores showed no evidence of undetected aggression or trauma. These suggest that, when using the aforementioned techniques, male mice can be successfully socially housed in acute and subacute toxicological studies.

P159 Socially Housing Male Mice in a Laboratory Environment: Paper Pulp Bedding vs. Hard Wood Chipped Bedding

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Mice are social creatures living together in groups in the wild. The industry standard has been to single-house male mice due to aggression towards one another as they reached sexual maturity. The goal of this project is to find a solution to social housed male mice. Not only is this beneficial for the mice, but it also has an ergonomic benefit to techs, reducing the number of cages opened per event. Success in this project can lead to a big leap in animal welfare for male mice by allowing them to engage in social activity while also making them easier to handle. This study is split into two initial phases. Phase-1 looked at CD-1 mice and tested the variable of type of bedding on the success rate of social housing. Animals were housed 3 per cage, in cages ~69 cm^2, in either hardwood chip bedding or paper-pulp bedding. Each cage had twice daily observations looking for signs of aggression with additional cage assessments to determine group dynamics. After a 3-week acclimation phase, animals were oral gavaged to simulate typical lab procedures. During the first three weeks of acclimation, there were more animals exhibiting remarkable observations or separated from partners on hardwood chip bedding compared to paper-pulp. When oral gavage was added, the number of observations and separations on paper pulp surpassed that of hardwood chip bedding. Due to the

results of observations, separations, and aggression, it was concluded that the paper-pulp bedding provided a better environment for socially housed mice. The findings of Phase 1 led to the proposal of Phase 2, which will test the effect of group (5 per cage vs. 3 per cage) and cage size on aggression in socially housed male mice all housed on paper-pulp bedding.

P160 Compassion Fatigue on Professionals Working with Laboratory Animals: A Bibliographic Mapping

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Compassion fatigue (CF) refers to feeling emotionally and physically drained due to being around others' pain for a long time and is increasingly recognized among caregiving professionals. Laboratory animal science professionals face ethical care challenges while managing emotional stresses. This study analyzed existing literature on CF among these professionals to improve their well-being and animal care quality. We conducted a bibliometric analysis using Bibliometrix and VOSviewer, analyzing data from Scopus, Web of Science, PubMed, and EBSCO. Keywords related to CF, laboratory animals, and workplace well-being guided the search. After removing duplicates and uncorrelated studies, 22 unique entries were analyzed. The analysis revealed increased publications on CF and mental health since 2019, reflecting growing awareness in the scientific community. The USA is the leading contributor, followed by Spain, Germany, and Canada, indicating certain regions are at the forefront of addressing CF in laboratory animal science. Prominent themes include CF, animal welfare, workplace environment, mental health, and social support, highlighting the nature of CF and its impact on professionals' lives. Key sources are "Frontiers in Veterinary Science," "Laboratory Animals," and "Animals." Influential authors are affiliated with universities and research institutions focused on veterinary medicine, animal welfare, and psychology. The study mapped the literature, identifying citation trends and co-authorship networks. Findings suggest a growing, robust body of work on animal welfare and professional wellbeing. To mitigate CF and promote a supportive work environment, several measures are proposed: develop accessible counseling services and mental health resources; encourage open communication, respect, and appreciation for professionals' demanding work; and raise awareness about CF, its symptoms, and mitigation strategies through education and training. These actions can create a compassionate, sustainable research environment, benefiting humans and animals. Future research should develop effective interventions, explore cultural differences in CF, and identify best practices for promoting mental health and well-being among laboratory animal professionals.

P161 Professional Quality of Life in Animal Research Personnel is Linked to Retention and Job Satisfaction

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Working with research animals can be both rewarding and challenging. Rewarding aspects include understanding the contributions of animal research to improvements in human and animal health and the knowledge that one can provide care and compassion for the animals. Challenges include witnessing stress/pain in animals necessitated by scientific requirements, end-of-study euthanasia, and societal stigma associated with animal research. These challenges could be compounded with more general workplace stresses, in turn impacting job retention and satisfaction. However, these factors have yet to be formally evaluated. Therefore, the purpose of this survey was to comprehensively evaluate professional quality of life's correlation with key workplace metrics. Six institutions were recruited to participate in a longitudinal intervention trial on compassion fatigue resiliency. A cross-sectional mixed methods survey was developed to evaluate professional quality of life, job satisfaction, retention, and factors influencing compassion fatigue resiliency. Quantitative data were analyzed via general linear models, and qualitative data were analyzed by theme. Baseline data was collected from 198 participants. Personnel who reported higher compassion satisfaction also reported higher retention and job satisfaction. Conversely, personnel who reported higher burnout also reported lower job satisfaction. In response to open-ended questions, participants said their compassion fatigue was impacted by institutional culture (70% of participants), animal research (58%), general mental health (41%), and specific compassion fatigue support (24%). In conclusion, these results show that professional quality of life is related to important operational metrics of job satisfaction and retention. Furthermore, compassion fatigue is impacted by factors beyond working with research animals, including institutional culture and general mental health support. Overall, this project provides rationale and insight for institutional support of compassion fatigue resiliency.

P162 IQ Consortium's Contract Research Organization Outreach Working Group - A 14-year Retrospective Look

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The International Consortium for Innovation and Quality in Pharmaceutical Development (IQ Consortium® or IQ) is a not-for-profit organization composed of pharmaceutical and biotechnology companies. The IQ Consortium aims to enhance the capabilities of member companies in developing transformative solutions that benefit patients, regulators, and the broader research and development (R&D) community. The reduction, refinement, and replacement (3Rs) of animal testing in drug development is one of their key initiatives.IQ Consortium's Contract Research Organization Outreach Working Group (IQ CRO WG) was one of the first working groups under the 3Rs Leadership Group and was developed to benefit the industry by aligning and addressing common expectations shared by CROs and member companies. The primary objective of the IQ CRO WG was to align contract research organizations (CROs) and member companies in their efforts towards implementing the 3Rs and advancing animal welfare. This poster presents a comprehensive retrospective analysis of the past 14 years of the IQ CRO WG's contributions to the field of 3Rs and animal welfare. The development of tools for assessing animal welfare risks in work performed with external partners in a uniform manner and several publications that have been produced by the working group that cover a range of topics, such as recommended dose volumes for laboratory animals, blood collection guidelines, preclinical research techniques in non-human primates, and opportunities for improved 3Rs practices. A webinar was also developed to discuss how changes to the study timeline impact animal welfare and study integrity. Finally, insights into the current activities of the IQ CRO WG are presented, specifically two ongoing publications. The first publication discusses considerations for rodent bedding selection in animal research, while the second publication provides an update on blood collection guidelines. Through this retrospective analysis, the poster portrays the significant contributions of the IQ CRO WG to the advancement of the 3Rs and animal welfare over the past 14 years.

P163 Introducing High School Students to Biocontainment Research Career Opportunities: Outreach Program by the RAV3N Network

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The Research Alliance for Veterinary Science and Biodefense BSL-3 Network (RAV3N) is a network of academic and government BSL-3 level biocontainment animal and laboratory research facilities within the United States and Canada. RAV3N was developed with the purpose of creating more interactive relationships and collaborations among the biocontainment research facilities focused on veterinary and emerging zoonotic diseases. A common challenge experienced by all RAV3N members is the difficulty in recruiting and retaining staff to work in high-containment laboratories and animal research facilities. Reasons cited for this include concerns about widely advertising these positions due to sensitivities or public misconceptions about animal research and a general lack of awareness of the careers and opportunities in biocontainment research. To address this challenge, the RAV3N Workforce Development Working Group developed a pilot outreach and training program to introduce high school students to biocontainment research and career opportunities in the field. The initial launch and evaluation of the pilot program were conducted at one of the RAV3N academic member institutions with a group of 22 high school students at its mock BSL-3 training lab. The program commenced with a PowerPoint overview of animal research and its importance to human and animal health, biocontainment principles, and career opportunities that was then followed by several hands-on activities, including personal protective equipment (PPE) donning and doffing, training on entry/exit procedures for BSL-3 laboratories and practicing their pipetting skills in a biological safety cabinet. The students were highly engaged in the activities, and several expressed interest in learning more about career opportunities in this field. Student feedback confirmed that this program successfully raised their awareness of careers in biocontainment and communicated positive messages about the benefits of animal research to humans and animals. The training curriculum and course materials are now available for RAV3N members to adapt and use in their outreach and recruiting activities.

P164 Working Together to Increase Openness to Animal Research Among Students

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AstraZeneca (AZ) is committed to openness and transparency in how, when, and why animals are used for research. AstraZeneca is the first Biopharmaceutical Company to be awarded Leader in Openness status by the internationally renowned organization, Understanding Animal Research (UAR). The collaboration with the director of STEM and community engagement, which began in March 2023, has been a transformative aspect of AstraZeneca's efforts in this area. This partnership was established to increase the number of high school and undergraduate students participating in AstraZeneca's initiative on openness to using animals for research. Since 2023, there has been a notable increase in students touring the vivarium and engaging with our team, with ten tours given to over 150 students aged 12 and over. Tours and veterinarian presentations are conducted during AZ events like Take Your Child to Work Day (TYCTWD), the summer RISE program, and the efforts of Emerging Leaders in STEM. In 2023, over 1,000 children participated in TYCTWD, and in 2024, over 1,200 children interacted with the AZ team. In 2022, only one university visited the vivarium, but since then, three universities have learned about animal research and different careers in the field. Each year, meetings are held to discuss the schools planning to visit, and the AZ campus events are scheduled to ensure availability. The program for each visit, the number of students per tour, and how to rotate from the tour to the veterinarian presentation are discussed at the meetings. The STEM team has played a pivotal role in bringing students from high school and undergraduate programs to the site for STEM activities, which has amplified AZ's outreach initiatives. These interactions have been fulfilling, and we are eager to continue our collaborative efforts to draw more students to the site and further enhance their understanding and appreciation of animal research.

P165 Development of an In Vivo Techniques Undergraduate Course & Digital Badge to Improve Institutional Collaboration, Animal Welfare, and Student Marketability

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The Rutgers University Animal Care Training Program has introduced a 3-credit undergraduate course, In Vivo Techniques with Laboratory Rodents (IVT), to offer practical experiential learning. In the current biomedical research job market, employers often seek experienced workers, but undergraduate students typically lack the necessary experience. Unlike veterinary or veterinary technician schools, Rutgers University's animal science program does not require externships for graduation within the lab animal track. The IVT course addresses this gap by providing hands-on experience and career opportunities, often giving students their first exposure to working with laboratory rodents. Students in the IVT course learn hands-on techniques with mice and rats, including injections, blood collections, minor surgery, and complementary topics such as animal regulations, behavior, diseases, and occupational health. The course emphasizes the 3Rs by using non-animal models when possible and promoting non-aversive handling techniques. By the course's end, students proficiently apply current best practices for rodent work, enhancing animal welfare and care. Upon completion, students receive an academic digital badge that highlights their skills, increasing their marketability to recruiters. Digital badges are digital confirmation of an individual's skills and competencies, which can be verified through the credential page linked to the digital badge. Graduates of the course have been highly sought after for laboratory positions at Rutgers and other career paths, including roles within the department. Here, we discuss the steps involved in creating a course from the ground up, including leveraging a non-academic veterinary team, challenges, creation of a training space, forging an academic partnership with one of the colleges at the University, utilizing digital badging, and outlining, implementing, and evaluating the course.

P166 The 3Rs in Training Laboratory Animal Professionals: Considerations for Non-Animal Models

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Animals are used in a variety of research that requires technical skills for collecting data, such as restraining animals, drawing blood, and giving injections, and various methods may be needed based on the species and study needs. There is growing interest in using alternatives to live animals (alternative models) for training laboratory personnel in the early stages of technical skill acquisition. The purpose of this study was to survey laboratory animal professionals on their understanding, use of, challenges, and attitudes towards using alternative models in training programs. Laboratory animal professionals in the US and Canada were recruited to participate in a 15-question survey. Additionally, personnel responsible for training laboratory animal personnel were recruited to participate in an interview to answer 12 open-ended questions. Survey results were summarized using descriptive statistics. Interview questions were transcribed and qualitatively

analyzed for themes and subthemes. There were 116 survey participants (46 US; 70 CA) and 14 interview participants (10 US; 4 CA). A total of 64.8% of respondents stated their facility is currently using alternative models for training purposes. The alternative methods that participants were most familiar with were cadavers (83.2%), simulated models (62.6%), and mannequins (50.5%). The perceived benefits of using alternative models were reducing animals used (87.9%) and animal welfare (88.9%). The perceived challenges of using alternative models were poor skill acquisition (53.3%), time to develop alternative training techniques (48.6%), and cost (33.6%). Live animals were still the primary method for technical skill acquisition, with 73.2% of respondents stating their facility maintains animal colonies of various species for training purposes. When asked about endpoints for training animals, 89.9% stated they have defined endpoints for training animals based on animal condition, size or age, and time or number of uses. The additional tools trainers would like to see developed are more species-specific models, especially for primates and rodents, models for different sizes or ages of animals, and more realistic or responsive models.

P167 Developing an Institutional Roadmap to Achieve a Sustainable Approach to the 3Rs at a Regional Australian University

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Examples of implementation of the 3Rs, along with animal research data, are reported annually to our institution's governing body as well as to its external State government regulator. In 2023, the university sought internal advice to promote both the importance of the 3Rs and to improve animal-based researchers' commitment to them. Two working groups of key stakeholders were tasked with developing a roadmap consistent with the *Australian Code for the care and use of animals for scientific purposes*, 8th edition (the Code). Each consisted of either six or seven participants who were either animal-based researchers, or professional staff working in the animal research space. These groups met monthly to review current and potential 3Rs outcomes in either clinical or wildlife research, with the aim of developing a pathway for the institution to invest in initiatives that would continue advancing the appropriate use of the 3Rs whilst maintaining high-quality research in the fields of human and animal health. Major outcomes of the roadmap project have included advice to the institution on requirements for infrastructure and resources to assist researchers; advice on practical training in the 3Rs for both animal-based researchers and the independent Animal Care and Ethics Committee who review and approve animal-based research at the institution in line with the Code; and the establishment of guidelines for an internal 3Rs Grant Scheme. This last outcome from the 2023 3Rs roadmap task has assisted in the awarding of funds for projects that promote animal welfare by addressing one or more of the principles of the 3Rs. To date, three projects have been successful in securing funding of AUS $20,000 each from the newly introduced ongoing internal 3Rs Grant Scheme.

P168 Embracing 3Rs: Combining Safety Pharmacology and Genetic Toxicology Endpoints when Scientifically Justified

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The 3Rs in animal research are defined as Replacement, Reduction, and Refinement. In this study, we investigated ways in which to apply these values, focusing on the reduction of animals needed to achieve the intended endpoints in a regulatory acceptable manner. In a typical micronucleus assay, 58 animals are generally used in the study: 18 animals for the range-finding phase (3/sex/treatment) and 40 animals for the definitive phase (5/sex/treatment). Sex differences aren't generally established prior to a micronucleus study; thus, treatment is necessary for both sexes. These sex differences, however, should be better understood prior to an Irwin assessment. Therefore, in the standard Irwin assessment, one sex is utilized, typically resulting in using a total number of 32 animals of one sex (8/treatment). Performing both studies separately results in the use of approximately 90 animals. However, it is an acceptable regulatory approach to add each study to an existing toxicity study, usually the one-month GLP study, when scientifically appropriate. Using the same scientific rationale to add these endpoints to a toxicology study, we evaluated and then combined the Irwin and micronucleus studies in a situation where we were unable to add the endpoints to an existing toxicology study. The range-finding phase was performed using one animal/sex/dose level (14 total rats) and was scheduled with sufficient time to review the data in between treatments. This allowed an evaluation of sex differences to be determined prior to the definitive micronucleus/Irwin phase. In the definitive micronucleus and Irwin phase, a total of 36 rats were used. Using a stepwise, scientific approach, the endpoints were successfully combined on a cohort of the same animals, and the team was able to perform this study using only 50 animals, a reduction of approximately 40 animals.

P169 Development of a Beagle R Program and the Effect in Japan

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As a commitment to continually improve animal welfare, a supplier of animal models for biomedical research established a "Beagle R Program" in 2016. The "Beagle R Program" offers three options: 1. The transfer of retired research animals from facility to facility; 2. Donating cadavers for educational programs; 3. Rehoming healthy animals as pets. The "R" signifies Reutilization, Responsibility, and Reduction. We supplied a total of 26 reutilized animals for studies, 167 cadavers for education, and rehomed 97 animals as pets by 2023. Most of the R animals are dogs. The cats totaled two reutilized, eight cadavers, and 19 rehomed. We expected the high quality of our animals to provide enough value at the next facility or as a home companion if the animal is healthy and that our program would contribute to animal welfare. In Japan, we are the only rehoming group connecting research facilities with society. Customers expect confidentiality of their company name and personal information. We expected our R program to have a positive effect on research facilities and investigated the effect through a customer survey in 2022. We received responses from 8 individuals in 6 facilities, with a survey satisfaction rate of 4.63 out of 5.0. Most effected by the R program were the animal caretakers. Although feedback was collected from a small group, we gained knowledge of the positive impact the R Program has on animal caretakers. Awareness of the R Program increased animal welfare, in-house communication, outreach, and employee engagement. We believe healthy animals that contributed to research can continue to provide important contributions and by sharing our survey results with customers, we hope they gain an understanding of the benefits of openness and communicating with rehoming owners.

P170 Transitioning Laboratory Dogs into Research Ambassadors: from Clinical to Household Settings

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The use of domesticated species in lab animal research is a necessary but sometimes difficult challenge for institutions to navigate. Dogs, in particular, hold an important role in this paradigm. Due to their omnipresence in daily life, it can be difficult for staff to manage the emotional burden that is a byproduct of the end of their studies. Concerns may include compassion fatigue, backlash from the public, and adherence to the tenets of the three Rs (replacement, reduction,

and refinement). One outlet to alleviate these concerns is the retirement of research animals. However, transitioning a dog from a clinical to a household setting can prove to be a daunting task, in part due to the lack of literature on this subject. Key everyday events, such as walking on a lead, interacting with conspecifics and wildlife, or learning where to void, can be intimidating for an animal reared without these experiences. During a recent study involving canines, we were able to retire a 2-year-old spayed female hound mix into the care of a staff member. From the time that the retirement was announced, we have continuously seen benefits such as increased staff engagement with the animals and reports of feeling a sense of group accomplishment. We have found that staff investment in the animals' wellbeing goes far beyond the walls of the vivarium, and retirement provides a morale boost when staff receive updates on her progress or have the opportunity to interact with her outside of the workplace. We have also found that retired research ambassadors can start a dialogue and aid in the education of the public. Additionally, we have demonstrated how these behavioral challenges can be overcome through methods of positive reinforcement, pre-planned interactions, and controlled exposures. Lastly, retirement is a refinement of our practices, reducing the number of euthanasias while providing welfare for the animal beyond their study. Retirement provides the opportunity to positively represent the research community with physical ambassadors, provides a full and enriched life beyond their services to the scientific community, and illustrates the attention paid to staff mental health.

P171 Comparison Between Two Animal-Free Sentineling Products

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The 3Rs of Research encourage efficiency with and purposeful use of valuable animal life. The research field has been successfully incorporating methods to reduce or replace animals as sentinels in keeping with the 3Rs. Since 2022, the University of Alabama at Birmingham (UAB) has utilized 100% artificial sentinels (AS) using a filter media in a sentinel cage versus live mice or rats for health surveillance. The UAB AS method has previously been demonstrated to be more sensitive than live animal sentinels. A study was performed for one sentinel period in four mouse housing rooms historically positive for MNV and *Helicobacter* spp to compare detection using two types of contact media on an IVC (individually ventilated cage) rack versus a collection box off-rack to determine if they would be equivalent in the detection of mouse pathogens. At each cage change, soiled bedding from ~50-60 colony cages was placed into one sentinel collection cage. The mixed soiled bedding was divided between the IVC cage and collection box, and manual agitation was performed for a minimum of 15 seconds. Bedding and media remained in the IVC cage between cage changes, but the bedding was removed from the collection boxes post-agitation to prevent mold growth. At the conclusion of the 12-week study, both types of contact media were collected in separate 50 mL conical tubes for PCR testing. Results show that the detection of infectious agents was generally equivalent to that of the two contact media types. One room of the four was an outlier, only detecting agents in the contact media from the IVC cage. We suspect staff did not follow the directions for exposing media in the collection box; this room is being repeated for accuracy. The results show that contact media exposed once at cage change in the collection box performs equivalently at UAB and that soiled bedding may not need to be in contact with the media for the entire sentinel period to provide accurate test results. We encourage any interested institution to perform in-house studies such as this to establish the success of animal-free sentineling.

P172 Comparison of Seed Starter Mat to Circulating Warm Water Blanket for Rodent Heat Support

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Heat support is often provided to rodents in a variety of situations, including anesthesia, illness, and young and old age, to help maintain body temperature. One piece of equipment that is commonly used in a laboratory animal setting for heat support is a heat pump with a circulating warm water blanket. However, there are some drawbacks to these systems, such as cost, maintenance, use of distilled water, and the potential for leaks or damage to equipment. Our laboratory was interested in investigating a novel heat support system, seed starter mats, which are commonly used in horticulture for germinating seeds. Seed starter mats can be set to a temperature between 5°C and 42°C, which is similar to the temperature range for circulating warm water blankets, 10-42°C. We evaluated the performance of seed starter mats to provide consistent heat support in comparison with circulating warm water blankets. We used an infrared thermometer to measure temperature across different zones of each device surface. Additionally, a clean cage was placed half on/half off both mats, and temperature was measured on the surface of the bedding. Our data concludes that seed starter mats have a similar time to reach maximum temperature as circulating warm water blankets and are able to consistently maintain that maximum temperature across various areas of the mat. The results of this study support the use of seed starter mats as external heat support for rodents.

P173 Evaluation of the Effectiveness of Thermal Regulation of Mice using Various Heat Therapy Devices

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Thermal regulation of anesthetized rodents is critical to a successful recovery and continued health. Devices for providing heat therapy during surgery range from circulating water blankets, electrical coil heated platforms, and infrared emitting platforms to various gel packs, all with varying amounts of heat delivery, which could be further affected by placing the material between the heating pad and the animal. The aim of this study was to investigate the effectiveness of the different heating options in their ability to maintain the temperature of the animal and the recovery cage as well as the impact on heat transfer from materials placed between the heat source and the animal. Temperature was measured at the surface of the heating element and at the surface of the material that the animal would be placed upon with an IR Thermometer. The cage environment temperature was measured with both an IR Thermometer and an Air Thermometer. These temperatures were then evaluated for heat transfer using an animal replacement of a water balloon of similar size and weight to a mouse to gather basic information before using animals in a follow-up study. This substitution was made to Reduce the mice needed for this initial evaluation. The heat transfer readings varied depending on the material placed over the heating element. The ability of any heating element to maintain a steady temperature was impacted by the type of material placed between the heating element and the balloon to varying degrees, depending on the type of heating element. These findings indicate that great care must be taken to ensure that the correct heating element and material on which to place the animal is appropriate for the application for which it is in use.

P174 Evaluation of a Commercial Forced Air Warming Cabinet as an Alternative Warming Chamber in Mice (*Mus Musculus*)

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Laboratory rodents are sensitive to their external environment and can be subject to cold stress, which can impact their physiological and behavioral parameters. In this study, we evaluated the post-operative and post-procedural whole-body warming capability of a commercial forced air warming cabinet that is labeled for mice,

rats, and hamsters. There is no literature describing the amount of time that rodents can be temporarily housed within this commercial cabinet. Here, we determined the time that female BALB/c and C57BL/6J mice (*Mus musculus;* n=40) needed to be present in the warming cabinet until optimal core body temperature in mice was achieved (>36°C) after manipulation (15-20 seconds) outside their home cage. We hypothesized there would be strain variation, with non-albino (C57BL/6J) mice demonstrating a faster onset to > 36°C due to tyrosinase gene (*Tyr*$^{+}$) and production of melanin. Individual mice were placed in the warming cabinet, and rectal temperature was measured at time points 0, 5, 10, 15, and 30 minutes. Overall, both strains of mice were able to achieve > 36°C in the warming cabinet: C57BL/6J at 5 minutes (99% probability for the average mouse) and 10 minutes (95% probability for individual mouse) while BALB/c at 10 minutes (97% probability for average mouse) and 15 minutes (87% probability for individual mouse). Additionally, no animals entered a hyperthermic state at the last 30-minute timepoint. In contrast, although the allocation of animals into control and treated groups was imperfect, it was observed that control animals, which were not exposed to the warming cabinet, were not able to achieve > 36°C for either mouse model in a short time period. These findings suggest that the air warming cabinet is a safe and reliable method as a warming chamber with average optimal core temperatures reached within 5-10 minutes after removal from their home microenvironment.

P175 High-Throughput Preclinical Pharmacokinetics Assay: Enhancing T Cell Detection Efficiency and Reducing Animal Use

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Traditional preclinical pharmacokinetics (PK) assay workflows for detecting engineered human T cell concentrations in mouse blood often require large blood volumes and frequent animal usage, leading to increased stress for the animals and extended processing times. To address these challenges, an optimized in vivo PK assay workflow was developed using flow cytometry, comparing variables such as reduced blood collection volumes, different routes of blood collection, and a high-throughput assay plate format. The optimized workflow achieved comparable T-cell concentrations across all tested variables. The reduced blood volume and flexible blood collection routes allowed for more frequent sampling and reduced the number of mice needed, as multiple time points could be collected from the same mouse. Additionally, the increased throughput enabled the simultaneous processing of over 90 samples, reducing the total processing time to less than 3.5 hours with minimal personnel involvement. This approach significantly improved the efficiency and refinement of the PK assay workflow while reducing animal usage and stress. These advancements provide valuable insights for optimizing PK assays, facilitating better drug development strategies, and aligning with ethical research practices. Future applications of this workflow could extend to other preclinical assays, further promoting the principles of the 3 R's.

Clinical Posters

P200 A Happy Tale/Tail: Treatment and Management of a Tree Shrew Tail Tip Injury

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An approximately 2-year-old female northern tree shrew (*Tupaia belangeri*) housed in an indoor research colony presented with an acute tail tip lesion of unknown origin. Physical examination revealed an ellipsoid degloving-type injury with a combination of ablated external skin and loosened, retractable distal skin overlying exposed distal coccygeal vertebrae measuring ~0.5 cm long. Treatment of tail tip injuries in other animals can include 1) medical management consisting of wound care, pain management, and antibiotics, 2) surgical intervention consisting of a tail tip amputation, and 3) humane euthanasia based on the severity of the lesion and clinical presentation of the animal. Differential diagnoses and general etiologies for tail tip lesions include trauma, self-injurious behaviors, and peripheral vascular necrosis. As this cohort of animals was specifically conditioned over time for study use and the affected tree shrew remained bright, alert, and responsive, medical management was first utilized to attempt secondary intention healing. The tail tip showed delayed healing with cyclical drying and re-freshening of the lesion foci over time; culture and sensitivity results were unremarkable. Serial observations of routine tree shrew behavior in captivity revealed normal ambulation consisting of repetitive, direct contact of the tail with environmental implements such as cage dividers, cage sides, and perches akin to "Happy Tail Syndrome" in dogs whose tail tips are injured by striking surfaces. A modified, duct tape-covered, Robert Jones-type tail bandage was placed to provide buffered barrier support for the lesion, which proved successful as it remained comfortably in place and intact prior to surgical amputation. Routine surgical amputation without post-op bandaging was completed to remove the non-healing segment; histopathology of the resected segment was also unremarkable. A revision reclosure of the amputation site was required 1d later as dehiscence occurred, likely secondary to the tail tip directly contacting cage implements without a bandage in place. A tail bandage was replaced and maintained for ~2 weeks. With a tincture of time and a combination of medical, surgical, and bandaging management of the tail tip lesion, it healed completely without remarkable incident. To our knowledge, this is the first reported tail bandage and surgical tail tip amputation of the northern tree shrew.

P201 A Syrian Hamster with a Left Axillary Wound Secondary to Mastitis

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A 6.5-month-old female Syrian hamster presented with a wound in the left axilla. This hamster had given birth to six pups ten days prior to presentation, and all pups were euthanized one day post-partum for study. The hamster was otherwise experimentally naïve and had no other clinical history. On physical exam, the hamster was BAR, in good body condition, and well hydrated. The left axillary wound was ~1cm in diameter and penetrated to the subcutis but not the body wall. There were seven erythematous swellings on the ventral thorax and abdomen along the mammary chain. Differential diagnoses, including lymphoma, mastitis, and abscesses, were considered. The hamster was euthanized, and a diagnostic necropsy was performed. At necropsy, a ~6 mm firm white subcutaneous mass was found on the right ventral abdomen adjacent to the mammary gland. There were no other significant gross findings. Histologically, both the right ventral subcutaneous mass and the left axillary region were consistent with necro-suppurative and histiocytic mastitis, which were partial to completely encapsulated. Multiple bacterial isolates, including *E. coli, Staph spp., and Strep spp.*, were cultured from the white subcutaneous mass. The epidermal ulceration observed in the left axillary region communicated with mammary inflammation and the wound is presumed to be secondary to self-trauma induced by the discomfort of the mammary lesion. These findings are consistent with mastitis in this hamster. Both coliform and streptococcal mastitis have been reported in the Syrian hamster, though the incidence in laboratory colonies is not described. Mastitis should be a differential for masses or wounds on the ventral thorax or abdomen of post-partum hamsters, especially as part of experiments in which pups are removed from lactating mothers in early lactation.

P202 Chronic Kidney Disease in Strain 13/N Guinea Pigs (*Cavia porcellus*)

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Strain 13/N guinea pigs, although rare in the United States, are a valuable small animal model for the study of hemorrhagic fever viruses due to their similarities in disease progression to that of humans. At our facility, the average lifespan of a strain 13/N guinea pig is abbreviated compared to that of other guinea pigs, with both sexes often only reaching three to four years of age. The small total population of this inbred line has resulted in sparse literature detailing common age-related lesions and other health conditions. Identification of key diseases impacting the strain's longevity will allow us to target areas of research to enhance clinical decision-making and colony management, ultimately aiming to improve health outcomes and data integrity for the research studies. We performed a retrospective review of 81 pathology reports from the previous four years to determine the most common post-mortem diagnoses in our breeding colony. By far, chronic kidney disease (CKD) was the most frequently reported diagnosis at the time of necropsy, with 69% of animals showing hallmarks of CKD at the time of death. Gross findings included but were not limited to nephrosclerosis, hydronephrosis, and metastatic calcification. Microscopic examination further revealed glomerulosclerosis, tubular atrophy, diffuse tubular degeneration, and renal pelvis dilation. Clinically, these animals typically present with progressive weight loss and cachexia. Antemortem bloodwork may be characterized by anemia of chronic disease (microcytic, hypochromic) and/or azotemia, but often, animals will not have any remarkable changes. Notably, the degree of azotemia was not correlated with the severity of kidney damage. Hyperphosphatemia and hyperglycemia may also be seen. Predisposing factors for CKD in strain 13/N guinea pigs have not yet been identified. Although calcium often increases with age in strain 13/N guinea pigs, the exact relationship of calcium to CKD has not been described. Other guinea pig strains may also develop CKD but will typically present later in life, often three years of age or older. Urolithiasis, with or without secondary hydronephrosis, is another common diagnosis that has not yet been linked to CKD but may indicate underlying causes affecting the urinary tract more broadly. Further studies are warranted to explore the role of husbandry practices and genetics into the development of this disease.

P203 Spontaneous *Paeniclostridium sordellii* Enteritis in a Dunkin Hartley Guinea Pig (*Cavia porcellus*)

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A 4-month-old, intact female, pair-housed Dunkin Hartley guinea pig (*Cavia porcellus*) was found dead without prior clinical signs of illness in April 2024. In February 2024, the animal was administered phosphate-buffered saline (PBS) subcutaneously as a control in a Herpes Simplex Virus 2 study. The guinea pig was monitored during routine veterinary rounds and monthly weight checks post-injection without incident. On necropsy, the carcass was in good body condition with ample fat stores and appropriate muscle coverage. The subcutis was diffusely tacky. There was moderate diffuse gas distension of the entire gastrointestinal tract with no overt evidence of torsion, volvulus, or impaction. The small intestines were diffusely hyperemic with red, watery contents, and the cecal serosa was diffusely purple-black. The presumptive differential diagnoses were an enteritis, an intestinal torsion, or gastric dilatation that replaced itself prior to necropsy. Sterile aerobic and anaerobic cultures of the affected small intestinal contents were collected for further diagnostics, and *Escherichia coli, Enterococcus faecalis, Proteus mirabilis, Staphylococcus aureus,* and *Paeniclostridium sordellii* were isolated. Histology of the small intestine revealed moderate, histiocytic, necrohemorrhagic, and edematous enteritis with abundant Gram-positive rods with terminal endospores, which was consistent with *P. sordellii.* Although this guinea pig had gross and microscopic evidence of mild autolysis, the presence of inflammatory cells, hemorrhage, and necrosis adjacent to clusters of bacteria was consistent with an antemortem infection. The origin of this infection is not known as there is not currently a thorough understanding of *P. sordellii* transmission in the literature. The conspecific remained asymptomatic, and an anaerobic fecal culture revealed no growth. This is the first reported case of spontaneous *Paeniclostridium sordellii* infection in guinea pigs, to the author's knowledge. This agent should be considered as a differential in cases of sudden death and enteritis in guinea pigs.

P204 Acute Neurologic Presentation in a New Zealand White Rabbit *(Oryctolagus cuniculus)*

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A ~24-month-old intact female New Zealand white rabbit (*Oryctolagus cuniculus*) underwent bilateral transcortical screw implantation in the distal femur and proximal tibia as part of an IACUC-approved protocol. One set of screws was dipped into *Staphylococcus aureus* media before implantation. Post-operatively, the doe was given enrofloxacin (10 mg/kg SID SQ for five days) and carprofen (4 mg/kg SID SQ for seven days), and supportive care as needed, including appetite stimulants, fluid therapy, and nutritional supplementation. Weight decreased from 4.77 kg pre-surgery to 4.45 kg four days postop, then stabilized until acute clinical presentation. Thirteen days post-surgery, the doe presented with acute lateral recumbency and severe neurologic symptoms. Physical exam at presentation revealed bilateral nystagmus, no pupillary reflex, left-sided head tilt, and cold hyperextended hindlimbs with no deep pain sensation. The animal was removed to a recovery cage where external heat was provided, followed by sedated radiographs with midazolam (1mg/kg IM)/ketamine (15mg/kg IM). No fractures were observed; however, due to a grave prognosis, the doe was humanely euthanized. Full postmortem diagnostics were performed to rule out septicemia or thrombosis from experimental procedures. CBC analysis showed mild leukocytosis with mild to moderate neutrophilia and mild lymphopenia consistent with a stress leukogram. Cardiac necropsy noted a nodule on the mitral valve; histology revealed myocardial atrophy with necrosis and fibrosis of cardiac tissue. No gross evidence of thrombosis was visualized within the aorta. Acute aortic necrosis was identified and attributed to cardiac decompression; however, a saddle thrombus cannot be ruled out. Gross necropsy also revealed nutmeg liver; on histology, this was hepatic lipidosis. Inadequate nutrient intake resulted in hypoglycemia and hepatocellular triglyceride accumulation. White foci on the kidneys' external cortex were noted to be acute renal infarcts, lacking findings of fibrosis or regeneration. The cause of death was diagnosed as a combination of congestive heart failure along with nutritional and metabolic imbalances with no direct link to the surgical event. This acute neurologic presentation in a bacterial challenge orthopedic model demonstrates the need for complete pre- and post-diagnostics to ensure a sound experimental model.

P205 Lethargy and Inappetence in a Postpartum New Zealand White Doe

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A 21-month-old, 3.58 kg, intact, multiparous, New Zealand White doe rabbit presented with lethargy, inappetence, and decreased fecal/urinary output two weeks after kindling ten kits, with three surviving kits at the time of presentation. Previous litter sizes ranged between 3-4 kits. On initial physical examination, the doe had a body condition score of 2/5. Carprofen, metoclopramide, subcutaneous fluids, and dietary supplementation were administered. Later the same day, the animal was found laterally recumbent, paddling, and nonresponsive to stimuli, necessitating humane euthanasia. Differentials for acute decompensation/sudden death after recent parturition included pregnancy toxemia (hepatic lipidosis, hypocalcemia, hypoglycemia), uterine abnormalities (metritis), or infectious (bacterial overgrowth, listeriosis, encephalitozoonosis). On gross necropsy, the animal had abundant visceral and subcutaneous fat stores. The liver was diffusely pale, enlarged, friable, and greasy, with rounded edges to all lobes. Histologically, sections of the liver were diffusely affected by severe hepatocyte vacuolization consistent with lipid accumulation. In sections of the brain, there were foci of spongey change and neuronal degeneration in areas of the cerebrum and brainstem. Bacterial cultures were negative for infectious causes. Additional diagnostics were not performed. Clinical, gross, and histological findings support a diagnosis of metabolic derangement secondary to hepatic lipidosis (i.e., pregnancy toxemia). Pregnancy toxemia most often presents in multiparous does with large litters and sudden decreased feed intake. The caloric demands of gestating large litters, parturition, and lactation can lead to a negative energy balance, leading to a rapid and excessive mobilization of fat stores. As a result, superfluous production of ketones and overwhelming accumulation of lipid in the liver may lead to liver failure. In animals with pregnancy toxemia, neurological signs may be related to hypoglycemia or ketosis from the metabolic derangement or from hyperammonemia related to liver failure (hepatic encephalopathy). Multiparous does that are older, over-conditioned, and/or carrying large litters should be monitored closely for adequate nutritional intake to prevent pregnancy toxemia.

P206 Submandibular Swellings in a Mouse Model for Hepatocellular Carcinoma

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A colony of genetically engineered C57BL/6J mice were produced for modeling hepatocellular carcinoma development, yet were consistently reported to veterinary staff for large submandibular swellings (n=20). In accordance with institutional policy and clinical welfare concerns, several mice were euthanized prior to the predicted study endpoint, thus preventing sufficient time for the development of the intended hepatic tumor burden the lab aimed to study. Mice in this cohort received intraperitoneal tamoxifen injections at two months of age and were then started on thioacetamide water to induce hepatic tumor growth. A 5-month-old, intact male mouse with genotype C57BL/6-*Gt(ROSA)26Sor*$^{tm5(Map3k14)Rsky}$/J presented on physical exam with multiple soft and fluctuant submandibular masses that did not hinder the mouse's ability to eat, drink, breathe, or ambulate. While under clinical monitoring, the condition progressed to the humane endpoint, and the mouse was subjected to full gross and histopathological evaluation. Gross necropsy revealed severe enlargement of the submandibular, parotid, and sublingual glands. Histologically, submandibular tissues were largely effaced by a poorly differentiated carcinoma. No other histologic abnormalities were appreciated. Grossly, the liver had no visible tumors, consistent with lab findings on past mice. The genetic construct engineered into these mice aimed to overexpress NF-κB-inducing kinase (NIK), specifically in biliary duct cells. NIK overexpression is implicated in cholangiocarcinoma development, and the use of a cytokeratin 19 (CK19) promoter was intended to provide the model tissue specificity. Unexpectedly, carcinoma developed in the salivary glands instead of the liver. After further analysis, this may have occurred due to CK19 expression in other tissues, inadvertently causing off-target carcinoma development. This case highlights the clinical importance of assessing gene promoter specificity, the need to ensure engineered mouse models accurately reproduce the intended phenotype, and minimize the occurrence of clinically detrimental off-target effects.

P207 Vasculitis and Ischemic Necrosis in the Tail a Collagen-Induced Arthritis Study Mouse

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A 3-month-old male DBA/1J mouse enrolled in a collagen-induced arthritis (CIA) study was examined for lesions on the tail. At 2.5 months of age, the mouse had been subcutaneously immunized on the tail with a type II chicken collagen emulsion in complete Freund's adjuvant (CFA). On physical exam, the mouse was bright, alert, and responsive, with a body condition score of 3/5. At 4 cm distal to the tail base, the tail had three small, multifocal red areas and was diffusely purple extending to the caudal tail tip, which was black. Differentials included trauma, infarction, and coagulopathy. Due to research and clinical welfare concerns, the mouse was euthanized. Throughout the cranial quarter of the tail, histopathology revealed moderate, focally extensive, pyogranulomatous panniculitis with mild hemorrhage, fibrin, necrosis, and clear spaces (presumed collagen emulsion with CFA). In the caudal tail, multifocal vasculitis with fibrin thrombi and diffuse ischemic coagulative necrosis with severe hemorrhage were observed. A possible pathogenesis for the purple tail in this mouse was that the inflammation associated with the collagen and CFA immunization resulted in vasculitis, creating a hypercoagulable environment resulting in fibrin thrombi occlusion and subsequent ischemic necrosis of the caudal tail. Vasculitis and ischemic necrosis have not been previously described in murine autoimmune arthritis studies or in postmortem studies on pathologic evaluation of routine procedures such as handling, blood collection, and intraperitoneal injections. Although rare, this lesion should be considered as a differential for researchers, clinicians, and diagnosticians working with CIA mouse models.

P208 Arachnoid Cysts in mT/mG Mice

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Three B6.129(Cg)-*Gt(ROSA)26Sor*$^{tm4(ACTB-tdTomato,-EGFP)Luo}$/J (mT/mG) E18 mice, of four in the litter, presented for increased cranial size noted at study endpoint. mT/mG mice are used to detect if and where ShhCre is present. ShhCre is the most potent lung epithelial-specific Cre driver, such that when Cre is present it will "knockout" an integrin subunit typically only found in the lung epithelial cells. The increased cranial size led to investigations for potential off-target effects with differentials including hydrocephalus, neoplasia, anencephaly. Mice were fixed in 10% neutral buffered formalin at room temperature for three days. The head was routinely processed, embedded, and stained with hematoxylin and eosin. Histopathology identified an empty space occupying cyst, which appears to be within the leptomeninges at the level of the cerebral cortex along the cranial aspect of the skull in two of three pups, which was not evident grossly. DAPI staining for DNA did not reveal Cre expression. Based on these histopathologic findings, these cysts were diagnosed as arachnoid cysts. Although there are case reports in dogs and cats, arachnoid cysts have not previously been reported in mice. In the human brain, these cysts are sporadic and, despite being space-occupying lesions, are often asymptomatic with no treatment required. Because this finding has not previously been reported in this genotype, our findings are unlikely to be an unintended consequence of the genotype and rather a potential genetic variation inherent to the breeders.

P209 Clinical and Histopathological Presentation of Natural *Burkholderia vietnamensis* Infection in Immunocompromised Mice

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A group of 20 two-month-old female NOD.Cg-Prkdcscid Il2rgtm1Wjl (NSG) mice were administered a single dose of Busulfan, a myeloablative agent used to lymphodeplete mice prior to humanization, via intraperitoneal injection. The mice were maintained on antibiotic water (Enrofloxacin, ~25 mg/kg/day) beginning three days prior to and for two weeks following the injection. Approximately three weeks after Busulfan administration and between 7-10 days following discontinuation of systemic antibiotic, all mice were reported either dead or for varying stages of weight loss, lethargy, and pallor. As the strain is considered extremely immunodeficient, there was concern that these mice were declining due to opportunistic infection or adverse drug effects. One mouse was taken for necropsy, and on gross exam, pinpoint to 1 mm discrete raised areas were noted throughout the liver and spleen. Given the findings, septicemia resulting from an opportunistic infection became the primary differential, and a portion of spleen was submitted for culture. The remaining tissues underwent histopathologic exam, and a pyogranulomatous inflammatory process was noted in the lung, heart, liver, kidney, and spleen with intralesionalgram negative rod-shaped bacteria. This was consistent with *Burkholderia vietnamiensis* being isolated from splenic culture. This bacterium is a member of the *Burkholderia cepacia* complex, which are ubiquitous in nature and are known to cause opportunistic infections in immunocompromised humans and mice. However, the clinical signs associated with this specific species of *Burkholderia* as a natural infection in mice has not been reported before. Our case study, along with other recent reports of unique presentations following infection from other members of this genus, underlines the sensitivity of these mice and the importance of periodic, systematic evaluation of all disinfection and sterilization processes used when housing and working with these mice since we suspect this arises from incompletely treated water or materials.

P210 Clinical Features of *Staphylococcus xylosus* Induced Scaly Dermatitis in an Immunocompromised Mouse

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A six-week-old, female, B6.Cg-Igrm1<tm1Gat>Tg(CAG-EGFP/M1lc3b)53Nmz mouse was reported for scaly exfoliative dermatitis. The mouse was singly housed in a sanitized, individually ventilated cage with autoclaved Sani-Chip hardwood bedding, acidified reverse osmosis deionized water, and an autoclaved, natural ingredient rodent diet (5k20). The mouse was weaned at post-natal day 21 and was experimentally naive. Initial assessment found the mouse hunched, lethargic, and mildly dehydrated. White-yellow crusts were noted on the dorsum, abdomen, face, and within the ear pinnae and canal. Upon handling, the fur easily sloughed off in large clumps. Multifocal, ulcerative lesions (~2-3mm) were scattered on the dorsum, neck, abdomen, rectum, and vulva. The mouse was euthanized via CO_2 inhalation and submitted for necropsy. Gross necropsy examination of other organs and tissues were unremarkable. Dorsal skin and ears were swabbed for aerobic and anaerobic bacterial culture. Tissues were collected in 10% neutral buffered formalin for histopathology. Differential diagnoses for scaly, exfoliative, ulcerative skin lesions in immunocompromised mice include infectious agents, parasitic infestations, environmental factors, allergic reaction, and nutritional deficiencies. Histopathology examination of the skin from abdomen, ear, and eye showed chronic dermatitis with epidermal hyperplasia, hyperkeratosis, and dermal fibrosis. Special stains were negative for bacterial and fungal organisms. PCR of skin scrapings, both in-house and by an independent laboratory, were negative for *Corynebacterium bovis*. Aerobic skin culture was positive for *Staphylococcus xylosus.* 16S rRNA PCR and sequencing confirmed the presence of *S. xylosus.* This female was singly housed, and the stress of being weaned may have resulted in an opportunistic infection with *S. xylosus.* No other animals of this strain, including litter mates, dam, or sire, have presented with dermatitis or cultured positive for *S. xylosus* since this case was reported.

P211 Pathological Control Data Analysis of rasH2 Mice Over a 26-week Experimental Period

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CByB6F1-Tg(HRAS)2Jic (rasH2) mice are genetically engineered and commonly used in 26-week carcinogenicity studies, adhering to the ICH S1B guidelines (testing for carcinogenicity of pharmaceuticals). The rasH2-Tg(tg/wt) mice contain the c-Ha-ras (HRAS) gene. This strain is produced by cross-breeding C57BL/6JJic-Tg(HRAS)2Jic (B6-Tg) hemizygous males and BALB/cByJJic (BALB) females. Here, we investigated the most recent pathological control data of rasH2-Tg and rasH2-Wt mice from the breeding facility. We examined 205 male and 205 female 34–36-weeks rasH2-Tg mice (tested for 26 weeks, starting at 8–10 weeks), 45 male and 45 female rasH2-Wt mice of the same age, 45 male breeding retired B6-Tg mice (51–60 weeks), and 45 female retired BALB mice (36–42 weeks). Body weight was lower for rasH2-Tg mice than for rasH2-Wt mice (male, rasH2-Tg; 33.8 g vs. rasH2-Wt; 40.6 g; female, rasH2-Tg; 27.6 g vs. rasH2-Wt; 30.1 g). The survival rate of rasH2-Wt mice was 100% for males and females than 94.4% for rasH2-Tg males and 97.2% for rasH2-Tg females. The most common spontaneous tumors in rasH2-Tg mice were bronchioloalveolar adenoma of the lungs (males; 12.4%, females; 11.4%) and hemangiosarcoma of the spleen (males; 4.7%, females; 3.4%). The incidence of other tumors was extremely low. Accordingly, our recent pathological control data showed a low incidence of spontaneous tumors and a very high survival rate in rasH2 mice. Thus, this prospective study showed no significant phenotypic changes from previously reported findings, instilling confidence in conducting 26-week carcinogenicity studies. Furthermore, this study demonstrated the animal model's robustness and minimal drift.

P212 Retrospective Assessment of the Incidence Rate of Ulcerative Dermatitis and the Effectiveness of Treatments in C57BL/6JNarl Mice

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Ulcerative dermatitis (UD) is a common skin disease in C57BL/6 (B6) mice and strains with a B6 background, clinically characterized by hair loss, pruritus, self-mutilation, skin erosion, and subcutaneous tissue exposure across various anatomical regions which is commonly manifested in the dorsal neck area. Severe UD cases need to be euthanatized for humane reasons, thereby leading to animal wasting and potential loss of valuable experimental data before study completion. The etiology of UD in B6 mice remains unclear, and it may be multifactorial, which may be associated with age, gender, season, diet, or fur mite infestation. Considering cost and time, there is still no treatment deemed 100% successful for UD. For animal welfare and to minimize animal wasting, we intervened immediately

upon discovering typical symptoms of UD in B6 mice. We tried various treatment methods in different periods to effectively improve the wounds. These included 0.005% sodium hypochlorite applied by using a sterile swab on lesions once daily or hind limbs toenail trimming once every case, both of which have been discussed in the literature for their therapeutic effectiveness. After trying different treatment methods, we retrospectively analyzed the incidence rate, onset age, and gender distribution of UD for approximately two years in a cohort of 1,609 C57BL/6JNarl (B6) mice aged 9 to 86 weeks (2 to 20 months). Our findings revealed an incidence of approximately 6.7% for UD, demonstrating an age-related escalation and a heightened incidence among female mice. In addition, we found that trimming the hind toenails resulted in a healing rate of 65.9% for UD, with an average complete improvement time of 14.8 days, which was better than 0.005% sodium hypochlorite (healing rate of 23.8%, average complete improvement time of 17.2 days). In conclusion, trimming the hind toenails significantly improves the course of ulcerative dermatitis in B6 mice. This therapy is simple, cost-effective, avoids concerns about the impact of drug use on experimental data, and ensures animal welfare and the maintenance of animal quality.

P213 Unexpected Mortality and Morbidity on BALB/c Mice after Orchiectomy Surgery

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Twenty 10-week-old BALB/c male mice underwent orchiectomy surgery; by the second postoperative day, five mice were found dead in their cages; one was moribund, and another appeared lethargic and hunched. This pattern resembled mortality seen in earlier cohorts, though no necropsies were conducted previously due to the carcasses' poor condition. The surgeries, carried out by an experienced surgeon, involved a 7 mm midline scrotal incision closed with tissue glue. Each mouse received a subcutaneous injection of meloxicam, diluted 1:10 with saline, at a dosage of 5 mg/kg intraoperatively. Post-surgery, all mice exhibited normal recovery and typical behavior. The differential diagnoses for the observed decline in these mice include surgical complications like dehiscence or hemorrhage, infection, drug toxicity from meloxicam use, and behavioral or stress-related issues such as aggression leading to injuries. The moribund mouse, presenting with moderate dehydration and cold extremities, was immediately euthanized; gross necropsy revealed only mild erythema near the scrotal incision. The lethargic mouse, although responsive, showed significant weight loss of 19%, moderate dehydration, and reduced mobility. Despite receiving warm fluids as supportive care, its condition declined with increased lethargy and further weight loss, leading to euthanasia the following day. The gross necropsy revealed a blood clot in the perineal area, an intact surgical site, and mild scrotal swelling. Histopathological examinations of both mice showed severe bilateral renal papillary necrosis and moderate acute tubular necrosis. Cultures from the incisions of both mice yielded *Staphylococcus xylosus*; however, gram stains indicated no bacterial infection at the surgical sites or within the kidneys. Although the direct cause of the renal papillary necrosis remains uncertain, it appears to be associated with the use of meloxicam. Meloxicam, a non-steroidal anti-inflammatory drug (NSAID) that selectively inhibits the cyclooxygenase-2 (COX-2) enzyme, has been linked to renal papillary necrosis due to inhibition prostaglandin production and subsequent vasoconstriction of renal afferent arterioles. However, renal complications have not been reported in laboratory mice administered meloxicam at doses of 5 mg/kg. In addition, dehydration may also play a role, as two other orchiectomy cohorts that were supplemented by gel food post-operatively showed improvement in mortality and morbidity.

P214 Detection of Helicobacter in African Spiny Mice (*Acomys cahirinus*)

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Helicobacter species exhibit a spectrum of interactions with their hosts, ranging from commensal to pathogenic. Immunocompetent mice infected with Helicobacter species typically remain asymptomatic; however, changes in the immune system, metabolic disruptions, and altered breeding performance can occur and may have a direct impact on research. Thus, maintaining an accurate and up-to-date health status of rodent research colonies is essential in management, breeding, and humane care and use. In addition to mice, Helicobacter species are known to infect other rodents, including Mongolian gerbils (*Meriones unguiculatus*) and Syrian hamsters (*Mesocricetus auratus*). We report here the detection of Helicobacter during routine pathogen surveillance of our colony of African spiny mice (*Acomys cahirinus*). The bacteria was initially detected by Helicobacter PCR in samples collected using direct cage swabbing. It was subsequently detected in 20% of direct cage swabbing samples (4/20) collected quarterly over a two-year period. After switching to a sentinel-free method of contact media exposure to soiled bedding, the bacteria was detected by PCR in 100% of samples (5/5) collected over nine months. Fecal pellets collected directly from a representative sample of colony animals were also PCR-positive for the bacteria. From these positive samples, a Helicobacter-specific 16S rRNA sequencing PCR assay successfully amplified a 600 bp product that was 100% identical to *H. ganmani* (MIT 95-2011, GenBank accession U96298). The route of introduction of this agent into our colony is unknown. Bacterial culture and isolation, biochemical analyses, and whole-genome sequencing to further characterize the Helicobacter may reveal a novel species specific to spiny mice. Contamination during sample collection and transmission from mouse colonies are also possible. The clinical significance of the Helicobacter detected in our colony of spiny mice has yet to be determined; however, potential research impacts on wound healing, metabolic disruption, and reproduction are of particular interest for future studies.

P215 Acute Toxicity Associated with Nyanzol-D Fur Dye in Weanling Marsh Rice Rats (*Oryzomys palustris*)

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Marsh rice rats (*Oryzomys palustris*) are an uncommon research species used to study periodontal disease and medication-related osteonecrosis of the jaw. We have maintained a colony of rice rats for 15 years. Animals are identified by laboratory staff using Nyanzol-D, a dark brown dye. Nyanzol-D is reported as a non-toxic hair dye commonly used to identify wildlife and zoo animals, including small mammals and primates. We report here a case of acute toxicity in weanling rice rats after topical application of Nyanzol-D dye. At weaning, twenty rice rats were separated by sex into new cages, and Nyanzol-D dye was applied topically for identification. Within four hours, seven animals (four female, three male) across five cages were found dead. No clinical signs were observed in the remaining animals. At gross necropsy, two animals had moderate edema in the ventral cervical region and pulmonary hemorrhages, and one animal had pulmonary hemorrhages and pleural effusion. All other affected

animals appeared grossly normal. The histopathological evaluation showed multifocal areas of myocardial degeneration characterized by foci of myocardial pallor, with increased clear space in the myofiber cytoplasm and swelling of the fibers. Based on the presentation, gross and histopathologic findings, and the lack of any additional findings, a presumptive diagnosis of anaphylaxis was made. Differentials included cardiac arrhythmias and associated causes. In response, researchers no longer use this dye in the colony. While a specific cause of death was not identified, the active ingredient in Nyanzol-D, para-phenylenediamine, is reported to cause angioedema, intravascular hemolysis, acute renal failure, hepatic necrosis, and death after ingestion in humans. Anecdotal reports also indicate acute mortality following Nyanzol-D use in meerkats and infant macaques. Researchers should be aware that acute death following the use of Nyanzol-D may occur, and an alternative identification method should be used.

P216 Effect of Maropitant Citrate Administration on Buprenorphine-Induced Pica in Rats (*Rattus norvegicus)*

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Rats (*Rattus norvegicus*) lack the ability to vomit when experiencing nausea. Instead, nausea in rats is often expressed as pica behavior (consumption of non-nutritional substances). Buprenorphine, an analgesic opioid commonly used in veterinary medicine to reduce post-surgical pain, has been found in previous studies to induce nausea-related pica when administered to rats. Maropitant citrate, an antiemetic neurokinin-1 receptor, is commonly used in veterinary medicine to prevent nausea in dogs and cats. The objective of this study was to evaluate the ability of maropitant citrate to decrease pica in rats when provided prior to buprenorphine administration. Eighteen naïve male CD (Sprague Dawley) IGS Strain Rats, approximately 8 to 12 weeks old, were randomly assigned to one of two groups with a crossover design. Non-toxic natural clay was used as a substrate to quantify pica in rats throughout the study. Clay consumption was monitored following no treatment, buprenorphine alone (0.05 mg/kg SQ), and maropitant citrate (1mg/kg SQ) plus buprenorphine (0.05 mg/kg SQ) treatment. Maropitant citrate was administered 2 hours prior to the buprenorphine to allow time to take effect. A 5-day washout period was used between treatments. Rats were naïve to pain throughout the study other than the subcutaneous injections. Administration of buprenorphine with or without maropitant citrate significantly increased clay consumption ($p \leq 2.0 \times 10^{-5}$). However, the results of this study show a statistically significant and quantifiable decrease in pica when rats were administered maropitant citrate in addition to buprenorphine, compared to the administration of buprenorphine alone ($p = 0.023$). These results suggest maropitant citrate has value in decreasing symptoms of nausea in rats, although it may not alleviate the symptoms entirely. This study shows that maropitant citrate has the potential to decrease buprenorphine-induced pica and expand the use of buprenorphine as an analgesic option in rats.

P217 An Overview of Common Diseases and of the Normal Anatomy of the Southern Giant Pouched Rat (*Cricetomys ansorgei*)

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Southern giant pouched rats *(Cricetomys ansorgei)* have an exceptionally well-developed olfactory system and are highly trainable, which makes them a valuable research model for studying mammalian olfaction. Despite their popularity, the normal anatomy, histology, and common pathologies of pouched rats remain poorly characterized. Because *Cricetomys spp.* are in the *Neosomyidae* family and just distantly related to rodents of the *Muridae* family, we hypothesized that *Cricetomys ansorgei* will have certain unique features when compared to species of the genus *Rattus and Mus.* We investigated organ anatomy *in situ* and obtained representative histologic sections and generated a preliminary overview of the normal anatomy of this species, and summarized common clinical pathologies that we have encountered over the years in 169 animals. For example, African pouched rats have large cheek pouches, no gallbladder, a large bi-chambered stomach, and a prominent olfactory bulb. Common pathologies in our colony included tooth and cheek pouch abscesses, chromodacryorrhea and conjunctivitis, non-alcoholic steatohepatopathies, diabetes mellitus, and tail injuries. Improved husbandry practices and clinical care for these common pathologies will be presented. The most unique is the rumen-like non-glandular part of the stomach that is lined by long epithelialized papillae. On histology, these papillae are covered by intricate mats of slender, filamentous bacteria that have not been described in the literature. To understand if these bacteria have a physiological role in the nutrient metabolism of this species, we further microbiologically characterized stomach samples. Aerobic bacterial colonization consisted mainly of clostridia, *Lactobacillus*, *Bacillus*, and *Enterococcus* species. Future work will focus on next-generation sequencing of the stomach to identify the full microbiota spectrum and will focus on understanding the physiologic function of these microbes. This will inform us about the best animal care for these animals and how to clinically manage pathologies.

P218 Lupine Alkaloid Neurotoxicosis in a Research Herd of Rafter 7 Merino Sheep

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On July 12, 2023, an outbreak of quinolizidine alkaloid neurotoxicosis occurred among Rafter 7 Merino breed sheep being grazed on an allotment of natural rangeland forage near Eureka, NV. A band of 846 yearling ewes was inadvertently trailed through a large patch of lupines along the route from a natural spring watering location. Within hours, several sheep began showing clinical signs of staggering locomotion, shaking, uncoordinated movements, collapse, and then death. On that day, 43 sheep died and 47 died the next day, another three died the following day, and four more died over the next two weeks despite the provision of supportive care, which included intravenous fluid therapy and atropine sulfate administered by the veterinarian. Varying signs of intoxication were apparent among all sheep that ingested the lupine and subsequently died. Necropsy evaluations by the veterinarian found lupine seeds and pods in their ruminal contents. All sheep were current in the program of parasite control and immunizations and no invasive procedures had occurred on any animal, further narrowing the differential diagnosis to poisonous plant toxicity. Representative specimens of the suspected plants were collected and sent to the USDA ARS Poisonous Plant Research Laboratory (PPRL) herbarium. The PPRL speciated the plant as *Lupinus argenteus* var. *utahensis* and measured high levels of lupanine (11.5 mg/mg) and spartiene (13.7 mg/mg) in collected samples. Laboratory methods for toxin content included gas chromatography (GC)-flame ionization detection and GC/mass spectrometry in comparison with a panel of authenticated alkaloids. Previous reports of sheep mortality from lupine ingestion had not been noted at other ranches in the area. An increased abundance of lupines-producing seed pods with high alkaloid content may have been associated with abnormally elevated precipitation during the winter-spring months prior, which was the seventh wettest on record (1895-2023). Population cycling of lupine abundance has been observed in association with climatic changes. The sheep herders

are from Mexico and Peru contracted through the Western Range Association and secured through the H-2A nonimmigrant visa program. Training in the recognition and avoidance of poisonous plants native to the Nevada Great Basin is provided as these species are unlikely to occur in their home country. In follow up to this event, all herdsmen have been re-trained in the identification of lupines and other locally occurring flora toxic to sheep. Grazing animals are at risk of poisonous plant ingestion, whether on open range or in pasture, so educating animal care personnel and confirming any implicated species along with their toxins can help maintain the expected framework of consistent, high-quality care in programs of all types.

P219 Management Strategies for Yorkshire Pigs (*Sus scrofa*) with Intermittent Vomiting

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A cohort of naive, mixed-sex ten-week-old Yorkshire pigs presented with intermittent vomiting consisting of small amounts of digested pellets and yellow, foamy bile 2-3 weeks after arrival at the animal facility. Similar clinical signs were presented in the following four cohorts. 29 out of 32 pigs were affected, which may be artificially elevated due to social housing. The animals otherwise remained bright, with normal vitals and no evidence of abdominal pain on physical examination. A subset of animals experienced intermittent episodes of hypersalivation, but instances of vomiting were limited to the morning before being fed. The animals were fed ad libitum at the vendor and switched to being fed twice daily on business days and once daily on weekends at the animal facility. The primary differential diagnoses included bilious vomiting syndrome (BVS), infectious causes, and toxin exposure. The clinical signs and medical management delayed research project timelines. Clinical interventions involved diagnostic tests and the administration of drugs, including maropitant citrate (2 mg/kg PO once or daily up to 5 days, 1 mg/kg SQ once) and omeprazole (20-40 mg/pig PO up to 14 days). Management strategies included adjusted housing density, increased afternoon feed ration, and changes in kennel sanitation schedules. Veterinary staff observed all animals with reports of vomiting every 24 to 48 hours and logged each instance of vomiting to identify trends. Diagnostic tests ruled out intestinal parasites, coronaviruses (PEDv, PDCoV, and TGEV), and clinically significant levels of mycotoxins in the feed. Animal housing rooms with increased density correlated with a higher incidence of vomiting. Neither the drug administration nor the altered sanitation schedule impacted clinical signs. The clinical signs resolved as the animals adjusted to the facility feeding schedule over three months. The presumptive diagnosis is BVS secondary to the change in feeding schedule, as a diagnosis of exclusion. We have not experienced these clinical signs within our other swine populations (Yucatan and Gottingen breeds). The next step to consider is an altered feeding schedule, such as increased frequency or adlibitum feeding for fast-growing breeds, such as Yorkshires.

P220 Rectal Stricture in Newly Arrived Domestic Pig: Differential Diagnosis to Consider

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Eleven CG36/Landrace cross pigs, 75-94 days of age, sourced from a commercial farm, arrived for biomedical research use. One barrow, 36.5 kg, had diarrhea on arrival, with otherwise normal physical exam findings for all pigs. A fecal flotation for parasites was positive for only Balantidium coli. Over the next seven days, the pig was active, eating, and diarrhea appeared to respond to Probiotic Gel (10g PO). On day nine post-arrival, the pig presented with diarrhea, decreased appetite, and abdominal bloating, which was non-responsive to a treatment course of carprofen (100mg SID) and enrofloxacin (90mg IM SID). Differential diagnoses included bacterial enteritis (e.g., intestinal Salmonellosis, Porcine Proliferative Enteropathy) or viral enteritis (e.g., Porcine Coronavirus, Porcine Circovirus) as parasite involvement was ruled out. The pig was sedated for abdominal radiographs and physical examination. Dilated gas-filled stomach and intestines were present. The gas-filled intestines appeared to decrease in size at the pelvic area, and no gas was detected from the mid-pelvis to the rectum. A digital rectal exam was performed with resistance approximately 5cm into the rectum, consistent with a rectal stricture. The pig was euthanized, and necropsy performed. A fibrous annular stricture was present in the rectum with an ulcerated area cranial to the stricture along with enlarged colonic lymph nodes and an encapsulated abscess. Histopathology confirmed lymphoid hyperplasia; chronic and diffuse ulcerative colitis; and severe chronic fibroplasia consistent with a rectal stricture. It is not uncommon in farm settings to see rectal stricture secondary to chronic enteric mucosal damage in growing pigs that leads to megacolon. It is often associated with chronic enteric Salmonellosis or rectal prolapse. The underlying cause appears to be ischemia injury to the rectum resulting from compromised arterial blood flow. This condition appears to be unreported in a biomedical research setting. It highlights the diagnostic challenges in non-purpose-bred animals, without known histories. The vendor was contacted and confirmed that no apparent clinical signs were detected prior to shipment. Communication with vendors on overall and individual herd health is critical with consideration of purpose-bred suppliers depending on specific research uses.

P221 Systemic Cytokine or Neurological Syndromes following Chimeric Antigen Receptor (CAR) T-cell Immunotherapy in a Non-Human Primate Model

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Chimeric antigen receptor (CAR) T-cell therapy is a novel cellular immunotherapy currently being used for the treatment of lymphomas and other liquid and solid tumors. Autologous T cells are engineered to express receptors that target antigens on malignant cells. Side effects can be life-threatening and have been documented to be secondary to antigen load, cell dose, the type of CAR construct, the preparatory regimen, and off-target effects. We will discuss our experience with an anti-CD20, B-cell-specific CAR T cell (CD20CART), which resulted in different clinical and immunological outcomes in macaques. Two macaques in a cohort of four demonstrated cytokine-related toxicities following infusion of CAR T-cells. A 6-year-old male rhesus macaque experienced facial erythema and swelling one week after infusion. Another animal, a 5-year-old male cynomolgus macaque, experienced different clinical signs, including dull mentation and focal neurologic deficits four days following infusion. Diagnostics included complete blood count and serum chemistry panels, neurologic scoring, flow cytometry, and measurement of cytokine levels. Both conditions were associated with a paroxysmal expansion of CAR T-cells observed in white blood cell counts and on flow cytometry and B-cell aplasia. Differential diagnoses included hypersensitivity reaction for the rhesus macaque and seizures, pain, or other neurologic events such as ischemia for the cynomolgus macaque; when considering the history and diagnostic results, however, these presentations were consistent with cytokine release syndrome (CRS) and immune effector cell-associated neurotoxicity syndrome (ICANS), respectively. Both cases were successfully managed with dexamethasone sodium phosphate (1-3 mg/kg intramuscularly), levetiracetam (18-25 mg/kg orally or subcutaneously), and/or tocilizumab (8 mg/kg intravenously), a monoclonal antibody that blocks interleukin-6 receptor, a potent inflammatory mediator. These studies reinforce the importance of macaques as models for immunotherapies, specifically for the

assessment of novel CAR T constructs, prediction of potential toxicities, and their relevance as clinical-translational partners by increasing the predictive value gained from preliminary rodent studies.

P222 Calcium Carbonate Urolithiasis in African Green Monkey (*Chlorocebus aethiops sabeus*)

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An adult male African Green Monkey (*Chlorocebus aethiops sabeus*), estimated age 8-10 years old, was reported for acute onset of anorexia and distended abdomen. The animal was wild caught from St. Kitts and had arrived in our AAALAC-accredited facility 5 weeks prior to presentation. The animal was fed a standard, commercially available high-protein NHP diet. The animal was sedated for veterinary examination and diagnostic workup. On examination, the caudal abdomen was noted to be severely enlarged due to a severely distended urinary bladder with cranial GI displacement, as confirmed by abdominal radiographs. Several small mineral opacities were observed along the lower urinary tract. Blood work was consistent with dehydration secondary to post-renal azotemia. Urinary catheterization through the penile orifice was attempted but unsuccessful due to complete obstruction of the urethra. The animal was elected for humane euthanasia. Differential diagnoses include urolithiasis, neoplasia, congenital anomalies, metabolic, and idiopathic etiologies. On gross necropsy, the urinary bladder was found to be markedly distended with purple mottling of the bladder mucosa. A 0.5-centimeter urinary calculus was found in the distal penile urethra and was identified as calcium carbonate by optical crystallography. The urine culture showed no bacterial growth. Urolithiasis is rarely reported in Old World Monkeys. To our knowledge, there are no previously reported cases of calcium carbonate urolithiasis in African Green Monkeys. Urolithiasis has been reported in macaque species and of those reported, calcium carbonate stones are one of the most common. However, calcium carbonate uroliths have been rarely reported in humans, with a cluster of cases found to be associated with hypocitraturia, hypercalciuria, and hypomagnesuria. In rabbits and guinea pigs, other types of calcium stones such as calcium oxalates are associated with increased intestinal or bone absorption of calcium and is often associated with alkaline urine and calciuria. A definitive cause of this animal's urolithiasis was not determined.

P223 Ectopic Pregnancy in Common Marmoset (*Callithrix Jacchus*)

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Ectopic pregnancy (EP) is a potentially life-threatening condition due to the implantation of a fertilized ovum outside the main cavity of the uterus. Depending on the implantation location, EP is classified into two types: 1) tubal pregnancy (in the oviduct), and 2) abdominal pregnancy (in the peritoneal cavity). A female common marmoset (3 years old, 491g, with two parturition experiences) (*Callithrix jacchus*) that was pregnant with one fetus exhibited a small amount of uterine hemorrhage approximately 4 months after conception (estimated based on biparietal diameter in fetal sonography). We performed gross examinations and fetal sonography to differentiate uterine hemorrhage from conditions such as miscarriage and uterine infection, and the results showed no abnormalities. However, gross examinations and fetal sonography showed no abnormalities. After two weeks, the mummification of the fetus was confirmed by sonography, and a caesarean surgery was carried out. A gestational sac (diameter 5 cm) was found in the middle abdominal cavity rather than the uterus. The gestational sac was connected to the maternal omentum by a serosa-like duct and contained a 30-gram fetus inside. The fetus showed no morphological or developmental differences compared to normally delivered fetuses, indicating it received sufficient nutrition from the mother. Considering the gestational sac's connection to the omentum and the absence of uterine injury, this case was diagnosed as an abdominal pregnancy. We considered this a primary abdominal pregnancy. Unlike rodents, common marmosets are similar to humans in terms of the structure and physiology of the placenta—the interhaemal barrier. The occurrence of EP is generally known to be 2% of all spontaneous conceptions in humans and has been rarely reported in non-human primates. This report may be the first EP case in a common marmoset and could serve as basic data for pregnancy research using common marmosets.

P224 Invasive Endometriosis in Rhesus Macaque (*Macaca mulatta*)

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Endometriosis is a medical condition defined by the presence of endometrial tissue outside the uterus, and it is commonly found in older female rhesus macaques. While the disease itself may not be surprising upon discovery, its manifestations certainly can be. In this case, a 19-year-old female rhesus macaque presented for decreased activity and vaginal bleeding. During the physical exam, a large multilobulated mass was palpated on the uterus. Complete blood count (CBC) and chemistry analyses showed an inflammatory leukogram, mild hypoproteinemia, mild hyponatremia, and a marked increase in triglycerides. A pelvic ultrasound showed a large, cystic, fluid-filled mass associated with the uterus. These findings made endometriosis or a uterine mass top differential diagnoses. Due to the poor prognosis and progression of clinical signs, euthanasia was elected and the animal was submitted for necropsy. Remarkable findings on gross necropsy included abundant adhesions between the urinary bladder, uterus, rectum, and abdominal fat that obscured the pelvic organs from view. A sagittal section through the organs revealed adhesions forming multilobular cystic spaces filled with hemorrhagic fluid. Additionally, a 1 cm diameter polypoid mass was protruding from the cervix, and there was a firm, tan, fibrous region along the ileocecal margin. Histopathology of the pelvic organs confirms the presence of endometrial stroma with transmural invasion of the endometrial tissue through the muscular wall and mucosa of the ileocecal junction. Histopathology also showed that the mass in the cervix was a benign polyp, and this finding was likely incidental. The clinical signs seen upon presentation are attributed to endometriosis, including invasion transmurally in the region of the ileocecal junction. This invasion is a much rarer manifestation of endometriosis and, therefore, is of note to contribute to the greater understanding of this pathology within the scientific community.

P225 Testicular Congestion and Vascular Abnormalities in a Cynomolgus Macaque Post Nephrectomy and Allograft Kidney Transplant

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A 12-year-old, 6.7 kg, intact cynomolgus macaque (*Macaca fascicularis*) presented with acute unilateral scrotal edema. Two days prior to presentation, the animal underwent a right-sided allograft kidney transplant, which required a right-sided native nephrectomy. It was noted during surgery that the animal's vasculature surrounding the native kidney was abnormal, necessitating a ligation of a secondary vein extending off the native kidney into the caudal vena cava. Otherwise, the surgery and recovery were uneventful. On physical examination post-surgery, the right scrotum was noted to be edematous, firm, and discolored. Ultrasound showed minimal blood flow to the right testicle with no venous return. The left

scrotum and the rest of the physical exam were unremarkable. Differential diagnoses included venous congestion, testicular torsion, strangulated inguinal hernia, trauma, venous thrombosis, orchitis, epididymitis, varicocele, and neoplasia. The animal was treated supportively with Meloxicam (0.1 mg/kg IM), but due to lack of improvement, an orchidectomy was elected. Gross examination of the right testicle post-orchidectomy revealed it to be diffusely hemorrhagic and congested. Although testicular complications can occur with kidney transplants, reports of testicular congestion in humans are rare and no reports in non-human primates were found. The animal continued on study until it was eventually euthanized for respiratory-related issues; at which time a full post-mortem work-up was performed. At necropsy, abnormal kidney vasculature was confirmed, as well as aberrant positioning of the testicular vein. The previous event of testicular congestion in this animal was likely due to inadvertent ligation of an abnormal testicular vein during the nephrectomy. This information adds to the knowledge of atypical anatomical landmarks in this species, and if seen, precautions should be taken to prevent testicular vein ligation. Because humans and non-human primates share many anatomical similarities, these findings may also translate to human medicine.

P226 Accuracy of Doppler for Measuring Blood Pressure in Adult Rhesus Macaques (*Macaca mulatta*)

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Blood pressure is monitored in animals undergoing anesthesia and surgery to ensure patient safety and circulatory stability. While monitoring direct blood pressure via an arterial catheter is the gold standard, placement of these lines in non-human primates can be technically challenging. Doppler uses a non-invasive method whereby an ultrasonic probe (crystal) is placed over an artery and a cuff is placed proximal to the probe. Inflation of the cuff occludes the artery, and once inflated beyond the animal's blood pressure, the audible sound of the pulsatile flow is lost. The pressure in the cuff is then released and the point at which the audible sound returns is equated to the animal's blood pressure. Previous studies have examined both direct and indirect (doppler and oscillometric) methods of obtaining blood pressure in rhesus macaques and have found that indirect blood pressure measurements vary based on the location and method used. Additionally, these studies have determined that like in dogs, doppler, while underestimating direct values, better predicts systolic blood pressure. In our study, we collected doppler and direct blood pressure measurements every 5 minutes from six rhesus macaques undergoing surgery to determine whether placement of doppler on the forearm (n=3) or hindlimb (n=3) would better align with direct systolic or direct mean blood pressure measured via saphenous arterial catheters. We used a Bland-Altman plot to analyze and visually compare our data. Like previous studies, we determined that placing a doppler on the forearm does better align with systolic blood pressure in this species. However, there was no clear determination when placing the doppler on the hindlimb whether this more closely measures systolic or mean blood pressures. Therefore, we recommend that when using doppler to monitor blood pressure to help guide clinical intervention during surgical procedures, anesthetists be mindful of where the doppler is placed to know what measurement of blood pressure they are actually observing.

P227 Comparison of Dexmedetomidine/Morphine and Xylazine/Morphine as Premedication in Isoflurane Anesthetized Sheep

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This study investigated the sedative and anesthetic effects of dexmedetomidine/morphine (DM) and xylazine/morphine (XM) in sheep. We hypothesized DM would provide profound sedation and maintain physiological parameter under anesthesia better than XM in sheep undergoing laparoscopic surgery. Nineteen male sheep were premedicated with either 1) dexmedetomidine (0.06 mg/kg)/ morphine (0.3 mg/kg) (DM) or 2) xylazine (0.1 mg/kg) /morphine (0.3 mg/kg) (XM). After DM or XM administration, three blinded veterinarians evaluated sedative scores [0 (no sedation); 1 (mild); 2 (moderate); 3 (severe)]. Sheep were induced with intravenous tiletamine/zolazepam (4 mg/kg), intubated, and maintained with isoflurane in 100% oxygen. Anesthetic parameters were monitored for 60 min included heart rate, respiratory rate, direct arterial blood pressure, %SpO2, ETCO2, body temperature, arterial blood gas analysis, and isoflurane requirement level. At the end of procedure, sheep were euthanized and lung pathology (pulmonary edema) assessed. Results were 1) sedative scores were not different between DM (0.8±0.2) and XM (1.1±0.2); 2) anesthetic parameters were not different between both groups, but the DM sheep isoflurane requirement was lower than that in XM sheep; 3) marked pulmonary changes, consistent with pulmonary edema, were noted in the XM group. In conclusion, DM and XM provided similar sedation and physiological parameters under isoflurane anesthesia, while DM had a lower isoflurane requirement and less evidence of pulmonary edema.

P228 Tiletamine/Zolazepam and Ketamine with Dexmedetomidine Cocktail Provides General Anesthesia as Effective as with Xylazine Cocktail in Pigs

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Tiletamine-zolazepam (Telazol) and ketamine with xylazine has been used in pigs. Here we investigated an alternative to xylazine in 5–7-week-old Duroc, Large White Yorkhsire and Landrace cross pigs. This study aims to examine general anesthesia induced by tiletamine-zolazepam and ketamine with dexmedetomidine (TKD, 0.05 ml/kg) or tiletamine-zolazepam and ketamine with xylazine (TKX, 0.05 ml/kg) in pigs undergoing both short-term and long-term anesthesia for surgical procedures. We hypothesized whether general anesthesia induced by TKD would be comparable to that induced by TKX in pigs undergoing surgery, both short-term and long-term anesthesia. The study involves 20 male, intact crossbred pigs subjected to castration for unilateral cryptorchid (short-term, 45-min) or bilateral cryptorchid (long-term, 90-min) castration. Duration parameters monitored were induction/recovery [time to/return of sternal recumbency or lateral recumbency; time to loss of/return of withdrawal reflex, jaw tone, and palpebral reflex]. Physiological parameters monitored were heart rate, respiratory rate systolic, direct arterial blood pressure (SAP, MAP and DAP), %SpO2), ETCO2, and body temperature. Isoflurane levels were recorded if used. Results were: 1) for duration parameters, there were no differences between TKD and TKX groups in both short and long-term anesthesia (induction time 1 min; recovery time 18-35 min); 2) for physiological parameters, during short-term anesthesia, MAP (at T30) and SAP

(at T30 and T40) were higher in TKD compared to TKX groups; average MAP were 59.8-74.5 (TKD) and 55-69.4 (TKX) mmHg. Other physiological parameters were not different; 3) isoflurane levels (0.1-0.6%) were not different. The results indicate that general anesthesia induced by TKD is comparable to that induced by TKX in pigs undergoing cryptorchid surgery both short-term and long-term anesthesia.

P229 Development and Utilization of a Burnout Method to Eradicate Endemic *Pneumocystis carinii* from Immunocompetent Rats

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Pneumocystis carinii is a fungal organism that causes species-specific infections in rats. Immunocompetent rats are typically asymptomatic whereas immunodeficient rats can develop fatal pneumonia. The prevalence of this pathogen in present-day laboratory rats has significantly decreased through the years, likely due to the use of individually ventilated caging (IVC) and advancements in commercial testing. Research facilities that have yet to eradicate this pathogen can experience difficulties exporting rats to other institutions that exclude it. Total depopulation with repopulation is described as the only effective method of eradication; this can be costly and may not be feasible. A non-depopulation burnout eradication procedure was developed for a conventional rat room endemic with *P. carinii* since 2016, as determined by quarterly soiled bedding sentinel health monitoring. The room housed approximately 120 cages of various immunocompetent strains of rats. Eradication procedures involved complete room decontamination via hydrogen peroxide fogging, transition to IVC, and implementation of practices to minimize environmental contamination and exposure of naïve animals. At the initiation of eradication (IoE), the prevalence of active pathogen shedding was determined by environmental PCR swabs of each cage. Two rounds of sentinel-free health monitoring (SFHM) kits (PathogenBinder™) were employed with rounds 1 and 2 having a 30-day and 120-day sampling period, respectively. Twelve naïve pups born after IoE were screened by serology to help confirm eradication success. At IoE, zero rats were actively shedding the pathogen. All SFHM results were negative for *P. carinii*. Pups born to previously seropositive dams tested seropositive for up to 12 weeks due to maternal antibodies but were seronegative thereafter. *Pneumocystis carinii* was considered successfully eradicated after receiving negative results for all serology and SFHM, totaling 32 weeks after IoE. Success was dependent upon total room decontamination and effective containment strategies to allow for burnout in immunocompetent rats.

P230 Effects of Buprenorphine Extended Release on Localized Ocular Pain Compared to Buprenorphine

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Intravitreal injections are an invasive ocular procedure often used as a delivery method for experimental compounds. In nonhuman primates, post-procedural pain is commonly observed following injection and requires palliative care. Buprenorphine is a synthetic opioid widely used in early development research facilities due to its safety and effectiveness in pain reduction. Buprenorphine extended release (ER) is a compound formulated to have a longer-lasting analgesic effect as compared to buprenorphine. Previously, buprenorphine intramuscular injections had been used as a primary form of pain relief for nonhuman primates, but recently buprenorphine ER has become the standard. A meta-analysis was conducted comparing the use of buprenorphine and buprenorphine ER in frequency of painful observations among nonhuman primates (eye rubbing and squinting). Data were collected from 10 separate studies over a period of two years, including 65 nonhuman primates. This analysis concluded that there was a 54% increase of instances of localized ocular pain (eye rubbing and squinting) with the use of buprenorphine ER compared to buprenorphine. These results may be attributed to a process change that increased documentation of these observations in concurrence with the use of buprenorphine ER. Regardless, buprenorphine ER can ultimately increase animal welfare by reducing frequency of animal handling, number of injections and stress of the animal. Overall, buprenorphine ER is an adequate treatment of breakthrough pain following intravitreal injections.

P231 Difference in Post-Operative Mortality in Mice Given Non-Steroidal Anti Inflammatory Drugs vs Sustained Release Buprenorphine

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Surgeons perform an average of 250 survival craniotomy surgeries per month on mice, which requires three days of analgesia. We had previously used non-steroidal anti-inflammatory drugs (NSAID), Carprofen at 5-10 mg/kg or Ketoprofen at 2-5 mg/kg, for analgesia, requiring five injections per mouse (one dose of NSAID at surgery and four doses post operatively). We decided to offer the option of sustained release buprenorphine (BPSR) at 3-3.5 mg/kg for post-operative pain relief, thereby decreasing the analgesic injections to two (one dose of NSAID at surgery and one dose of BPSR post-operatively), to decrease the total number of injections per mouse. Before we implemented the BPSR regimen, mice had an average of 2-6 % post-operative mortality after surgery per month. In the three months after we implemented the BPSR option, mortality in the BPSR group averaged 9.5% (N = 73), while the NSAID group averaged 2% (N =68). Mice receiving BPSR received, on average 1.23ml less fluids in the immediate post-operative period because the fluid volume of the NSAID is higher than the BPSR. We hypothesized this could be why the outcomes with the BPSR group were worse. Fluid couldn't be added to the BPSR because it would interfere with the sustained release action of the drug, and we didn't want to add supplemental fluids in an additional injection. Instead, we reformulated the post-operative antibiotic injection so that the BPSR mice received a similar total fluid volume as the NSAID mice. After this change, we observed the post-surgical mortality for the BPSR group (N = 86) improved to a level comparable to the NSAID group (N = 59, an average of 2-6% mortality per month.) We concluded the difference in fluid support after surgery was the most likely cause for the difference in mortality between the two analgesic regimens.

P232 Elimination of Murine Chapparvovirus in Immunocompetent Mice by Selective Breeding

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Murine chapparvovirus (chaphamaparvovirus-1, murine kidney parvovirus) is a single-stranded DNA nonenveloped parvovirus that produces no clinical disease and mild to sub-moderate kidney pathology in infected immunocompetent mice but produces progressive renal disease and failure in immunodeficient mice. A colony of immunocompetent C57BL/6-Tg(Uchl1-EGFP)G1Phoz/J (UCHL1-EGFP) and CD-1 mice were found to be infected with murine chapparvovirus during routine soiled bedding sentinel monitoring

by fecal PCR testing. Further, pooled PCR testing of experimental animals in the affected room identified the UCHL1-EGFP strain as the only positive animals. Procedures were put in place to limit the spread of infection and the UCHL1-EGFP colony of 38 mice were subsequently moved to a quarantine facility. Cross foster rederivation was not an option. Over the next year, 32 pairs of UCHL1-EGFP mice were bred at eight weeks old and culled at approximately 14 weeks old when offspring were weaned. This strategy of breeding and culling was performed four times successively to try to eliminate the infection. Initially, soiled bedding sentinels were placed at the time of quarantine and then again six months later. Sentinel testing by fecal PCR was performed by a commercial diagnostic laboratory at approximately 6-month intervals for one year. After two cycles of breeding and culling, the first 6-month sentinel test was negative for the virus. Repeat sentinel testing by fecal PCR, kidney PCR, and serology in 6- and 12-month-old sentinels was negative for the virus. At this time, 8-week-old offspring were released from quarantine and returned to conventional housing. Subsequent testing of approximately 6-month-old sentinels and 10-month-old offspring that had been released from quarantine was negative for virus by fecal PCR, kidney PCR, and serology. Our observations and results suggest that murine chapparvovirus may be eliminated through a selective breeding scheme by breeding of young adult mice and culling them after weaning their offspring for a total of two breeding cycles. More work in other mouse strains is necessary to support this observation and these results.

P233 Enteric Disease in Immunodeficient Mouse Strains Infected with a Clostridial Species Phylogenetically Related to *Clostridioides celatum* and *C. cuniculi*

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Mouse strains deficient in adaptive and innate immune functions, such as NOD-scid gamma (NSG) or its variants NOD.Cg-Prkdcscid Il2rgtm1Wjl Tg CMV-IL3,CSF2,KITLG (NSG-SGM3) and NOD. Cg-KitW-41J Tyr+ Prkdcscid Il2rgtm1Wjl/ThomJ (NBSGW), are highly susceptible to opportunistic infections. Over a period of four months, 1,003 mice were observed with perineal staining and mild to moderate loose stool in several rooms housing these mouse strains, with minimal weight loss or mortality. Roughly 10% of them were tested utilizing standard bacteriology and expanded multiplex PCR-based sentinel testing that was negative for known opportunistic agents. However, anaerobic fecal culture and identification revealed a poorly characterized clostridial species. Intestinal contents from the affected mice were negative for *C. perfringens* toxins (beta, epsilon, and CPE) and *C. difficle* toxins (A and B). Further characterization using mass spectrometry narrowed the species identity to *C. celatum/disporicum/saudiense*. Histopathology revealed cecal epithelial degeneration with brush border bacterial adherence in most of the mice, with a subset also showing small intestinal bacterial overgrowth with mucosal hyperplasia, vacuolation, and erosion. Naïve NSG mice confirmed to be negative for the clostridial species of concern were exposed to dirty bedding from affected cages; at 8 days post-exposure, 2/4 mice exhibited loose stool, and all four mice became culture-positive for this bacterium. Whole genome metagenomics on feces from this study indicated a significant difference in beta-diversity post-exposure. Relative abundance and linear discriminant analysis revealed the presence of *C. cuniculi* in post-exposure mice consistent with bacteriological findings. Due to the incomplete genome for *C.cuniculi* in the metagenomics database, we pursued complete genome sequencing on our isolate to further species level characterization. Preliminary analysis indicates a close identity with *C. cuniculi* and *C. celatum*. This investigation provides significant insights into the spectrum of clostridial infections in mice and lay the groundwork for exploring the sources of such infections in immunodeficient mice.

P234 Granulomatous Arteritis/Aortitis Associated with *Mycobacterium Genavense* in Zebra Finches (*Taeniopygia guttata*)

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Mycobacterium genavense is a common cause of mycobacteriosis in passerine and psittacine birds. In a research colony of approximately 150 zebra finches, twenty birds were either found dead or euthanized due to abnormal clinical signs, followed by necropsy over a two-year period. Clinical signs included respiratory distress, lethargy, feather loss, and ataxia leading to a potential differential diagnosis of bacterial, fungal, parasitic, or neoplasia in origin. No apparent gross findings were present. Histopathological examination in all seven birds revealed perivascular inflammation of the aorta and/or other great vessels. The tunica media was expanded by a moderate number of foamy macrophages mixed with flocculent eosinophilic material containing aggregates of acid-fast bacilli. The myocardium showed multifocal necrosis and cardiomyocyte vacuolation with small numbers of macrophages. Of these twenty birds, seven were diagnosed postmortem with *Mycobacterium genavense*. Four of the seven cases included histiocytic inflammation and acid-fast bacteria in additional organs. PCR of pooled fecal samples from the colony confirmed the presence of *Mycobacterium genavense*. Mycobacterial aortitis/arteritis has not been thoroughly described in zebra finches and should be considered a lesion of latent or active mycobacteriosis.

P235 Identifying Estrus in Ossabaw Island Swine through Behavioral Observations and Vaginal Cytology

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Ossabaw Island swine have become an important animal model for cardiovascular, diabetic, and ocular research. There are currently limited sources/vendors that produce Ossabaws for research purposes. Very little information is available concerning the successful management of a breeding program for these animals. Specifically, information on the detection of estrus is lacking. To meet research needs, we were challenged with attempting to artificially inseminate three genetically modified Ossabaw gilts. To accomplish this we required a better understanding of their estrus cycle to ensure successful timing of insemination. Estrus was synchronized by daily oral administration of 7 mls/gilt of atrenogest (Regu-Mate Merck™) for 18 consecutive days, followed by a 5 ml/gilt subcutaneous injection of PMSG/HCG (PG-600 Intervet™) 24 hours after the last altrenogest dose. Behavioral and anatomical changes typically seen in domestic swine as well as vaginal cytology were tracked twice daily for 10 days post the administration of estrus synchronizing agents over two cycles. Behavioral changes monitored included response to the introduction of a mature boar, mounting, and a standing reflex when pressure was applied to the lower back. Observation for anatomical changes included vulvar swelling and discharge. Vaginal cytology was collected by gently rolling a sterile cotton swab moistened with saline along the anterior wall of the vagina. The cells were immediately transferred to a microscope slide and stained using safranin and crystal violet. Cytology slides were assessed for the ratio of epithelial cells to WBCs. All observations and sample collections were stress-free. The ossabaws showed little to no behavioral or anatomical changes associated with estrus. Vaginal cytology changes were profound specifically in the ratio of epithelial cells to WBCs. Epithelial cells predominated diestrus with a complete reversal and WBCs becoming the predominant cell type in estrus. Unlike domestic swine, behavioral and anatomical changes were not present in the ossabaw gilts in association with estrus. Vaginal cytology was determined to be the only parameter demonstrating measurable change and thus the most predictive method of estrus detection in Ossabaw Island swine.

P236 Use of Intranasal Midazolam in Swine During Minor Procedures to Supplement Physical Restraint

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The most prevalent concerns during any procedure in an animal research facility are animal welfare and human safety. In our research facility, minor procedures are done on a regular basis to swine and a few other species. A minor procedure is one that could inflict minimal pain, if any at all, but does NOT require full anesthesia. Some of the minor procedures that our group of technicians perform include blood collection, telemetry chip insertion, and clinical evaluations. Our goal was to find a way to lessen the amount of physical restraint needed to reduce the risk of injury to the technicians and provide a calmer experience for the swine in particular, one of our facility's most used and fractious animals. In this clinical research study, we chose to evaluate benzodiazepine (midazolam 5 mg/mL) as a form of supplemental chemical restraint. Multiple trials were run using a total of 11 Yorkshire swine, weighing 35-80 pounds (16-27 kg), and midazolam doses ranging from 0.1mg/kg to 0.4mg/kg. A commercial atomizer device was used to administer a single dose of midazolam intranasally. After waiting 10-15 minutes (dependent on how quickly affects set in) for the drug to take effect, the swine were either restrained by hand for telemetry chip insertion or roped for jugular blood collection. During this time, the swine were evaluated for level of sedation and reaction to restraint and pain. We found that 0.35 mg/kg gave us the correct amount of sedation and analgesic response to safely insert telemetry chips or collect blood, without the swine being too sedated and requiring anesthesia monitoring. The results of this clinical study will be used in future studies that require minor procedures to be performed on swine.

P237 Refinement Strategies to Mitigate Adverse Events During Anesthetic Procedures in Lagomorphs

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Lagomorphs are a common laboratory animal used in orthopedic studies. However, they can prove to be challenging patients when undergoing anesthesia. One of our recent orthopedic studies utilized nine New Zealand White lagomorphs that were anesthetized weekly for up to 13 weeks. The animals underwent treatment with a novel therapeutic administered intraarticular at the stifle joint post tendon resection. To evaluate the efficacy and pharmacokinetics of treatment, serial blood collections and seven Tesla MRI sequences were performed during the experiment. Our MRI-compatible monitoring equipment was a significant limiting factor, as it was not designed for a species with such an elevated resting heart rate. Due to the strength of the MRI magnet, the anesthesia machine remained outside of the room, creating 30 feet of dead space between the patient and the isoflurane vaporizer. Other obstacles we encountered included hypothermia, apnea, cardiopulmonary arrest, and dislodgment of supraglottic airway devices. A total of 41 sedation events that lasted for a minimum of 60 minutes were evaluated to compare the recovery times between an intramuscular injection of Ketamine and Midazolam that was maintained with vaporized isoflurane versus the use of only vaporized isoflurane. The average recovery time when sedated with Ketamine and Midazolam was 51.54 minutes. Compared to the average recovery time of 13.52 minutes when only vaporized isoflurane was used. Showing a 73.71% decrease in the time it took the lagomorphs to recover fully. There were three adverse events that occurred when induced with intramuscular sedation that resulted in apnea and cardiopulmonary arrest. No such adverse events occurred when only vaporized isoflurane was used. Lastly, there were five more sedation events that persisted longer than 60 minutes induced by intramuscular sedation where the lagomorphs never fully recovered prior to the use of subsequent anesthesia needed for blood collection. These five events were not included in the data comparison as a full recovery time was not achieved. The veterinary team collaborated and developed refinement strategies, such as the cessation of intramuscular anesthetics and sedatives, increased heat support, and improvement in blood collection techniques. These changes greatly improved the efficiency of the procedure, mitigated adverse events, and optimized animal welfare. The study was completed successfully and the veterinary team has now permanently implemented these changes for their lagomorph procedures.

P238 Successful Elimination of *Corynebacterium bovis* Infection in African Grass Rats (*Arvicanthis niloticus*) with Amoxicillin Water Treatment

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Asymptomatic *Corynebacterium bovis* infection was identified in African grass rats (*Arvicanthis niloticus*) imported to Michigan State University in August 2021. Considering the value of this unique rodent species to ongoing research efforts, an antibiotic water treatment trial was implemented. Adult colony animals that tested PCR positive for *C. bovis* on pelt swab were transferred to a restricted access quarantine facility, underwent a 3-week acclimation period, and were then placed in breeding pairs (n=13). The breeding pairs received amoxicillin-treated water (1 mg/mL) and their standard rodent diet. The water was provided to breeding pairs from initiation of breeding until pups were weaned (1.5-4 months). A strict traffic pattern was followed when working with these animals. PPE requirements for personnel working in *Arvicanthis* housing areas included face masks, hair bonnets, disposable coveralls, shoe covers, and two pairs of gloves. All PPE was disposed of in fiber barrels for incineration. All weanlings were PCR-tested for *C. bovis* and received treated water until negative test results were available. When negative results were received, weanlings were moved to a separate room, given non-treated water, and tested again 30 days later. Following a second negative test result, weanlings were returned to the original colony housing facility. Breeding pairs produced a total of 144 viable pups. All pups tested negative for *C. bovis* at weaning. 119 pups were negative on subsequent PCR testing and were returned to the original colony housing location (25 pups were removed for study purposes prior to the second test). No animal health complications were identified during the antibiotic water treatment. Routine health monitoring of the *Arvicanthis* colony, including quarterly testing of sentinel animals exposed to dirty bedding and filter paper methods, has not detected any presence of *C. bovis* over the past 18 months. Amoxicillin water treatment of *A. niloticus* breeding pairs, in combination with increased PPE and adherence to traffic flow patterns, was 100% effective in producing negative offspring for return to standard housing conditions.

P239 Using Filters in the Sump for Monitoring Health of Laboratory Zebrafish

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Zebrafish (*Danio rerio*) are useful in scientific research due to their close genetic similarity to the human genome and fast reproductive lifecycle. Their increased use in scientific research calls for improved methods of monitoring their health, as current methods involve multiple types of testing, including submission of whole fish to identify various pathogens. This study developed a novel sampling technique by exposing nitrocellulose filters to sump water over 30, 60, or 90 days. Previously, we performed a pilot study to test nitrocellulose filters in sump water of one rack using a diversion

tank. Since then, we performed a safety study and were now able to place the filters directly in all sumps at two campuses, giving this study in depth, realistic value. The filter was compared against other known testing methods of swabbing biofilm from the sump, passing sump water through a vacuum filter, and whole fish PCR. It was hypothesized that the nitrocellulose filter would identify more pathogens over time, reducing the need for multiple testing methods. PCR testing was conducted to detect *Mycobacterium chelonae, Mycobacterium fortuitum*, Zebrafish picornavirus, *Myxidium streisingeri, Pseudocapillaria tomentosa, and Pseudoloma neurophilia* at each timepoint. *P. neurophilia and P. tomentosa* were not detected by any of the three environmental sampling techniques. Test filters were most sensitive at detecting *Mycobacteria* spp. and Z. picornavirus, while water filtration was most sensitive at identifying *M. streisingeri*. Swabs of the sump biofilm were highly variable in identifying pathogens. Testing the filters at 60 days yielded the highest pathogen detection. Nitrocellulose test filters may be a less labor-intensive method for health monitoring of laboratory zebrafish colonies at 60 days of sump exposure and may reduce the need for alternative pathogen detection methods.

P240 Comparison of Different Formulations of Extended-Release Buprenorphine in Perioperative Pain Management in Common Marmosets (*Callithrix jacchus*)

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Common marmosets are increasingly used as animal models to study human diseases and often undergo surgeries as part of IACUC-approved protocols. Pain control is essential to their clinical management and welfare, and long-acting buprenorphine is a valuable analgesic as it can maintain plasma concentrations above therapeutic levels (0.1 ng/mL) for up to 72 hr in this species. However, no validated efficacy model is available to verify the analgesic effect of buprenorphine in marmosets. Therefore, this study compared the efficacy of buprenorphine-ER-LAB (Bup-ER-LAB) at 0.15 mg/kg and two doses of Ethiqa XR (EXR) at 0.15 and 0.1 mg/kg administered subcutaneously in marmosets undergoing oocyte collection (OPU) (n=12 females) and vasectomy (n=9 males). We hypothesized that these formulations would provide similar analgesia during the 72-hr postoperative period. A composite measure pain scale was designed for semi-quantitative assessment of postoperative pain, focusing on appearance, activity, body posture/integument, respiration, surgical site, and social interactions. A total pain score of 0-1 corresponded to no or minimal pain; 2 to mild-moderate pain; and 3 to severe pain. Animals were also assessed for drug injection site reactions and hyperactivity. All pain scores were either 0 or 1, and no marmoset needed rescue analgesia. 56% of the males and 25% of the females showed hyperactivity which could last up to 48 hours. Hyperactivity occurred in 57% of the Bup-ER-LAB group, 43% of the EXR 0.15 mg/kg group, and 14% of the EXR 0.1mg/kg group (3 males and 4 females per group). Injection site reactions (erythema and/or swelling) occurred in 57% of the Bup-ER-LAB group, 29% of the EXR 0.15 mg/kg group, and 14% of the EXR 0.1mg/kg group. Based on these results, these long-acting buprenorphine formulations provide effective postoperative analgesia in marmosets, and EXR at 0.1 mg/kg provides adequate analgesia with the least hyperactivity and injection site reaction.

Husbandry and Management Posters

P300 Emergency Management of a Recirculating Aquaculture System Shutdown at a Zebrafish Facility

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A cyberhacking event occurred at a large academic research institution that was housing zebrafish (*Danio rerio*) for biomedical research purposes. The programmable logic controller (PLC), the control unit that maintains the recirculating aquaculture system (RAS), was infiltrated and became non-operational due to the actions of the cyberhacking organization. Tanks housing approximately 11,000 zebrafish were no longer connected to the RAS, and an emergency management plan was enacted to maintain basic life support of the juvenile and adult fish on this system. Due to lack of sufficient labor, approximately 60% of the original population was culled. The veterinary team worked in collaboration with researchers and husbandry staff to implement a plan that involved an altered feeding schedule, manual water changes, and daily water quality evaluating and reporting. After regaining functional control of the PLC, the process of restarting the RAS and biological filter began. Tanks of fish were gradually introduced back on the RAS seven days from the initial event. There was no significant morbidity or mortality associated with the fish housed in a static configuration after the PLC went down and before being re-introduced onto the recirculating system. Water quality values were closely monitored by husbandry and veterinary staff daily and adjusted accordingly to ensure health of both the biological filter and fish colony. To resolve this issue in the future, the PLC was replaced with a new system and located behind an institutional firewall, contrary to the original configuration of this system. The event underscores the critical need for enhanced cybersecurity measures within all aspects of an academic institution. This scenario is example of an acute response to an unexpected event that was managed appropriately and collaboratively by research, husbandry, and veterinary staff to maintain animal health in an emergency situation.

P301 The Impact of Bryozoans on Zebrafish Systems and Possible Mitigation Solutions

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In most freshwater systems, bryozoans can provide evidence of good water quality and can be a welcome addition; however, they can have serious negative impacts, especially when found in research aquatic systems. We examined the risks bryozoans pose to zebrafish systems and husbandry methods to help reduce these risks. Bryozoans are microscopic aquatic invertebrates that live in colonies. They have adaptations to marine and fresh-water systems and can reproduce both sexually and asexually. The most unique method of reproduction is through statoblasts, a hard seed-like pod created in their bodies that can withstand the variable conditions of freshwater systems. Statoblasts can survive years in unfavorable conditions, allowing them to restore the colony when conditions improve. While bryozoans themselves do not pose any known clinical risks to zebrafish, they may serve as a vector for other pathogenic species. Bryozoans are also a fouling species notorious for clogging pipes, filters, and pumps. This can cause system-wide failures if left unchecked and is exacerbated by the fact that they are fast-growing, with some species known to double in size within four to seven days. The continual growth of bryozoans introduced to a single stand-alone zebrafish system within our facility was controlled through diligent cleaning efforts, the use of hot water and chemical disinfection to kill live colonies, tailored feeding plans to reduce overfeeding, and additional filtration to catch and remove statoblasts. These methods have also been successful in containing their growth to the affected stand-alone system, with no observable spread to any adjacent systems.

P302 A Simple Method to Increase Zebrafish Production Parameters Using Decapsulated *Artemia* Embryos

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Vendor decapsulated *Artemia* embryos (decaps) can replace in-house hatched *Artemia* nauplii as a primary feed source for zebrafish colonies after past research showed replacement with decaps in a standard diet (*Artemia* nauplii plus pelleted feed) found no significant difference in mean survival, mean weight at 90 days post fertilization (dpf) or mean embryo production. Our group expanded on this experiment and tracked embryos raised to 150dpf. When comparing the vendor decap group to the standard *Artemia* group, we found no significant difference in survival, mean group weight, mean group length and embryo production supporting past research findings. In a second trial, we doubled the concentration of vendor decaps and found a significant difference in the decap mean group weight, mean group length, and embryo production. We provide a method of feeding decaps to zebrafish colonies that can have a significant difference on production parameters.

P303 Evaluation and Proposed Integration of a Commercially Available Sampling Device for PCR Diagnostics on a Zebrafish Recirculating System

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The use of zebrafish as an animal model continues to expand substantially among research vivaria. As with other laboratory animal species, health monitoring of pathogens plays an important role in animal health and research data reproducibility. Accurate animal health assessment at the colony level ensures the integrity of information for reporting in research-related findings, as recommended by the ARRIVE guidelines. Most standalone recirculating zebrafish systems have centralized drainage that collects water from all attached tanks. This water can be diverted to a dedicated tank to expose sentinel animals to any potential pathogens, but unfortunately, sampling of these animals is a terminal process. Sump sludge/detritus has been proposed as a replacement for sentinel animals, however, historically, not all pathogens are detected via this method, nor do all systems provide easy access to a sump tank. A commercial filtration device exists that can collect draining water from standalone systems; however, the efficacy of this device has not been published. Our study sought to evaluate the sensitivity and utility of the commercially available sump-water filtration device to detect infectious agents compared to standard fish and sump detritus samples. Over five quarterly health monitoring periods, we collected sentinel fish, detritus, and filtration sampling among select racks (n=1-2 per period) for PCR infectious agent screening. Five different pathogens were detected among the enrolled racks: Zebrafish picornavirus (ZPIC), *Mycobacterium fortuitum* (MYFR), *Mycobacterium chelonae* (MCHE), *Mycobacterium gordonae* (MGOR), and *Pseudoloma neurophilia* (PSN). ZPIC was detected in all samples, with least frequent detection in detritus. MYFR was detected in filtration devices, but not in any other samples. MCHE and MGOR were detected reliably in detritus and filtration devices but intermittently via sentinels. PSN was only detected in sentinels. Based on these results, a proposed recommendation for health monitoring would include a combination of sentinel animals with the commercial filtration device. If PSN is not excluded, quarterly testing could alternate between sentinel testing and the filtration device, leading to a 50-75% reduction in animal numbers.

P304 Evaluation of Chemical and Mechanical Methods of Zebrafish Net Disinfection

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Nets used to capture and transfer zebrafish have direct contact with fish as well as organic material within the tank and, therefore, may act as a fomite for pathogens if the nets are not adequately cleaned and disinfected between uses. A commonly used commercial benzalkonium chloride product used for net disinfection, whose effectiveness for net disinfection had been evaluated previously, was recently discontinued making it necessary to identify an alternative. We first evaluated the impact of residual net disinfectant on water quality by assessing three different disinfectants with and without post-disinfection treatment with a water conditioner that detoxifies ammonia, chlorine, and chloramines. Nets were exposed to effluent water from a recirculating zebrafish housing system for 2 minutes before subjecting them to a 5-minute disinfectant soak in 7% hydrogen peroxide (HP), 1% potassium peroxymonosulfate (PPM), or 0.27% accelerated hydrogen peroxide, followed by a 10-minute exposure to either neutralizer or reverse osmosis (RO) water before soaking in pH and conductivity buffered reversed osmosis (RO) water for 10 minutes. The pH, ammonia, and chlorine of the post-soak RO water was evaluated, with none having a negative impact on water quality. A previously published study demonstrated that PPM effectively reduced bacterial burden on zebrafish nets. Therefore, this product was chosen for further investigation. Nets were exposed to effluent water for 2 minutes, then exposed to PPM for 2-, 10- or 30 minutes, followed by a 15-minute soak in RO water. Disinfection effectiveness was evaluated by reduction of ATP bioluminescent to 11 reactive light units. Adequate disinfection was only observed in the 30-minute soak group. We also evaluated the efficacy of an under-counter washer with and without the use of HP. Soiled nets were exposed to either effluent water or water from a dirty tank with recently removed fish for 2 minutes and subjected to an 82°C wash cycle with or without HP. Pre- and post-wash ATP swabs were collected, which revealed that mechanical washing with and without HP were effective. Together, these findings reveal that a 30-minute PPM soak and rinse and mechanical washing at 82°C are safe and effective methods for disinfecting nets.

P305 Photography of Live Fish in a Laboratory Setting

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Unlike land-based animals, photography of live aquatic animals poses several unique challenges (water hazards, glare from reflective surfaces, hazy containers, etc). It is also a challenge to accurately capture the form, depth, color, and texture of the three-dimensional animal in a two-dimensional image. Even so, a collection of equipment to form a mini studio and image processing setup can be assembled with a modest investment of funds and space. This enables laboratories to capture quality images and videos of fish for presentation, journal publication, or in-house materials.

P306 Characterization of Occupational Murine Allergens in Stanford Research Facilities

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On average, 10-30% of individuals who work in laboratory animal facilities develop symptoms of laboratory animal allergy (LAA),

with 5-40% of workers developing LAA symptoms within the first two years of exposure. The most common animal species associated with LAA is mice due to mice being the dominant species in genome-based research in most countries. The primary mouse allergen that causes allergic sensitization and allergic respiratory disease is *Mus m* 1. The *Mus m* 1 proteins in fur and bedding are readily aerosolized by activities of animal care technicians and scientists performing cage-changing activities or other procedures. The objective of this research project is to measure the amount of dust particulates and protein allergens from personal and area samples to see if there is a correlation between total dust particulate levels and protein allergen levels. This would help evaluate whether dust particulate levels could be used as a measure of protein allergen exposure. Being able to estimate protein allergen exposure by measuring dust levels would make personalized allergy risk assessment simpler and more cost-effective to obtain for laboratory animal workers. Two air sampling pumps measuring personal dust and personal allergen concentrations were attached to the breathing zone of an individual performing laboratory animal-related work, while two other air sampling pumps were used to measure area dust and allergen concentrations. All measurements for the personal dust and area dust samples were below the limit of detection (LOD). Therefore, the allergen and dust concentrations should be determined to have no correlation and suggests that using total dust measurements to test for allergen exposure is not a valid method. Overall, this study suggests that personnel who work with animals, even under a cage-changing hood, may still be exposed to high amounts of allergens.

P307 Utilizing Innovative Methods to Improve Safety of Vivarium

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Safety is paramount in our daily work routine, necessitating regular assessments of our work environments to maintain a secure workplace. Lean lab projects prove invaluable in identifying bottlenecks and involving staff to propose efficiency and safety enhancements. Through collaboration with our husbandry and cagewash staff, we implemented several safety improvements based on lean lab ideas. Notably, we changed from autoclaving all mouse cages and housing racks to only those for immunocompromised mice and utilized bulk trucks for cage transportation and storage rather than housing racks, thus reducing equipment deterioration and the risk of injury from broken wheel casters caused by repeated autoclaving. Additionally, we installed nonslip mats in our cage wash areas, preventing slips, trips, and falls while providing antifatigue comfort. For movement between floors, a cover was made to insert in the elevator floor door track prior to rolling racks on/off to stop wheels from being caught in gaps and prevents racks from tipping over. In the cagewash areas, yellow boundary lines were painted around the drains as a "no transport zone" for the same purpose. These measures act as visual cues and physical barriers, ensuring rack stability and preventing accidents. Furthermore, we acquired lighter and more maneuverable water dollies, streamlining the transportation of water bottles and reducing physical strain on our staff. Finally, the implementation of an Ergo Mobile Desk allowed for an all-in-one mobile desk to be used throughout the vivarium for updating door logs and cage card labels, improving ergonomics, reducing back strain, and enhancing organization. Through the active implementation of lean lab ideas and robust collaboration, we have successfully achieved significant safety enhancements in our workplace.

P308 Utilizing Rodent Behavior Testing in an ABSL3 Setting

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Behavior testing in mice can be an invaluable tool for assessing motor phenotype and monitoring the development of anxiety-like behaviors and neurodegenerative symptoms in infection studies. Open field, rotarod, and tail suspension tests have all been proven to be effective testing modalities that can monitor progression of symptoms in mice suffering from neurologic illness. Working with Select Agents in a high biosecurity research setting presents numerous constraints in space and maneuverability when working with infected animals. For these reasons, behavior testing in ABSL3 and ABSL4 facilities in the United States is incredibly rare. Here we describe the use of open field, rotarod, and tail suspension testing in the Laboratory for Infectious Disease Research, an ABSL3 laboratory at the University of Missouri, using a murine intranasal infection model of brucellosis. All tests were modified for use in a biosafety cabinet and utilized for the characterization of a novel neurobrucellosis model. Rotarod and open field tests were reliably predictable in diagnosing neurologic symptoms prior to the development of overt disease. These findings demonstrate the importance and value of behavior testing in mice infected with Select Agents in a high biosecurity setting.

P309 Animal Biosafety Level (ABSL) 2 – It's Not One Size Fits All

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The changes in the 6th edition of the Biosafety in Microbiological and Biomedical Laboratory (BMBL) presented new challenges for doing ABSL-2 rodent work. This provided an opportunity to refine our processes, leading to changes in the way we manage our ABSL-2 housing and transport/process of supplies. The first step taken was to significantly consolidate ABSL-2 cages into designated areas. This decreased the total number of ventilated racks and blowers needed, decreasing costs for annual certification. In line with that change, a special cost center could be created and applied at the room level, eliminating a need for manual cage counts and ensuring the increased costs associated with hazard use were captured. Feedback from staff working in such areas reported the challenges with transporting dirty supplies to cage wash due to the need for secondary containment. To minimize labor costs, a metal trolley fitted with a cover, was modified with a tray in the bottom to allow for transportation of cages (approximately 150 cages and 200 wires). Once cages reached cage wash, autoclaving occurred immediately, leading to difficulties when the bedding, now baked on, needed to be removed. Now, the bedding is removed prior to autoclaving, and we have additionally developed an alternative chemical decontamination protocol that can be used in the absence of a working autoclave. Such changes have improved our efficiencies by reducing the amount of time spent processing supplies and billing cages, allowing for real-time billing of hazard cages, and has reduced costs associated with annual blower certifications. Considering this, I would recommend this type of approach to other facilities to streamline their management of ABSL-2 cages and improve overall staff wellness. Such changes have allowed our technicians to better utilize their time while still meeting the recommendations in the BMBL.

P310 How Low Can You Go?: Using Bottle Volume as an Alternative to Time-Scheduled Water Bottle Replacements for Mice

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The use of sterile disposable cage components has led to an increased need for performance-based standards. The *Guide* specifies that sipper tubes require weekly sanitization. However, the gravity-drip design of the water bottles our institution uses should minimize contamination, suggesting water quality and cleanliness may be

maintained for longer durations. Weekly changes for disposable bottles yield abundant plastic and water waste: bottles are typically greater than half full at seven days, even in densely populated cages. Caps must be removed and bottles emptied in order to recycle—a labor-intensive and ergonomically challenging process. A volume-based schedule is a visual alternative to weekly changes, reducing the number of bottles processed and facilitating operations by allowing spot changes without the need to track bottles. We hypothesized that extending bottle duration from weekly to volume-based would not adversely affect mouse health or water quality. 100mL was chosen as the lower limit, as this represents an approximate 4-day supply of water in densely populated cages. We collected baseline (7-day) data from 15 cages of C57BL/6NCrl mice at various housing densities (ranging from 1-5 mice per cage) for changes in animal weight (measured weekly) and average water consumption (measured every 2-5 days). At bottle replacement, we measured packed cell volume (PCV) as a marker of clinical dehydration and assessed water quality by visual inspection, plating for *E. coli* and coliform bacteria, and total microbial count. We repeated these assessments, leaving bottles on the cage to 100mL (13-47 days, depending on cage density). No difference was observed in animal body weight, body condition score, average water consumption, or PCV, regardless of cage density or the amount of time the bottle was deployed. Average water consumption was maintained at an average rate of 3.9 ± 3.5mL/mouse/day for both seven-day and volume-based changes. Microbial analysis showed no bacterial growth in any bottle, and visual inspection showed no turbidity or cloudiness. We conclude that, at our institution, pre-filled disposable, acidified water bottles can be extended beyond seven days using a volume-based replacement at 100mL, with no adverse effect on animal welfare.

P311 Monitoring Automatic Watering for Vivarium Animals

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Research institutes that utilize animals must provide water to them, and they often do so by various methods. One method is automatic watering through a distribution line. The visibility into real-time close monitoring can help reduce the dangers to animals. Managing the vivarium watering distribution system with various methodologies for alerting, such as a phone call, voice message, or SMS message, keeps piece of mind that animals are safe from drowning, hypothermia, and dehydration. Water monitoring is a growing concern throughout the research industry. Institutes can monitor all aspects of their water, from water pressures, water flows, schedules of daily water line flushes, and acknowledgment of alerts. Having appropriate standard operating procedures, detailed record management of the alert cause and remedial action, and trained system staff to confirm alerts are responded to in the allotted time range all assure that water dangers are minimized. An intuitive monitoring system available to the industry has allowed us to monitor over 119 targeted points per our needs. Manual operation and controls of watering solenoids and multiple rooms can be controlled on one pressure station. Close monitoring of the watering system enables us to distinguish alerting areas, such as broken caging causing leaks, the drinking habits and playful nature of the animals left open drains on the animal equipment, and pressure station component malfunctions. Investigations into the alerts provide staff with the awareness to thoroughly check drain valves or repair caging to help identify actual life-threatening alerts. This information is visible to management, veterinary staff, and the technical staff as information that can be utilized in risk management. AmplifyBio tested and validated the watering system during a vigorous installation process. Operational qualification and performance qualification testing of the system before animals were brought in provided security that when animals are received, they are provided superior care, have access to water, and water dangers are being monitored. In the future, monitoring a single rack can pinpoint an alert cause. Real-time pressure station monitoring will determine which components may need to be replaced in that station.

P312 Assessing Bedding, Diet and Wire Bar Lid Contributions to Flood Occurrence in Automatic-Watering Mouse Cages

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Automatic watering systems deliver potable water to laboratory rodents and simplify husbandry operations by reducing labor costs and ergonomic risks of water bottle handling. However, cage material such as bedding, feed waste, and nest enrichment can embed within and aberrantly activate the water valve, causing devastating cage floods. To assess flooding in mouse cages on automatic watering, we prospectively compared approximated average flooded cage occurrence (% flooded cages/average total cage census/month) using 1) 3 corncob bedding products: ⅛″ corncob, ¼″ corncob, or ¼″ corncob including paper nesting product; 2) 2 diets: standard or trimethoprim-sulfamethoxazole (TMS) feed; and 3) 2 commercially available wire bar lids: one standard and one modified with horizontal cross-hatching. For six months, we recorded flooded cage occurrence in 9 research colony rooms, averaging a cumulative census of 4,459 cages/month. In months 1-4, cages on standard feed had a flooded cage occurrence of 0.2% (0-0.98%) with ⅛″ corncob bedding and 0.02% (0-0.15%) with ¼″ corncob bedding. Cages on TMS feed and ¼″ corncob bedding had a flooded cage occurrence of 0.59% (0-3.03%). In months 4-5 with standard feed, there were zero flooded cages with both ¼″ corncob bedding and ¼″ corncob bedding including paper nesting product. Cages on TMS feed and ¼″ corncob bedding, with or without paper nesting product, had a flooded cage occurrence of 0.28% (0-1.68%) and 0.87% (0-1.74%), respectively. We frequently observed excessive feed waste with TMS feed, which readily crumbles, and its accumulation in water valves. In month 6, to explore whether reducing TMS feed waste would further decrease cage flooding, a subset of TMS feed cages with modified wire bar lids (n=112) were compared to all TMS feed cages with standard lids, finding zero flooded cages with both ¼″ corncob bedding and ¼″ corncob bedding including paper nesting product. Rather, cages with standard wire bar lids had a flooded cage occurrence of 0.39% (0.25%-0.54%). In conclusion, cage flooding and its detrimental impact on mouse health can notably be reduced with larger diameter bedding and modified cross-hatched wire bar lids, which respectively mitigate bedding and TMS feed waste contributions to automatic watering-associated flood occurrence.

P313 Better Together: Evaluation of a New Bedding with Enrichment Substrate Based on Mouse Interaction Scores at a Commercial Vendor

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Commercial vendors develop new products to continue improving the lives of laboratory research animals. These products, such as bedding or enrichment offerings, undergo safety and efficacy reviews before they are available to the industry. The recent bedding with pre-mixed enrichment offers a solution to reduce manual labor and storage space dedicated for enrichment. This study examined the animal utilization of a bedding with pre-mixed enrichment product to have these end users demonstrate how they interact with it. Environmental enrichment is necessary, with common rodent enrichment being classified as social, structural, or manipulable. Foraging enrichment is most often associated with providing edible food enrichment via seeds or formulated treats but are underutilized given the unknown interactions of treats on study results. To better understand how mice interact with a novel approach of mixing enrichment into the bedding, an initial study was conducted to evaluate nest building and enrichment utilization in mice at a commercial vendor. This study utilized socially housed, age-matched

male and female C57BL/6NHsd and Hsd:ICR (CD-1) mice (housed by sex and model) and provided water and feed *ad libitum*. Animals were housed on either bedding with enrichment added by hand or with enrichment pre-mixed into the bedding. Bedding substrate was either corn cob or paper pulp and the enrichment evaluated was a Diamond Twist or ½"-cut Diamond Twist nib. The animal's behavior at the cage level was assessed to conclude the utilization of the pre-mixed bedding and enrichment as compared to bedding with the addition of enrichment. Nest, utilization, and utilization quality scores were assigned to evaluate animal interaction with the bedding and enrichment substrate. This team proposes that mice will utilize provided enrichment material regardless of if it is pre-mixed into or added by hand. However, the animals did not used the 1/2"-cut Diamond Twist nibs as anticipated. Instead, these nibs served as a forage-like enrichment as opposed to the expected shred-able nesting material. Future research into animal utilization of novel or existing enrichment products is needed to improve caging enrichment options for animal research models. This study has been approved by the facility's IACUC.

P314 A Comparative Assessment of the Effect of Animal Bedding on Tumor Condition in Nude Mice

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Ensuring the quality and integrity of tumor specimens is vital to the successful collection and analysis of samples for cancer research. One important factor in tumor integrity is the environment in which the tumor is grown, both its microenvironment and macroenvironment. An animal being used for cancer research spends the majority of its time in contact with cage bedding, making it a significant portion of what can impact tumor integrity externally. Pressure sores and ulcerations from prolonged contact and surface necrosis may be impacted by the quality and type of bedding used in animal cages. In order to minimize potential environmental damage to tumor quality, a comparative study of the effect of two different types of bedding on tumor condition was conducted. Female athymic nude mice were subcutaneously injected with cancer cells and randomly placed into cages with either uniform virgin paper pulp cellulose or 1/8-inch corncob bedding. Tumor growth and condition were measured three times per week using 3D and thermal imaging until the termination criteria of 1000 mm^3 LWH was reached. Preliminary results indicate no statistically significant differences in the overall integrity and condition of xenograft tumors grown in nude mice between the two bedding types. This finding indicates that both bedding types are likely equally suitable for studies with tumor-bearing nude mice. Additional research to replicate and further support these conclusions is ongoing.

P315 A Successful Management Strategy for Mitigating Intra-Cage Aggression in Research Mice (*Mus musculus*)

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Agonistic interactions among research mice can have far reaching implications on animal well-being and scientific results. It is well-documented that mice are a social species and should be housed with compatible conspecifics. However, certain strains of mice, including FVB and BALB/c, may be prone to agonistic behavior when socially housed in a research setting. Various strategies for managing agonism are employed across laboratories, including the practice of reducing cage enrichment to lessen fighting. Research suggests that a reduction in cage density, or smaller numbers of animals per cage, and an increase in enrichment providing visual barriers should have a positive effect on socialization. In our facility, mice were housed in groups of up to 5 animals with nesting material (8g crinkle paper) in standard ventilated microisolator mouse cages (77.5in2). Clinical cases involving fight wounds in a breeding colony of FVB mice became a concern for animal welfare and negatively impacted research goals. Separating mice into smaller groups after fighting and nest transfer at cage change did not eliminate agonistic behavior or wounding. As a new management practice, FVB mice were consistently weaned into groups of 2-3 mice per cage and were provided two cardboard tunnels (4x2in) with nesting material. The implementation of this strategy was highly successful and expanded to other strains prone to agonistic interactions in our facility, BALB/c and PRP (prion protein knockout). When not bred in our facility, groups of no more than 2-3 mice were established upon arrival. Age, sex, cage density, wounds, and mortality from fighting were tracked over a 3-year period and demonstrated a marked decreased incidence of fighting, resulting in clinical injury and increased long-term stable socializations in the three mouse strains. Our data confirms that providing mice more space by reducing cage density to less than four animals per cage and increasing physical and visual barriers in the form of multiple opaque cardboard tunnels is an effective and preferred practice for housing research mice prone to agonism.

P316 Extending Cage Change Intervals for Ventilated Cage Housed Rat Species

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The Guide notes that ventilated cages (VC) may justify decreased cage sanitation frequency depending on the conditions (strain, density, sex, bedding, and species). Longer cage change intervals will decrease animal stress and animal care costs. Cotton rats (CR; Sigmadon) are routinely single housed due to aggression with breeder cages housed on aspen chip (AC) for nest building. CR were single housed in VCs on corncob (CC; n=6, 3F and 3M) or AC (n=9, 4F and 5M). Two rat (Rattus) strains: Sprague Dawley (SD; n=38, 20F and 18M) and Lewis (LEW; n=20, 10F and 10M) were housed 2 to 5 rats per VC on CC bedding. Animals were checked daily for signs of stress (not grooming, isolation, excessive porphyrin staining). Ammonia (NH3) was measured daily starting on day 7 using Hydrion NH3 paper and a Draeger gas analyzer. Cages were changed when NH3 levels reached 20ppm for CR and 25ppm for Rattus. Animals were weighed at the final cage change to determine total biomass. NH3 levels for all CR housed on CC remained ≤20ppm until day 60, while only 86% and 43% of CR, with no sex difference, housed on AC had NH3 levels ≤20ppm at 8d and 14d, respectively. Rattus NH3 levels correlated to cage biomass with SD male being the largest (1423gm±50SE) and LEW female the smallest (525gm±3 SE) ($p<0.001$). SD had higher NH3 levels sooner than LEW rats, with 100% of female LEW having NH3 <25ppm out to 14d. These findings show that CR housed in VC on AC bedding requires cage change every 7d while cage change for CR on CC bedding could be extended to 60d. Cage change interval for group-housed Rattus could be extended depending on biomass within the cage with intervals up to 14d for female LEW on CC bedding in accordance with their size. Reducing the frequency of cage change can decrease stress and improve animal care efficiency.

P317 Comparison of Ammonia Levels and Latrine area in Individually Ventilated Cages from Two Different Manufacturers

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We conducted a comparative study of ammonia levels in individually ventilated cages (IVC) from two different manufacturers, housing 3-5 animals per cage. Ammonia levels were measured on Days 5-14. On Day 14, after the ammonia measurements, the cage was removed from the rack and a picture

of the underside of the cage was taken. The picture was used to measure the latrine area and to determine if it could be used as a secondary indicator of ammonia levels. Ammonia sampling was performed with all cages remaining on their racks to maintain uninterrupted interior air flow. The blower units on both racks were set to the manufacturer's recommendations (Rack A 75 ACH, Rack B 50 ACH). An ammonia concentration of 50 PPM was used as the threshold for acceptable exposure. Our findings indicate significant differences in ammonia level trends between the two vendors over time. Generally, ammonia levels increased with the number of animals per cage, with one vendor's racks exhibiting notably higher NH3 concentrations, particularly in cages with 4 and 5 mice. Several cages of 4-5 mice on both racks exceeded the 50 ppm threshold. The latrine area increased with the number of mice and time for both vendors. Consequently, at our institution IVC cages containing 4-5 mice will be changed on a 10-day interval to minimize cages with ammonia levels exceeding 50 ppm. The shift to a 10-day interval significantly affected the total number of cages changed per week, accounting for 30% of the overall weekly cage changes. Cages on the 10-day schedule are identified by a green cage flag placed behind the cage card. Cages with three mice will continue to be changed at a 14-day interval. A latrine size of >3000 square millimeters (approximately the size of a credit card) will be considered in determining if a spot change is necessary.

P318 Considering Disinfectants, Changing with the Times

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Disinfectants are an integral part of sanitation practices for animal facilities. Spor-Klenz® Ready-To-Use cold sterilant (SK) was the primary disinfectant for biosafety cabinets (BSC) and counters for many years. SK has a strong odor and requires a BSC or eye protection and skin protection to avoid irritation or ocular damage. Selection of a new disinfectant requires careful evaluation of effectiveness, cost, and safety. Coordination, education and training of animal care and research teams is essential for the successful implementation of widespread change. PeroxigardTM Concentrate (PG) was evaluated for use in facility cleaning. Diluted PG is very safe, does not require any specific personal protective equipment (PPE), produces less odor, and has a contact time of five minutes compared to ten minutes for SK. Consideration of the cost for changing to PG included the installation of dilution stations (1 station per 2,500 cages), plastic bottles, and gallons of usable product. The initial cost for the switch to PG (26 stations, 200 bottles) was $6215. The annual disinfectant use was 808 gallons across ten facilities with a cost of $33,189 for SK and $2,169.64 for PG. This represents a cost saving of 74.7%, with an additional saving of 18.7% after the first year. Education and communication with research teams involved the posting of signs and the removal of SK from all areas. Animal care teams were notified during monthly meetings and in small group discussions. All users appreciated the decreased odor and lack of safety concerns. The use of concentrated formula aligned with the University's "green initiative" by creating less plastic waste compared to purchased gallon jugs. PG requires less storage and less transportation as it is delivered on the same schedule and truck with routine supplies. Change on a large scale is complicated. Periodic evaluation of current practices can yield meaningful physical rewards.

P319 Effect of Different Disinfectants on Rodent Cage Integrity Following Repeated Autoclaving

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Effective sterilization of animal caging is required prior to their removal from high containment facilities to ensure biosafety. However, repeated sterilization events can lead to cage cloudiness and loss of integrity. Maintaining good visibility of plastic cages is critical for performing health checks and ensuring the welfare of laboratory rodents. Previous application of disinfectants can exacerbate the negative effects of sterilization on cage integrity, and removal of disinfectants from cages prior to autoclaving is preferred to slow or prevent these effects. However, washing rodent caging prior to autoclaving in high containment facilities is not often feasible, and so selecting disinfectants that effectively inactivate experimental pathogens but have minimal effects on cage transparency and integrity is needed. The effect of three different disinfectants commonly used in animal biosafety level 3 (ABSL3) facilities on polycarbonate cage integrity following autoclaving was evaluated, with the hypothesis that corrosive disinfectants, specifically phenolic solutions, would lead to impaired transparency at a faster rate than other non-corrosive disinfectants. Cages were sprayed with one of three disinfectants: 1) a phenolic disinfectant, 2) a hydrogen peroxide-based disinfectant, and 3) an alcohol-based disinfectant, or were left untouched. The disinfectants were allowed to fully dry, and the cages were autoclaved using standard sterilization settings of 121°C at 30 minutes. The cages were allowed to cool completely, at which time the disinfectants were reapplied, and the autoclave cycle was repeated. No change in transparency was observed after repeated disinfectant application-autoclave cycles in any of the disinfectant groups. However, etching was observed on cages sprayed with the hydrogen peroxide-based disinfectant after just one cycle, and full-thickness breaks in the plastic were observed in this group after three cycles. This study highlights the importance of balancing experimental pathogen inactivation effectiveness with reduction of adverse effects on caging integrity when choosing disinfectants for high-containment animal facilities.

P320 Enhancing Environmental Sustainability through Innovative Vivarium Cage Wash Updates

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Environmental sustainability is a crucial aspect of vivarium operations, and optimizing cage wash processes plays a vital role in minimizing the overall environmental impact. By implementing refinements such as reducing the temperature of the tunnel washer's final rinse, adopting selective autoclaving practices for immunocompromised animal caging, and decreasing the wash times of the rack washers, we were able to see a reduction in our carbon footprint. The temperature of the tunnel washer's final rinse was reduced to 165°F (74°C) from the conventional 180°F (82°C), achieving considerable energy savings without compromising sanitation, validation of sanitization was confirmed using ATP (Adenosine Triphosphate) swabs. In addition, we modified the historical practice of autoclaving all mouse caging and racks to only equipment for immunocompromised mice, further reinforcing environmental sustainability goals. By limiting the autoclaving process to only the equipment used for immunocompromised animals, the energy and water requirements associated with sterilization are reduced. This approach not only optimizes the use of resources but also minimizes staff workload, resulting in operational efficiencies. Lastly, the final rinse rack washer cycles were shortened, enhancing the speed of wash times while ensuring efficient utilization of resources, such as water and electricity. Reducing the time required for cleaning and decontamination resulted in minimizing the consumption of resources. All these changes directly contributed to reducing the carbon footprint associated with cagewash operations. By aligning vivarium cage wash updates specifically focusing on the environmental impact, researchers and facility managers can contribute to a more sustainable approach to animal research. The optimization of cage wash processes minimizes the overall carbon footprint, water consumption, energy usage, and cost while making a positive environmental impact.

P321 Mop-timizing Wall and Ceiling Sanitation in a Research Animal Facility

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Sanitation of all components of the animal facility is required, yet little information exists about sanitation of walls and ceilings. This study validates a new disinfection method using a microfiber mop (MF) and *Peroxigard*™ (P) for walls and ceilings in rodent housing rooms. MF+P was compared to a doodlebug fiber pad mop (DB) with Process NPD® detergent (PNPD). We hypothesized that the MF+P process would be effective while being faster and more user-friendly. Ten participants were asked to sanitize a housing room once using either MF+P or DB+PNPD and again six months later using the alternate method. All participants were novices for the MF+P sanitation process. Participants were timed from start to finish, including set up, cleaning, and break down. Times were normalized per square foot of space, and participants were compared to themselves to normalize for faster or slower workers. Sanitation efficacy was measured using Replicate Organism Detection and Counting (RODAC) plates and ATP monitoring system. RODAC pass level was defined as 0-15 colonies and set the standard for ATP pass level of ≤ 17 RLU's. Samples were taken from two wall types (smooth and grooved). To evaluate cleaning efficacy, a water-based invisible ink marker was used on walls and photographs were taken before and after sanitations. A survey was administered to determine user preference. Both sanitation methods were effective. MF+P was significantly faster than DB+PNPD due to easier preparation ($p < 0.0001$) and breakdown ($p < 0.0001$). There was no difference in time to clean rooms ($p=0.356$). The decreased time represents a potential 58% labor cost saving. Survey results indicated participants considered ease of preparation, mop type, and disinfectant characteristics as important, and 90% preferred the MF+P method. MF+P is an effective, fast, and easy method for the sanitation of rodent housing rooms.

P322 Improving Functionality and Biosafety Methods in Specialty Areas Utilizing Decontamination Floating Mats

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Decontamination floor mats are a highly effective solution for controlling and preventing cross-contamination in both clean and BSL2 areas in vivariums. These mats have been proven to be 99% effective in inhibiting contamination by trapping and retaining particulates from shoes and wheels while also minimizing dust and charged particle accumulation. In addition to their contamination control capabilities, they provide a clear visual dedicated space for individuals to properly don and doff personal protective equipment (PPE). In our vivarium, we historically used traditional methods such as imaginary lines on the floor and extra PPE in these areas (double shoe covers, Tyvek suits, different color lab coats). However, they proved to be inadequate in effectively controlling foot and wheel-borne contamination while also contributing to increased waste. We implemented an alternative solution to address this issue and promote better cleanliness practices while reducing waste with the installation of decontamination floor mats. These mats have enabled LAR to create a separation of specialty areas from common spaces, and its non-permanent installation allows for the benefit of easily removing and adding locations with the ever-changing needs of the vivarium. The vibrant color and design of the floor mats serve as a clear indicator of the perimeter for special gowning requirements in these specialty areas within the vivarium. This visual cue helps ensure that researchers and staff are always aware of the specific precautions and procedures that need to be followed while working in these areas. Additionally, providing a dedicated space for donning and doffing PPE enhances worker safety and, contributes to the overall cleanliness and containment goals of the facility, and reduces the constant need for PPE changes, which can be time-consuming to put on and take off. This ultimately improves overall facility cleanliness, worker safety, and adherence to biosecurity control protocols.

P323 Prepare to be Floored

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Ensuring a clean and safe housing environment is a cornerstone of husbandry for laboratory animals. Flooring styles used for large animals have an impact on the ability to maintain cleanliness and may pose a risk of injury. The two most used floorings are kennel decking (KD) and expanded metal (XM). KD is made of plastic resin with textured slats. While safe due to the flat, textured surface, KD can retain waste material and moisture on top of the flooring increasing animal exposure to feces, cleaning times, and promoting moisture-associated dermatitis. XM is created from a sheet metal that has been cut and stretched to create a diamond-shaped pattern. The flooring is dipped in a polyvinyl coating to cover the surface, protecting the animal from direct exposure to the metal and promoting cleanliness. XM remains cleaner than KD but may result in injury if digits are caught in the openings. Because of these risks, we evaluated a self-supported, poly-vinyl coated woven wire (WW) flooring with 2in x 0.625in openings with a 0.625in wire diameter. WW has rectangular openings with the animal contact surface being rounded due to the woven-wire design. We compared WW to our older flooring which included a combination of KD (1.625in wide slats) and XM (1.375in x 0.75in openings) in each run. We utilized 48 ft^2 runs with single-housed mongrel dogs, using the same dogs for each trial. We hypothesized WW would reduce cleaning times and digit entrapment. The older style flooring required 112.5 ± 26.6sec for routine daily cleaning. WW cleaning time was reduced to 87.4 ± 8.8sec ($p<0.0001$), and there were subjectively less feces present in the cage between cleanings. In runs with XM, there was about one episode every two months in which a digit became stuck; no injuries occurred, but the risk of injury was a concern. Since making the transition to WW over six months ago, no digits have been stuck. The use of the WW resulted in a significant reduction in time spent cleaning due to the ease of flushing waste material away and has avoided the probability of injuries caused by digit entrapment. Staff preferred WW due to reduced cleaning times and the ability to maintain cleanliness between cleanings.

P324 Daylight Saving Time Recommendations for the Management of Laboratory Zebra Finches (*Taeniopygia guttata*)

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Spring Daylight Saving Time (DST) is a practice that optimizes longer summer daylight wherein clocks are set forward one hour so darkness begins at a later time. Adopted during World War I to conserve energy, DST is still used in most of the USA, Canada, and Europe. At our institution, the light cycle is shifted in most animal rooms biannually for DST, including a room housing a zebra finch (*Taeniopygia guttata*) breeding colony used in neuroscience research. However, light cycle shifts can perturb animal circadian rhythm and physiology, and studies show that the circadian clock controls song production in zebra finches. Light adjustments also increase work complexity for husbandry/facility staff. An acute, transient increase in zebra finch offspring mortality was observed after DST in March 2023, hypothesized to be due to stress from the one-hour light change. To test this hypothesis, DST was simulated in January 2024, with the lights in a zebra finch cubicle turning on and off one hour earlier. Pooled fecal samples were collected noninvasively one week prior, the day of, and one week after

the light change (stressor) for fecal corticosterone metabolite concentrations measured via an ELISA as a physiological stress biomarker. During the same weeks, as well as two weeks prior, the week of, and the week after true DST in March 2024, offspring population data was collected. No significant increase in aggression or offspring morbidity/mortality was noted. A time*stress two-way ANOVA revealed no statistically significant increase in corticosterone concentration at any time point, but there was a transient increase 30 minutes to two hours post stressor. Although no repeatable morbidity/mortality was seen in 2024, the cohort was smaller than in 2023. Another study with a larger sample size of offspring may recapitulate the 2023 conditions. Additionally, stress measurement from females and breeding males may display a more significant response compared to adult males for which the ELISA was validated. Therefore, we recommend to forgo the biannual DST change. This will simplify husbandry practices, reduce potential animal stress, and lessen confounding effects on research. Future studies evaluating the utility and effects of DST in other laboratory animal species are warranted.

P325 The Establishment of Cephalopod Standards and Protocols at the University of Chicago in Response to Growing Use in Neuroscience Research

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Cephalopod research has precipitously increased over the past century across a variety of disciplines, namely within neurological research. Researchers have focused on developing the cephalopod connectome, transcriptome, genome, and more to understand the unique neuroanatomy and physiology of a highly complex invertebrate model. Currently, organizations within the United States have not established regulations regarding these animals despite a new biological understanding of cephalopod responses to negative stimuli. Initially, it was thought that invertebrates are not capable of detection nor reflexive avoidance of negative stimuli; however, scientific studies reveal that cephalopods do exhibit nociceptive behaviors and likely experience pain and distress. Interest has gained traction to establish guidelines and policies, and other countries have begun to publish guidance for the use of these animals. Without complete knowledge of the standards of care and management of cephalopods, uninformed guidelines may present strong challenges for researchers to achieve their scientific goals. It is imperative that continued exploration occurs to gain a better understanding of critical topics. Our institution has made progress toward creating our own standards of care for this species, such as the establishment of animal care and husbandry SOPs, as well as the creation of octopus care and the use of IACUC protocols. These defined standards and regulatory expectations have encouraged proper water quality maintenance, the addition of enrichment, and the development of health assessments. These efforts have contributed to the increased welfare of these animals here as demonstrated by the decreased morbidity and mortality rates of developing hatchlings as well as increased species-specific behavior amongst adult octopuses. It is our goal that these efforts will also contribute to the creation of informed regulations that enhance welfare while bolstering scientific achievement.

P326 Care of Opossums Post Import

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There is little information out there about opossums. Most institutions do in-house breeding and have no acclimation plan. Our institution wanted to refresh the colony by working with four other institutions, both Domestic and International. A more species-specific plan was needed. My plan was to start with a five-day acclimation, as was our standard across covered species. During this time, weight is checked, and overall health and preventative medicines are given. Also, various forms of identification are given to create a medical history. Then, slowly increase this acclimation time upon each import while observing with minimal disturbance and providing environmental & food enrichment. Upon unpacking, the opossum was placed in a cage with bedding, bedding discs, jars, and ceramic bowls. They were also provided with items from the crate they arrived inside, providing familiar smells, along with a small amount of previous feed, bedding, and gel pack. Finding out later, providing another form of gel also in the cage helped transition to a water bottle and provided enrichment. Moistening new feed & giving a mealworm helped transition and give protein. In the beginning, we observed dehydration, stereotypical behavior-circling, possibly self injury-to tail & feet and flipping, while also up during the day. We found the longer the time given to acclimate, without disturbance, while providing a variety of enrichment helped improve their overall health. This time helps transition to a new feed source, resolve stress during transit, and adapt to a new environment.

P327 Out for Recess: An Innovative Housing Design for Macaques

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The Guide for the Care and Use of Laboratory Animals emphasizes that animal welfare is improved through environmental enrichment that promotes natural species typical behavior. To enhance our environmental enrichment program, two large enclosures were custom-designed to fit an unused animal room, giving primates additional opportunities to express species typical behavior. From inception to implementation, this project took approximately two years and involved contributions from multiple stakeholders, including the Veterinary Sciences, Engineering, and Facilities departments and an external caging Vendor. A remote video surveillance system was installed for each enclosure, allowing staff to monitor primates without observer interference and make modifications as needed. The process required intradepartmental collaboration to develop an animal selection schedule and procedural guidelines outlining best husbandry practices. This housing resulted in improvements to the program of veterinary care through the promotion of psychological well-being by increasing the environment's complexity and allowing additional space for species typical behavior. Approximately 58% of the colony was placed in the enclosure during the initial six months of the project, with each cohort spending five consecutive days in the enclosure. Six animals were rotated through an additional time to address behavioral concerns or to accommodate for assignment to new study protocols, resulting in improved outcomes for those animals. The extent of engagement with the room varied widely among individuals, with singly housed animals requiring additional enrichment more often than pair-housed animals. Husbandry during the week was not affected by the addition of this room, with the most labor-intensive day occurring at the end of the week due to the sanitization of the room in preparation for the next cohort. The enrichment room was well received among veterinary, technical, and animal care staff, with an increase in requests for remote camera access. This project has improved animal welfare, increased opportunities for behavioral observation, and introduced questions for further study.

P328 Per- and Polyfluoroalkyl Substances (PFAS) in Natural Ingredient Rodent Diets

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Per- and polyfluoroalkyl substances (PFAS) are highly stable fluorinated compounds with widespread commercial and industrial use. Due to their stability, these "forever chemicals" are found throughout the environment. Human exposure to these compounds occurs via ingestion (water and food), inhalation, and dermal, with ingestion being the most relevant route. Exposures have been linked to multiple adverse health effects, including hypertension, pre-eclampsia, decreased immune function, developmental effects, and cancer, and greater than 98% of the US population has detectable PFAS blood levels. The US Environmental Protection Agency (EPA) recently finalized drinking water regulations for five PFAS compounds with enforceable Maximum Contaminant Levels (MCLs) at 4.0 parts for per trillion (ppt) for Perfluorooctanoic acid (PFOA) and Perfluorooctanesulfonic acid (PFOS), and ten ppt for Perfluorohexanesulfonic acid (PFHxS), Perfluorononanoic acid (PFNA), and Hexafluoropropylene Oxide (HFPO) Dimer Acid and Ammonium Salt (GenX Chemicals). Published PFAS studies in animals have reported measurable concentrations of these compounds in non-dosed, controls without addressing the possible source. In the controlled setting of laboratory animals, the most likely sources are water and feed. This study was performed to assess rodent feed as a possible source. Our initial analysis of a panel of 36 PFAS compounds in natural ingredient rodent diets was performed by a commercial testing laboratory, which found significant levels in diets containing fish meal. However, the laboratory applied a "modified EPA 537.1" method not developed for solid matrices such as feed. This method resulted in false-positive results due to the presence of taurdeoxycholic acid (TDCA), a bile acid, confirmed by high-resolution mass spectrometry. Testing using a modified EPA method 1633 for non-potable water and solids found PFAS concentrations well below the current EPA water MCLs. Our results indicate that natural ingredient rodent diets are not a source of significant PFAS compounds.

P329 Specialized Husbandry Practices and Management of Grasshopper Mice

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The southern grasshopper mouse, *Onychomys torridus,* is used in neurophysiology studies due to its adaptations to chemically defended prey. The grasshopper mouse is a carnivorous rodent with a geographical range from the western United States into northern Mexico. In these prairies and brushlands, the mouse feeds primarily on arthropods, including the lethal Arizona bark scorpion. The housing needs of these mice emphasize their aggressive nature and adaptations to desert environments. They do well in static rat cages with a reduced cage change frequency. They have highly concentrated urine to reduce water loss in their natural environment and it reduces the amount of urine and ammonia production in static cages. Individual housing is the standard due to conspecific aggression. Breeding of wild-caught grasshopper mice can be successful. However, breeding pairs housed together are relatively short term, approximately 2-3 small-sized litters, before aggressive behavior is noted requiring separation. Enrichment items include routine rodent enrichment (nesting material, hiding huts and destructible items) and extend to include a running wheel and various feed items. Freeze-dried protein sources are an important addition to their diet and live prey is given as a form of enrichment. The handling of wild mice is focused on reducing stress for the animal and handler. Restraint devices and tunnel handling are the most frequently used methods. Wild rodents are extremely active during cage change. A specialized changing station was designed in-house to keep mice and care staff safe. The changing station, a large secondary container, allows the care staff to gently move animals from cage to cage without fear of escape. Animals that jump outside of their cages are contained in the changing station. Open bottles are strategically placed in corners to take advantage of the thigmotactic behavior of the rodents. The wild mice readily walk into these bottles and are returned to their clean cage. This process reduced total time for grasshopper mouse cage changing and reduced stress for both mice and animal care staff.

P330 Repurposing Rabbit Caging as Enhanced Environmental Enrichment for Rats

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Enrichment is vital in animal laboratory programs to improve animal welfare and reduce stress. High-stress environments can compromise experimental data, making it important to provide meaningful and beneficial enrichment to the animals while not compromising research aims. This is also true for breeding animals or those not yet on an experimental protocol. To provide enhanced enrichment to breeding rats, we set up rabbit primary housing cages as "play cages." Animals are in the play cages 30m a day for up to 5d a week and are continuously monitored to ensure no fighting or stress-related behaviors are exhibited. Each cage is set up with manipulanda including destructible enrichment (e.g., paper towels), crinkle nest, and toys (e.g., balls). Perforations between cages allow for singly housed animals to socialize with each other if desired. The home cage is placed inside this play cage, which provides a choice to interact with the new environment or stay in the home cage if desired. The home cage also acts as a retreat for animals if needed. Biosecurity is managed by every-other-week sanitization in a cage washer and restricting the play cage to a rat room during its use. Natural behaviors such as jumping, playing, running, digging, standing, climbing, and exploring are observed, suggesting that the rats are interacting positively and with curiosity to the enrichment. Average litter sizes and survival rates do not differ or are higher with this enrichment when compared with breeding rats of the same line at another institution who received a standard enrichment environment. This provides evidence that breeding is not negatively impacted when rabbit caging is used as an enhancement to breeding rat enrichment. We conclude, based on behavioral observation and breeding data, that rabbit cages can be successfully repurposed to provide enhanced environmental enrichment to breeding rats in our program.

P331 Scratching the Surface of Swine Enrichment

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Environmental enrichment programs are intended to provide for species-typical behaviors and decrease animal stress; reduction of stress may lead to decreased variability and better scientific results. Any enrichment item should be safe for the animal, easy to sanitize, easily incorporated into an existing program, and provide novelty to the animals. As naturally social animals, swine generally enjoy manipulanda, food, treats, and seek to interact with human caretakers. Enrichment programs should be reviewed and updated as new or advanced devices or methods are indicated. This poster will describe a novel and cost-effective device we incorporated into our own swine enrichment program that we now use daily. Toilet brushes were originally designed for cleaning purposes; however, these brushes can also be used as a scratching implement between the caretaker and swine. They are easily sanitized, readily available, and inexpensive. We have introduced this item as a new way to provide socialization between our swine and technicians. The thin, soft bristles of the brush are durable yet not too abrasive to the integument of the animal. We found this device brings much joy to the animals regardless of swine species, along with the caretakers providing the interaction. At our facility, we house two different species of swine, and both responded positively to these enrichment interactions. Also, while it is not its main purpose, it does gently exfoliate the animal's skin in dry areas. Positive responses from the animals also influence the ease of handling/restraint. We found that using the brushes as back and flank

scratchers elicited consistent positive interactions time and time again. The device is used in locations of tougher integument. It is not to be used in sensitive areas where the skin is naturally thinner or mucous membranes (e.g., eyes, ears, snout, abdomen, hoof pads, and genitalia). We take pride in the improved new methods of enriching our animals. Our enrichment program plays a vital role in the welfare of our animals daily. At our state-of-the-art facilities, which include both biomedical research and swine breeding sites, we strive to provide the best possible care and attention to our enrichment program for the best possible quality of our animals' time spent with us.

P332 Exploring Enrichment Options for CD-1 Mice

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Abstract-Environmental enrichment is a key factor to the quality of life for our animals and essential for any animal work. Food grinding behavior in rodents may be stereotypic or compulsive, stemming from lack of stimulation, or may have a genetic component. This is prevalent in the CD-1 mouse strain. We have been working on decreasing the amount of shredded food pellets because excessive shredding causes a buildup of feed, bedding, and fecal material that can reduce movement in the cage, as well as block access to fresh water sources. We designed a study to look at how additional enrichment options could prevent CD-1 mice from shredding food. To help stimulate the mice, we tried three different types of enrichment. Our study design included: manzanita sticks, wooden chew blocks, and a running wheel, as well as a control group with just our normal diamond twist enrichment. We evaluated the mice over 16 days. We took food weights to measure the loss of feed by consumption and shredding per day. We also used a scoring system for the enrichment to gauge each cage's activity per day. It ranged from a scale of 0-5 (0= no use to 5 = Highly used) We took the average score per group and plotted this in our end point data. We concluded that any one of the extra enrichment items reduced feed shredding, with the wheel and manzanita sticks being the best option. In consultation with study leads, we chose the manzanita sticks for our enrichment option as they met our storage and cost needs.

P333 Trash Panda's Unite! One Person's Trash is an Animal's Treasure

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Enrichment is an essential part of any animal care program because it promotes natural behaviors in animals, but the cost to maintain can be an obstacle. Single-use, destructible enrichment is an ideal option to promote these behaviors, and we wanted a cost-effective way to provide this enrichment. Our Trash Panda committee was born. We gathered paper-based materials from our facilities and from staff donations of recycled items such as glove boxes, packaging supplies, egg cartons, and feed/bedding bags. We have never had a shortage of donated recycled supplies, as staff have always been very eager to donate items. With these donations, we craft various items that we give to some of our animals. All the items are sterilized by autoclaving and made safe for animal use by removing tape/staples and stickers. In organizing this program, we realized that this was a great way to help relieve compassion fatigue for our staff by enriching them while crafting for our animals. We schedule regular Trash Panda Craft Parties for our staff to gather, socialize, and create. These events are not restricted to just our animal husbandry staff but are open to all groups within our department and we consistently have participation from each group. Our staff are very creative and enjoy watching the animals interact with the items and find it is more meaningful that they made the enrichment. Staff feedback showed that the "downtime" at work to connect with each other while creating items to benefit our animals helped alleviate their compassion fatigue. The enrichment has given our animals opportunities with novel items to promote their species-specific behaviors. We give these new items to approved species, including rats, rabbits, ferrets, cats, hamsters, sheep, deer, and muntjacs. The animals engage with the enrichment by foraging, nesting, shredding, and scent marking. Our behavioral husbandry manager feels this has vastly improved our enrichment program. This committee has excited our employees and encouraged them to think of new ways to enrich our animals, sparking the expansion of this varied enrichment to include more animals under our care with approval from different labs. Overall, this program is a fun, low-cost way to engage staff by providing new enrichment for our animals. Giving our staff a creative outlet alleviates compassion fatigue while providing our animals with novel enrichment. Every facility has the tools to be a Trash Panda and we encourage you to implement this into your animal husbandry program.

P334 Genetically Engineered New Zealand White Rabbit Kit Development: Kindling to Weaning

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Genetically Engineered New Zealand White (GENZW) rabbits are increasing in demand as a translational model for biomedical research and drug development. However, the establishment and maintenance of GENZW rabbit lines can be challenging. Confirmation of proper neonatal development from kindling to weaning is crucial to ensure both clinical welfare and research success. Routine assessment of parameters that include kit physical appearance, body weight, developmental stage, and behavior are critical to the generation of a successful GENZW rabbit breeding colony. Data and photographs were collected on days 0-14, 20, 30, and 42 from 20 distinct GENZW litters containing 4-8 kits. The photographs create a visual reference of the standard kit physical appearance. Daily body weight measures are graphed over time and in relation to the developmental stage. Behavioral changes were tracked through structured visual observations. Kit physical appearance was temporally comparable across all litters. Individual body weight significantly varied in relation to total litter size. Therefore, only data from litters with 4-8 kits was utilized. Behaviorally, a significant decrease in nursing was noted on day 14, in conjunction with a stark increase in consumption of dry chow, hay, and water. By day 21, kit diets were nearly completely modified to standard food products, with negligible nursing observed. Kit fecal production transitioned from soft, unformed material to firm fecal pellets by approximately day 18. Photographs display normalized pellet size changes from days 18, 30, and 42. Comparable behavioral observations of GENZW kits were observed across varying genotypes. The visual guides generated from this project work to standardize the physical and developmental stages of GENZW kits from kindling to weaning. The application and use of these informational visual guides will serve as an asset for new facilities seeking to establish and manage a colony of GENZW rabbits.

P335 Cross-Fostering to Mitigate Poor Maternal Care from Mutant Mouse Dams, and its Effect on Strain Phenotypes

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Laboratory mice have long been a model organism for studying development, physiology, and disease. Genetically modified (GM) mouse strains modeling human disease have now become an important part of preclinical research. Modifications are meant to

mimic disease pathologies or phenotypes. Consequently, however, they can also impact breeding performance and maternal care. The resulting low number of GM pups reaching weaning age can compromise the outcome of drug efficacy studies carried out during the early days after birth. Here, we investigate the use of cross-fostering as a means of increasing the survival of neonates from a GM strain with poor maternal care. Additionally, we will study whether cross-fostering changes the established relied-upon phenotype of the GM progeny. The impact of fostering on the phenotype progression was studied in pups of B6.129P2(C)-Mecp2<tm1.1Bird>/J (JAX stock# 003890), a knock-out (KO) model for human Rett syndrome, whose husbandry is made challenging by poor maternal care from the natural dams. Mecp2 is X-linked, and hemizygous male mice develop severe Rett syndrome-like phenotypes in the first nine weeks of life. FVB/NJ (JAX Stock# 001800), a common inbred laboratory strain, was used to cross-foster Mecp2 KO neonates. FVB/NJ dams tend to provide excellent maternal care. Females from both strains were mated with same-strain males. Within 24 hours after birth, the Mecp2 KO neonates were fostered to either a heterozygous Mecp2 KO or FVB dam. Progression of the Rett syndrome-like phenotypes was documented for eight weeks in Mecp2 hemizygous male cross-fostered to FVB. These phenotypes were compared to the ones of Mecp2 hemizygous males raised by their natural, heterozygous dams like in previous benchmark natural histories. We found cross-fostering Mecp2 KO neonates to FVB dams does increase survival through weaning age by over 20 percent. The increase in Mecp2 pup survival comes without any loss of key disease phenotypes up to weaning age.

P336 Successful Hand Rearing of Orphaned African Green Newborns to Promote Health and Social Skills

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At the National Institute of Health Animal Center (NIHAC), first-time mothers within our African Green (*Chlorocebus pygerythrus*) breeding colony have a high frequency of newborn rejection, requiring the hand-rearing of infants by research staff. We have found the reasons for rejection in primiparous African greens can be caused by either inexperience or traumatic birth. To address inexperience, efforts are made to place potential new mothers in the room with visual access to a more experienced female with a baby for observational learning. In the case of traumatic birth and rejection, attempts are made to re-introduce the infant to the dam, if unsuccessful, the NIAID research staff initiates the orphan AGM protocol. A 24-hour feeding schedule is implemented, with feedings initially every two hours for the first two weeks. Observations are taken at each feeding, including weight, temperature, volume of formula consumed, and stool/urine production to monitor overall health. Timing between feedings is gradually extended, and soft food in the form of rice cereal is offered at three weeks to encourage independent feeding. Supportive care while hand-raising includes heat support viaan incubator, blankets, and appropriate toys within the enclosure, visual stimulation with TV during the day, and sound machines at night. A surrogate mom in the form of a stuffed plushie is used during feedings as a perch for the infant to reduce human contact. Overall, long-term analysis of infants naturally reared versus hand-raised shows little discrepancy in weight, with hand-reared infants returned to the colony in the care of a foster mother within six weeks. The combination of hand raising and fostering at NIHAC has shown a reduction in significant long-term behavioral abnormalities in infants. Over the last decade, five African Green infants have been hand-reared and successfully integrated into the colony by NIAID using the above methods.

P337 Don't Cry Over Spilled Milk

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Neonatal piglets are commonly utilized in biomedical research models for developmental and perinatal studies. Historically, working with piglets necessitated the procurement of the sow to provide nutrition for the piglets. Housing, husbandry, and medical care for the sow had to be considered, and at study completion, a disposition method had to be identified. In our experience, a significant amount of time and resources were spent caring for the sow. To minimize the resources needed for the sow and to reduce the number of animals used, we sought to identify an alternative method for piglet care that did not require a sow. Early attempts utilized commercially available gravity waterers filled with milk replacers. This method was effective in providing milk but resulted in significant waste due to spillage soiling of the enclosure and required frequent bathing of piglets, in turn leading to dermatitis and dry skin. Each gravity waterer could only feed two piglets simultaneously, requiring multiple feeders for larger studies. To address these concerns, we created an expandable gravity feeder using supplies purchased from a hardware store and lixits available in our facility. The unit holds up to five gallons of prepared milk replacer and can feed five piglets simultaneously. The total cost of the supplies was less than 70 USD, with the ability to add additional feeding locations for 1.75 USD each. In our experience, 4 – to 6-day-old piglets learned to use the feeder in less than 24 hours. There was minimal waste of the milk replacer; piglets and their enclosure remained cleaner and dryer, minimizing bathing needs. It took less than 15 minutes per feeding, reducing the amount of time required by staff to care for the piglets. The feeder can be sanitized by flushing the unit with a diluted bleach solution and rinsing it with fresh water. This method avoids the need to acquire a sow and improves all aspects of piglet husbandry, resulting in healthier pigs, minimal waste, and improved personnel time management by minimizing the need to bath and clean the piglets.

P338 Institutional Environmental Sustainability Goals: My Green Lab Initiative

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Environmental sustainability in the research field can be difficult. Our institution is committed to achieving net-zero greenhouse gas emissions by 2040. We have collaborated with a non-profit third-party sustainability agency, My Green Lab, to assist in reaching this goal. My Green Lab has five levels of certifications ranging from Bronze to Green. Initially, we scored below Bronze (45% sustainable actions) with 25% of sustainable actions in place. To improve our certification status, we began a weekly educational program for colleagues on current sustainability measures, as well as communicating newly developed initiatives. Proceeding with our new educational program and initiatives, the Comparative Medicine team obtained the second highest level certification of Platinum, with 70% of sustainable actions. This was done in a multitude of ways, including, but not limited to, proper disposal of aerosolized canisters and batteries, load testing equipment to assess electricity expenditure, separate bins for waste, recycling, and composting, and daily monitoring for water leaks throughout the facility. Just through the waste sorting efforts of our cafeteria staff and colleagues, over 40 tons of compostable material was collected and sent to an anaerobic digester to produce clean energy in 2023. After implementing these and other operations in our facility, we have seen significant improvements in sustainability.

P339 Who Let the Mites In?

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Beginning in June of 2021, our institution noticed an increase in feral rats (Rattus norvegicus) due to the decreased activity caused by Covid. Rats captured inside and around the vivarium tested

positive for the zoonotic mite, Ornithonyssus bacoti, known as the Tropical Rat Mite. Within three months, rats were seen frequently inside the cagewash area. By September, staff reported seeing bugs on the research mice (Mus Musculus) in our largest vivarium. Sentinel results confirmed the presence of O. bacoti in nine mouse rooms. A plan to eliminate the mites was needed. Four objectives were implemented to eliminate the pests from our research colonies and vivarium. First, stop the spread of mites to other buildings while continuing operations in all vivaria. Next, eliminate the O. bacoti from the research animals, and the environment, and facilitate research among the affected investigators. Then, block the feral rats from accessing the vivarium. And finally, implement a comprehensive pest control program. Permethrin & Ivermectin were chosen to treat the mice & environment. Electronic traps with digital notifications were deployed. A lack of preventative maintenance mandated the removal of the tunnel washer to stop the rats' free access to water. Dirty caging was absorbed by our remaining three tunnel washers while handling contaminated caging separately. Spot changing was executed in all areas. A robust pest control strategy was implemented utilizing electronic traps, monthly treatment of all cage washes & vivaria; and immediate intervention when pests were seen. In total, 4299 cages of mice were infested. It took ten months to eliminate the mite. It took two years to replace the cage wash & return to regular cage changing. Failure to perform preventative maintenance decreased vigilant upkeep of building access to vermin, and a lack of a well-defined pest control program led to the mites getting in.

P340 Evaluating a Reduction in Treatment Duration of Ivermectin Diet for Fur Mite *(Radfordia affinis)* Eradication in Mice

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Murine fur mites are commonly excluded in modern research animal programs, yet infestations continue to persist due to challenges in detection and control. Because all diagnostic methods and treatment options have limitations, programs must make many operational decisions when trying to eradicate these ectoparasites. The primary aim of this study was to assess various durations of treatment time with an ivermectin-compounded diet in eliminating *Radfordia affinis* in mice as determined by PCR testing and pelt examination. A shorter treatment duration would be highly advantageous as compared with the current regimen of 8 wk as it would minimize cost and time for animal management programs, impediments to research, and ivermectin drug effects on infested animals. Five experimental groups of *R. affinis*-positive mice received dietary ivermectin for 0, 2, 4, 6, or 8 wk. A fur mite-negative, naïve mouse was added to each group every 8 wk to perpetuate the infestation and amplify any remaining populations of fur mites. At 16 wk after the respective treatment end, PCR testing was performed for all treated groups in conjunction with the positive control group (no treatment). Visual examination of pelts for mites and eggs via direct microscopy was also performed at each time point. All treated mice were free of *R. affinis* at 16 wk after the end of treatment as confirmed by both PCR testing and pelt examination. These findings indicate that a dietary ivermectin treatment duration of as little as 2 wk is effective in eliminating *R. affinis*, making successful eradication initiatives more achievable.

P341 Management of a Booklice Infestation in Rack Mounted Exhaust Blowers

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During rack change out, a large number of 1-2mm insects were found inside an exhaust blower mounted on a ventilated mouse rack. Insects were grossly identified as *Psocoptera sp.* (booklice). Booklice were confined to the space between the exhaust hose-to-blower connection and the exhaust pre-filter inside the blower housing. Checks of all rack blowers in the facility revealed that 9/38 exhaust blowers were affected. No booklice were found inside animal cages, rack plenums, animal transfer stations, feed bins, or in any support spaces including feed storage or break rooms within the main vivarium. A lab space with direct access to a housing room on another floor of the facility was also found to be affected. Initial cleanup efforts included replacing affected blowers, hoses, exhaust pre-filters and racks, complete cage changes, increased sweeping and mopping frequency, and increased PPE requirements. Exhaust blower hoses and pre-filters were changed weekly. Sticky insect traps were placed inside the exhaust blower housing and were monitored weekly. For two months after implementation, no further booklice were found. Over the following months, booklice were found on traps in three exhaust blowers. While efforts remain ongoing to eliminate the booklice infestation, active screening using traps and exhaust hose and pre-filter change outs have reduced the booklice infestation to 3/38 blowers (a reduction of 67%). Rack mounted exhaust blowers appear to be a viable space for booklice to populate, and staff should be aware that this is a possible location for infestation. Utilizing sticky insect traps inside the exhaust blower housing appears to be an effective method to detect booklice, allowing for rapid surveillance and response. Effects of booklice infestation on exhaust blowers have not been reported. However, infestation of exhaust blowers may serve as a potential source of contamination for other equipment or spaces inside the vivarium.

P342 Zipper Top Bags are Used for Bedding Container in Contact Media Testing

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Environmental Health Monitoring (EHM) has been greatly expanded by using contact media exposed to soiled bedding from rack systems with filtration at the individual cage level. This approach has increased the number of rodent health surveillance programs that can now avoid reliance on live sentinels. One of the challenges we faced with this method was storing the soiled bedding collection containers in our animal holding rooms (some with up to 12 racks) because of limited space. Storing this many soiled bedding collection containers would require roughly the same floor space as one animal holding rack. To minimize soiled bedding container storage space, we tested the novel use of labeled zipper top plastic bags for each rack for dirty bedding collection. The prelabeled bags were stored in a single container, requiring less storage space and less biosafety cabinet (BSC) space when sampling the dirty bedding. The soiled bedding samples are collected in zipper top bags every two weeks by husbandry staff, then dropped off at a central collection site in the facility and picked up daily to prevent mold contamination. The contact media is then exposed to the dirty bedding bag in a BSC in a lab outside of the animal rooms, thus using less Personal Protective Equipment and freeing up the BSC in the animal holding room. We compared PCR results of zipper top bags and container soiled bedding samples. Once we confirmed that the results were comparable, we felt confident using the zipper top bags for all racks throughout our facilities (N=209) for our quarterly rodent health surveillance program. When compared to the previous year's test results the contact media in zipper top soiled bedding bags showed the same if not better detection of the persistent infectious agents known to be present. Of the tests that were included in the panels for both years, the filter testing showed improved results in detecting *Rodentibacter*, *Staphylococcus aureus*, *Tritrichomonas,* and *Klebsiella pneumoniae*. These results demonstrate that zipper top bags are a suitable alternative to bedding collection containers.

P343 Where the Fantastic Pathogens are: A New Approach to Health Monitoring at a Portuguese Mouse Facility

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A well-defined and active Health Monitoring Program (HMP) is an essential requisite for any functional animal facility to maintain and ensure its health status. There are different methods of Health Monitoring, with the most common being Soiled Bedding Sentinels (SBS). However, new alternative methods that allow for better pathogen detection but also allow for the implementation of the 3Rs Principle, have been developed in recent years. The main new method that stands out is the Environmental Health Monitoring (EHM) method. EHM can be further divided into two different approaches: the Sentinel Free Soiled Bedding (SFSB) and Exhaust Air Dust (EAD). While the former is a replication of SBS, with the animals being replaced by filter paper or sterile flocked swabs, the latter is based on the application of specific filters into the ventilator exits in the ventirack. EAD can only be used with ventiracks, while SFSB is of more general use. A Portuguese Mouse Facility has had a well-defined HMP from the beginning: until 2012, using the SBS method that sentinel animals were sent to an external company for health analysis (SBS_A method). In the same year, the program underwent an update, with the adoption of the SBS_S method - sent animal samples for analysis instead of sent animals. The program was then reviewed at the end of 2022: in parallel with the SBS_S method, alternative approaches of EAD and SFSB were tested. The new approaches have shown significant improvement in pathogen detection compared to the traditional SBS approach, which can be justified by the increased sensitivity of the current PCR techniques. Examples of this increased performance are the cases of Staphylococcus aureus – detected for the first time by the EAD method - and Helicobacter spp, with increased taxonomical resolution using the new approaches. Quantitatively, the implementation of EAD and SFSB methods resulted in a 12% increase in pathogen detection rates compared to the SBS method. In September 2023, we started the exclusive implementation of EHM, abandoning the use of animals as sentinels. This transition has led to a reduction in the number of animals used for surveillance by more than 230 mice annually. EHM is undoubtedly an improvement with considerable advantages, not only in the maintenance and/or production of animals necessary for sentinel purposes but also in improved pathogen detection.

P344 Improved Detection of Murine Pathogens Using Sentinel-Free Media Compared to Live Animal Sampling

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Routine health monitoring of rodent colonies has traditionally used live animal sentinels, with the associated expenditure of labor, supplies, and animals. In the spirit of the 3Rs (reduction, replacement, refinement), sentinel-free approaches are becoming more common. Individually ventilated cages (IVC) that exhaust at the cage level cannot use exhaust duct sampling but are ideal for sampling of in-cage media. We hypothesized that media exposed to pooled soiled bedding within IVC would be as effective as live animals in detecting several enzootic organisms of mice, *Mus musculus,* in our facility. Commercially available media were placed in IVC and exposed to pooled soiled bedding from all cages on a rack side at biweekly cage changes during two consecutive three-month periods. Feces, pelt swabs, and oral swabs from similarly exposed sentinels, along with 8-10 randomly sampled colony animals, were obtained from the same rack side over the first of these three-month periods. Media and live animal samples were submitted to a commercial laboratory for testing by polymerase chain reaction (PCR) for a standard list of murine pathogens. Two organisms (*Murine Norovirus and Staph xylosus*) were detected by PCR at similar rates in sentinel-free and live animal samples. Five organisms (*Proteus mirabilis, Rodentibacter heylii, Staphylococcus aureus, Klebsiella oxytoca, Klebsiella pneumonia*) were detected in sentinel-free samples but not in live animal samples. *Demodex musculi*, *Entamoeba, Helicobacter, Proteus mirabilis and Rodentibacter heylii*, were detected at significantly higher rates in sentinel-free samples compared to live animal samples. The PCR detection rates and comparisons of sentinel-free derived samples were similar in two consecutive quarters. Exposure of media to pooled soiled bedding results in PCR detection of several rodent pathogens at equal or greater rates than live animal sampling. The use of in-cage media represents a promising sentinel-free approach to routine health monitoring in rodent colonies.

P345 3D Printing to Create Inexpensive Quality Control Tools

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Quality control tools in the animal care industry are challenging to find as most processes are unique and specific to each animal care program and/or facility. The use of 3D printing technology in the animal care industry has led to significant benefits. By creating custom tools and devices, facilities can enhance operational quality and improve animal welfare. Unlike traditional machining, 3D printing allows for complex designs that conventional manufacturing cannot achieve, and 3D printers aren't as expensive as one might think, a good quality 3D printer costs less than $5000. You can also solicit user input and feedback and quickly make adjustments on the fly. For instance, we developed a 3D-printed bottle capper to help prevent leaks and biosecurity risks. Its intricate design leverages the capabilities of 3D printing, allowing for custom shapes and efficient production. After some user feedback, we adjusted the size of the handle to better fit in a hand. This modification ensures ergonomic usability and enhances overall functionality. Additionally, we created a bedding depth measurement tool to help improve accuracy and consistency, ensuring that depths align with our internal specifications. This approach minimizes variability and enhances overall animal welfare. We also designed an ergonomic bottle cap removal tool, which prevents damage to bottles and seals. Our Environmental Health & Safety Services Team is excited to have a more ergonomically sound tool. The cost to 3D print these tools, not including cost of the printer, is $0.38-$1.00 per tool. Once the protypes were optimized and approved by users, we created final designs made of stainless steel and more durable materials to enhance the longevity, ensuring they withstand the demands of animal care facilities. Overall, 3D printing promotes safety, innovation, quality control, and cost-effective solutions in animal care facilities.

P346 Keeping Research Clean - Development of an ATP-Based QC Program for Hand-Washed Items

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Ensuring the integrity of animal research involves several key practices, one of which is stringent sanitation protocols. Many devices used in research cannot be sanitized in the traditional Cage Wash Facility due to electronics, irregular shapes, and temperature-sensitive components and must be hand-washed. At our facility, we created a quality control program to show the effectiveness of our sanitation process with those devices that come in direct contact with mice. Our Process Quality Control Department (PQC) partnered with our Comparative Medicine & Quality Department (CMQ) to collect data pre- and post-sanitation using bacterial culture methods (CFU) and adenosine triphosphate (ATP) swabbing technology. We conducted three live challenge rounds per material type, with a correlation between ATP relative light units (RLUs) and colony-forming units (CFUs). The sampling plan was based on ASQ-ANSI Z1.4, general inspection level 1, with an acceptable quality level

(AQL) of 2.5%. We observed very high RLU and CFU levels pre-sanitation and low to zero post-sanitation. Using the ANSI Z1.4 sampling plan to determine our sample size, we swab every quarter. Due to the aseptic nature of 'swabbing,' there was training around the use of the ATP and CFU swabs, which helped engage the staff. If an RLU limit is above our cut-off limit, the item is re-swabbed. If it comes back above our cut-off limit a second time (it has not), the item would be re-washed and re-swabbed. If it failed again, we would meet as a group to go over the process and how it changed since we validated it. The PQC and CMQ teams review the data each quarter and help with suggestions on improvements for sanitization. While the ATP swabbing methodology is not new to the field, we cannot stress enough the positive effects we have seen since using it. It provides an assessment of the sanitation process on the spot, in seconds, and is easy to use. It helped create engagement with our personnel, which has improved sanitation and enthusiasm. Remember, a motivated and engaged team contributes to a cleaner and safer research environment.

P347 Care of Animals when Using Cerebral Open Flow Microperfusion to Study Neurodegenerative and Neurological Diseases

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Cerebral implants are crucial in studying neurodegenerative diseases, brain cancer, and other neurological disorders. However, it is inevitable that implants cause local damage to brain tissue and disrupt the blood-brain barrier (BBB), so pharmacodynamics and pharmacokinetic results are skewed by the implantation trauma and the ensuing leakage. Cerebral Open Flow Microperfusion (cOFM) is a long-term sampling technology that allows tissue trauma healing and BBB re-establishment after surgery and thus makes it possible for researchers to collect samples from the brain interstitial fluid with an intact BBB. cOFM can be used in a range of species but this poster will discuss the application in small animals such as mice and rats. To care for and maintain health of animals with long-term cerebral implants, special husbandry and handling considerations are necessary. This poster will explore changes like additional animal care steps to maintain probe functionality between multiple study days by using the healing dummy, specialized observation and monitoring for infection at the skull implant site, modified handling for activities like cage changes to prevent undue stress on the implant, modifications to standard animal housing and enrichment to provide more vertical space for the implant, and modified husbandry to avoid disturbance of implant and continuous sampling. Implementing these animal care modifications makes it possible to study neurological disease progression and treatment over longer periods of time for up to 40 days, as well as providing the potential to reuse animals for multiple studies.

P348 Novel Method of Corticosterone Collection to Assess Stress at Different Population Densities in *Xenopus laevis*

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Xenopus laevis, a fully aquatic amphibian species, has been extensively used in research. Yet, despite nearly a century of X. laevis use in research, a gap remains in our understanding of this species response to stress resulting from population density. The laboratory animal field currently has no standard population density recommendations supported by objective, evidence-based research. To that end, we developed an enzyme immunoassay to validate non-invasive corticosterone measurements from skin secretions (mucus) in Xenopus. Using this method, we assessed stress in variable population densities in X. laevis. We hypothesized that stress, as reflected in elevated corticosterone levels, would be the greatest under increased population density. We compared stress levels resulting from three population densities. In addition to corticosterone levels, weights and behaviors were assessed. We obtained data from 76 frogs which were subdivided into tanks of three densities of 6, 12, and 20 frogs each. Each frog was subjected to each housing density over three repetitions. For each frog, we recorded seven corticosterone levels in mucus at different time points. Our analysis revealed no statistically significant differences among the three tank density groups (Kruskal-Wallis test, $p = 0.68$) or between any pairs of tank densities (6 vs 12 frogs/tank: $p = 0.41$, 6 vs 20 frogs/tank: $p = 0.9$, 12 vs 20 frogs/tank: $p = 0.51$). The remaining frogs showed no significant corticosterone level differences across the different tank conditions, and there was no consistent pattern in their corticosterone level changes under different stress levels. Lastly, we performed a generalized estimating equation analysis to compare the corticosterone levels of the frogs in the three conditions, adjusting for frog-specific variabilities. There were no significant differences between the corticosterone levels of frogs from tanks with 20 frogs compared to 12 frogs ($p = 0.807$) or from tanks with 6 frogs compared to 12 frogs ($p = 0.305$). Thus, there were no differences in stress at the various population densities. We concluded that frogs may be housed at any of these densities with no impact on research data or egg production.

P349 Promoting Positive Rodent Behavior

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The animal research industry has had many advancements in animal handling since its inception in the 1940s. Handling laboratory animals during technical procedures can be a source of stress that may impact the reliability of study results. Traditional mouse handling methods are considered aversive, mimicking the experience of being captured by a predator. We are introducing refined methods of handling mice by approaching them at their level rather than from above. Some of these non-aversive mouse handling methods include letting mice walk into a tunnel or onto a technician's hands for removal from their home cage. These new methods encourage species-specific behavior that promotes positive interactions between technicians and animals. The goal is to give the mouse the opportunity to be handled with the least amount of stress possible. Advancements in animal handling can also be used to promote behavior that is conducive to what we want to see in rats. Rats show reduced stress levels when they form close social bonds with humans. This can be achieved through rat tickling which is a positive reinforcement method that is used to resemble aspects of how rats play with each other. Our goal is to promote positive interactions between rodents and technicians. By minimizing stress during handling, data can be collected more easily, avoiding the complications of handling a fearful animal and ensuring a smoother experience for both the animal and the technician.

P350 Reducing Adeno-Associated Virus Seroconversion in Non-Human Primate Colonies

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Adeno-associated viruses (AAVs), a group of single-stranded deoxyribonucleic acid (DNA) viruses, are often employed as

vectors for gene therapies to deliver specific DNA sequences to target effector cells. AAVs are endemic in both human and animal populations, but populations can remain seronegative when proper conditions are met. However, humans and animals can seroconvert, or develop neutralizing antibodies (NAbs) to various AAV serotypes, which hinder the vector's ability to transduce genetic material into the host cell. Prevention of non-human primate (NHP) seroconversion is key to efficient gene transfer from the viral vectors. Prior to study assignment, we screen NHPs for NAbs of different AAV serotypes. Once the NHP serostatus is known, efforts to protect these statuses are taken. We have developed strict engineering controls which include heightened cleaning procedures and extensive personal protective equipment (PPE) in addition to mediated traffic flows to reduce potential human to NHP transmissions. Additionally, our procedures take measures to minimize housing disruptions and ease stress levels to reduce conspecific viral transmission. Through these engineering controls, processes, and efforts to reduce stress levels, we have observed reduced seroconversion. Overall, these procedural processes have enhanced our ability to reduce the rate that the NHPs are developing NAbs to the AAV vectors. Thus, being able to protect the serostatus of animals creates a larger pool of animal models available for the utility of AAV products to advance gene therapy. Our animal care, husbandry, cleaning, etc. that have been implemented and discussed in the abstract are specific to our institution's SOPs and procedures.

P351 The Fight Against Laboratory Hoarding

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A systematic and well-organized approach to vivarium cleanout was established in preparation for an AAALAC site visit. This was achieved by following a series of steps, starting with creating a comprehensive room list outlining all areas within the facility that was subject to inspection, including animal holding areas, supply rooms, and procedure rooms. A SharePoint list was created to streamline progress tracking and task assignment. This centralized platform enabled the allocation of specific rooms to individuals or teams responsible for their preparation. A designated holding space for misplaced or unwanted items and equipment served as a temporary storage location until appropriate action could be taken (remove or repurpose). By standardizing holding room cabinets with consistent organization and labeling systems, we created efficiency for quick retrieval of items. To ensure compliance with internal organization requirements, a specialized inspection team was formed to sign off on completed rooms. Furthermore, to sustain order and cleanliness, monthly walkthroughs were scheduled to promptly address any issues, thus keeping the rooms clean, decluttered, and inspection-ready. By implementing this systematic approach to storage and standardization, our facility experienced a reduction in the number of laboratory supply orders, a decrease in the discovery of expired items, and an overall improvement in the efficient utilization of laboratory space.

P352 Using a Voice-Enabled Digital Assistant in a Gnotobiotic Mouse Vivarium Aids in Accurate and Efficient Data Recording under Sterile Conditions

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Working in an animal research facility requires detailed record-keeping that is both accurate and accessible. Those working in a gnotobiotic mouse vivarium face an added challenge when collecting animal data, as it must be recorded while working in a sterile hood or isolator. Current practice for data recording under gnotobiotic conditions requires either additional personnel to transcribe for the researchers in the sterile environment or extra personnel time to manually document after observations are made. Moreover, researchers must later transcribe all observations into digital files, thus requiring more personnel time and adding another opportunity where errors can occur. This collective process is time-consuming, inefficient for staff, and has the possibility of inaccuracies. To address this problem, a system that utilizes voice-to-input data in real-time to online spreadsheets was acquired. The system consisted of a low-code online designer as well as a mobile app for use when collecting voice-acquired data. The online designer was used to build step-by-step workflows based on our facility and individual study needs. After designing a custom workflow, we used the mobile app in our gnotobiotic mouse vivarium to acquire data. As animal staff and researchers worked, data was automatically recorded to a cloud-based storage system. A hands-free headset was used for ease of communication between the digital assistant and the researcher. Quality control prompts were also added to allow the digital assistant to speak back data to ensure accuracy was maintained in our sterile, hands-free environment. Workflows developed for husbandry practices and specific animal studies enabled our staff and researchers to collect data independently. Simultaneous digital transcription of data allowed real-time updating of online spreadsheets and increased efficiency in our facility. In addition to efficiency, this system has also shown cost savings to the facility. We have experienced a staff time saving of 10 hours per week, which is the equivalent of 5 times the return on investment. The use of a voice-enabled digital laboratory assistant eliminated the need for manual data entry, enabled real-time animal data updates, increased overall staff productivity, and provided cost savings in our gnotobiotic mouse vivarium.

P353 Real Change & Real Results: Improving Colleague Safety as part of our Culture of Care in a Nonhuman Primate Program

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As a part of a Culture of Care, colleague wellness includes not only compassion science, but also colleague safety. Comparative Medicine, therefore, set out to reinvigorate our culture of colleague safety within the department. With a sharp focus on addressing acute and ergonomic injuries related to nonhuman primate (NHP) handling, we reduced colleague injuries by greater than 75% in a three-year period while increasing our census and workload. A review of common root causes led us to focus on a change to our historical pole and collar method and habituation program, an investment in upgraded equipment, including custom restraint chair designs and accessories, a fully revamped NHP husbandry and handling training plan, and the incorporation of a behavior team. Animal care technicians were inventive throughout the transition. They designed new ways to improve safety directly with vendors, such as a hands-free method for oral gavage head restraint and custom puncture-resistant gloves. To mitigate the risks related to a high census of sexually mature males, we were able to work with scientific partners to change our historical colony management practices (e.g., we now utilize naïve NHPs for colleague training purposes prior to placing them on terminal GLP studies). In 2024, corporate safety personnel from other companies visited our program to benchmark and to learn how we used innovative solutions to achieve tangible outcomes.

P354 Incorporating Openness & Transparency from the Very Start: At the Time of Facility Design

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Openness and transparency within animal research institutions and with the public is a key component of a Culture of Care.

As part of a multi-year vivarium modernization project, our institution had the opportunity to renovate our nonhuman primate facility. As part of the Must Have user requirements, we emphasized the need for enhanced opportunities to share Who We Are and What We Do with non-animal users. Although institutions with outdoor housing may have successful approaches for visitors, as a fully indoor facility, we were determined to design a space for safe visitation for both the people and our animals. Comparative Medicine worked with our facilities group and design team on room locations, traffic patterns, and innovative equipment concepts to increase opportunities to support visitors without the need for personal protective equipment or medical testing. One example includes our Nonhuman Primate Immersive Experience, which has been open for over a year now with hundreds of visitors from within our site and from the public, including school students. The room was designed in a location where we were able to break directly into a nonhuman primate housing room from an office hallway. Although some footprint was lost from the housing room to create a large smart glass viewing space within the room sharing the same wall, floor, and lighting features without sharing the HVAC support, the outcome was immediately worth it. We will also review how we maintain the security of the space, acclimate the nonhuman primates to the viewing, and advertise the experience. Other design concepts that will be covered include large window doors in airlocks, windows into animal corridors with removable shading, and windows with smart glass technology in strategic locations. We will share our experience of moving from an institution with a rare opportunity to share our nonhuman primate work to one that prioritizes it with the goal of further promoting a Culture of Care.

P355 Strengthening the Workforce: Employing Workers with Unique Assets

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If your institution could employ someone who possesses excellent skills, has exceptional attendance, and increases the overall production of the team, would your institution hire them? Most employers in today's workforce struggle to obtain applicants with the aforementioned characteristics. There is a potentially untapped segment of our population that would welcome the opportunity to fill positions within our respective organizations. Our department currently employs three individuals with intellectual or developmental disabilities who possess assets that others in the institution may not. This program was started due to a shortage of husbandry staff. Our group identified different duties/jobs that were always falling behind such as laundry and cage wash. It was identified that these shortfalls were due to personnel being promoted, personnel leaving due to boredom of the job with limited advancement opportunities, or personnel prioritizing these tasks last. The incorporation of personnel with intellectual or developmental disabilities into these positions fills a gap in our workforce to accelerate its ability to provide excellent care to our animals and provide PIs with outstanding customer service. Although there are some challenges with hiring personnel with intellectual or developmental disabilities, such as behaving in ways that seem odd or do not follow social norms, experiencing sensory sensitivities, or becoming overwhelmed, their attributes outweigh these challenges. For example, this subset of personnel consistently reports to work promptly with minimal call-ins, they successfully perform tasks with a high level of ownership, and they are loyal with long-standing years of service within our organization. Our goals are to highlight this workforce opportunity, explain how our institution has benefited from employing this unique population, and assist with the implementation of hiring these incredibly talented applicants.

Laboratory Investigations Posters

P400 A New Mouse Model Lacking Murine Fc Gamma Receptors Designed for Improved Efficacy Evaluation of Antibody-Based Drugs

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Fc gamma receptors (FcγRs) on residual murine immune cells in humanized immune system (HIS) mice can interact with human IgG-based therapeutics and confound preclinical results. We assessed impact of murine FcγRs on anti-PD1 efficacy in HIS mice engrafted with lung adenocarcinoma cells treated with pembrolizumab or vehicle. We also present humanization results for the newly generated FcγR knockout NOG-EXL. Expt A. HIS NOG (huNOG) or HIS FcγR knockout NOG mice (FcResolv™ huNOG) were made using identical protocols with CD34+ cells (3 shared donors, A-C). Reconstitution was evaluated in naïve animals (n=5-6/strain/donor) and HCC827 cells ($10x10^6$ cells in 200 µl RMPI 1640 via subcutaneous injection) were inoculated in remaining animals (n=58 huNOG, n=79 FcResolv™ huNOG). Animals were randomized on day seven post-tumor implantation into 12 groups (n=9-14 x 3 donors x treatment/vehicle). Daily clinical observations and twice weekly body weights and tumor growth were measured and recorded. Mice received treatment (pembrolizumab 10 mg/kg IP, 10 ml/kg) or vehicle (0.9% NaCl IP, 10 ml/kg) from D7, dosed twice weekly for four weeks, and were then euthanized for FACS analysis collecting blood, spleen, and tumor samples. Expt B. HIS NOG-EXL (huNOG-EXL) or HIS FcγR knockout NOG-EXL (available as the FcResolv™ huNOG-EXL mouse) were created using identical protocols with CD34+ cells from three human donors (D-F) shared across both strains (total n=118/strain). Animals were evaluated for chimerism at 10 WPE. Humanization was equivalent between strains. Pembrolizumab treatment showed significant tumor growth inhibition in 1 donor in FcResolv huNOG, but not in donor-matched huNOG. Human TILs in pembrolizumab-treated mice were significantly different between the strains for all donors, with more CD8+ T cells and fewer TAMs in FcResolv huNOG compared to vehicle-treated mice, and no significant differences in huNOG. Murine TIL analysis showed differences in murine macrophage populations between strains. Anti-PD1-treated FcResolv huNOG mice show expected pharmacodynamic changes and donor-dependent efficacy, whereas pembrolizumab-treated huNOG mice showed neither, demonstrating the impact of murine FcγRs on antibody IgG-based therapeutics.

P401 Development of a Cecal Ligation and Puncture Induced Acute Lung Injury and Acute Respiratory Distress Syndrome Mice Model

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Acute Lung Injury (ALI) and its more severe form, Acute Respiratory Distress Syndrome (ARDS), occur when an event, such as pneumonia, sepsis, inhalation injuries, or transfusion reactions, triggers a chain reaction of inflammation in the lungs. This cascade involves increased permeability, inflammation, and surfactant dysfunction. Commonly used ALI/ARDS mice models include sterile injury (LPS) models and infectious (bacteria) models. The LPS model offers control and standardization but lacks the complex immune response and organ dysfunction seen in ALI/ARDS. On the other hand, the bacteria model, while more clinically relevant, exhibits greater variability due to differences in bacterial virulence and mouse susceptibility, and logistical challenges around using live cultured

bacteria. The Cecal Ligation and Puncture (CLP) model is the gold standard rodent sepsis model due to its clinical relevance. It involves ligating and puncturing the cecum, leading to a polymicrobial infection in the abdomen that generates a strong immune response and multiple organ dysfunction, including inflammatory lung injury. While relatively simple to perform, CLP can be challenging to reproduce and vary in severity. We established a mild severity CLP model (23G double-puncture; no antibiotics; 2–3-month-old male C57BL/6NJ mice housed in static caging) to induce ALI/ARDS. The lung perfusion procedure was optimized by pumping PBS into the inferior vena cava and exiting from the abdominal aorta to minimize Evans blue dye contamination during the harvest of lung samples. Python imputation and feature engineering techniques were utilized to establish equivalent timepoints and identify key characteristics within the datasets. Throughout the seven-day study, we closely monitored several datasets to determine the humane endpoint. This included tracking body temperature and activity using UID RFID System, as well as assessing Murine Sepsis Scores. The survival rate of the mice subjected to our CLP model was approximately 75%, and the mice experienced decreased body weight in the days following CLP. Endpoint samples were harvested on day seven post CLP. Significant changes in biomarkers were observed in the mice subjected to our CLP model. These changes included elevated levels of plasma IL-1β and IL-18, increased pulmonary edema, lung permeability, neutrophil infiltration (assessed by lung MPO activity and expression), as well as reduced expression of VE-cadherin (correlated with increased degradation of VE-cadherin in the lung). Overall, the successful establishment of a CLP-induced ALI/ARDS model provides a valuable foundation for future research.

P402 Establishment of Single Cell-Derived Murine Pleomorphic Rhabdomyosarcoma Model System

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Human pleomorphic rhabdomyosarcoma (PRMS) is a rare tumor, predominantly arising in adult skeletal musculature and usually associated with a poor prognosis. The urgency of developing an animal model system for this rare tumor cannot be overstated, making my research particularly relevant and important. 1) My study aimed to investigate the potential involvement of ras and p53 in tumorigenesis. To do this, I meticulously generated an oncogenic K-ras knock-in mouse. This involved a detailed process of introducing a 5′-CAG promoter–LoxP–Stop–LoxP-K-rasG12V-IRES–LacZ–pA–Neo-3′ into the first intron of ryanodine receptor type 2 (Ryr2) by homologous recombination using RW4 ES cells. The KI-mouse could conditionally express oncogenic K-ras in adult skeletal muscles by the Cre/LoxP system on a background of p53 alteration. This led to the generation of tumors in the tissues, with incidences of 100% (11/11) at ten weeks and 40% (8/20) at 15 weeks, in p53−/− and p53−/+ backgrounds, respectively. The tumor histology was PRMS with characteristic bizarre giant cells, positive for desmin and α-sarcomeric actin and exhibiting a remarkable increase in total and phosphorylated extracellular signal-regulated protein kinase (ERK)1 and ERK2. Loss of the wild-type p53 was detected in K-rasG12V-expressed tumors of p53−/+ mice. Early lesions three weeks after tumor induction consisted of proliferating populations of myogenic progenitors, including stem cells positive for ScaI antigen, immature cells positive for desmin, and neural cell adhesion molecule-positive myotubes. 2) Next, I backcrossed the KI mouse to C57BL/6J and established the strain (N10+) and established single cell-derived murine cell lines, designated as RMS310 and RMSg2, by limiting the dilution of cells from a lung metastatic tumor colony. They were positive for various cancer stem cells and activated skeletal muscle-resident stem/progenitor cell marker genes. All cell lines stably grew in vitro and recapitulated the histological characteristics of human PRMS in vivo. All subclones of the RMSg2 cells, by the limiting dilution in vitro, could seed PRMS subcutaneously, and as few as 500 RMSg2 cells were sufficient to form tumors, indicating that the cell line had the properties of cancer stem cells. Overall, this model system will help develop therapeutics to treat PRMS.

P403 Effects of Irradiation on Tumor Growth in Various Xenograft Models in NSG-MHC I/II DKO Mice

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NSG-MHC I/II DKO mice are a preferred strain in preclinical research to assess the efficacy of human immune-therapeutics for cancers. To evaluate the effects of irradiation on xenograft tumor growth, three cohorts of 20 DKO mice each were randomized into non-irradiated and irradiated (100cGy) groups for testing three cancer models, respectively. Two luciferase-labeled cancer cell lines, human B cell lymphoma Raji-Luc and human breast cancer MDA-MB-231-Luc, were injected intravenously at 2 and 1 million cells per mouse, respectively. Non-labeled MDA-MB-231 cells (5 million cells per mouse) were mixed with Matrigel and orthotopically implanted into mammary fat pad. Luciferase-labeled tumor growth was monitored by IVIS imaging three times weekly for the Raji-Luc and two times weekly for the MDA-MB-231-Luc. Solid tumor growth was measured by caliper twice a week. Body weight and clinical observations were performed two to three times a week to monitor mouse conditions. Data was analyzed by 2-way ANOVA. Irradiation had different effects on tumor growth. For the two IV-implanted tumor models, irradiated mice showed faster tumor growth and significant body weight loss when compared to non-irradiated mice. In contrast, solid tumor growth was faster in non-irradiated mice than in irradiated mice, but the error bars were smaller for the irradiated mice. Our findings suggest that irradiation may influence tumor growth, depending on the cancer cell types and routes of tumor implantation.

P404 Characterization of hPBMC-ASID and ASID B2m models for Immune-Oncology Studies

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The human immune system (HIS) mouse models are the preferred preclinical platform to evaluate novel immune-oncology (IO) therapeutic candidates *in vivo*. The immunodeficient mouse strains such as NSG (NOD.Cg-*Prkdc*^{scid}*Il2rg*^{tm1Wjl}/SzJ) and NOG (*Prkdc*^{scid} *Il2rg*^{tm1Sug}) allow for human CD34+ hematopoietic stem cell (hCD34^{+} HSC) or human peripheral blood mononuclear cells (hPBMC) engraftment and multilineage immune cell development, which facilites studies of immunomodulatory agents. Compared with the 12 to 16 weeks taken by hHSC-HIS models, the hPBMC-HIS model provides a more rapid model for permitting T-cell engraftment within 2 to 3 weeks. However, the limitation of hPBMC-HIS model is that mice suffer xenogeneic graft-versus-host disease (xGvHD) within 3 to 5 weeks post-implantation. Thus, the aim of this study is to optimize the protocol of hPBMC-HIS models using the immunodeficient mice strains. It's known that MHC class I deficiency, as occurs in beta-2 microglobulin knockout in ASID B2m, results in resistance to xGvHD. In this study, we transplanted several different cell numbers of hPBMCs into ASID (NOD.Cg-*Prkdc*^{scid}*Il2rg*^{tm1Wjl}/YckNarl) or ASID B2m (NOD.Cg-*Prkdc*^{scid}*Il2rg*^{tm1Wjl}*B2M*$^{-/-}$/YckNarl) in both genders, either alone or in combination with irradiation, with five mice in each group. In order to monitor the development of multiple immune cell subsets, we collected peripheral blood via the facial vein in K2EDTA tubes at least every two weeks and analyzed the cell subsets by flow cytometry. We also concluded that the most obvious clinical symptoms of xGvHD mice were weight loss and scaly skin. According to the severity of these clinical symptoms, we found that

the xGvHD incidence rate of ASID mice was higher than that of ASID B2m mice. In addition, to address the effects of HIS model in tumor growth, the co-engraftment and growth kinetics of cell line–derived xenograft tumors in HIS mice and recovery of tumor-infiltrating lymphocytes from growing tumors also were analyzed in this study. Thus, through this comprehensive analysis of HIS models, we could potentially provide a more practical HIS model for evaluating IO therapeutic candidates.

P405 Characterization of Effect of Enterovirus D68 in 129S2/Sv Mice Deficient in IFN-Alpha/Beta and/or IFN-Gamma Receptors

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Enterovirus D68 (EV-D68), a respiratory RNA virus belonging to the picornaviridae family, has been proposed as a potential causative agent of acute flaccid myelitis (AFM) in young children. However, the absence of an effective treatment against EV-D68 necessitates the development of an appropriate animal model. The AG129 strain, characterized by interferon α/β and γ receptor double knockout on the 129 genetic background, has been suggested as a mouse model for EV-D68 infection. In this study, we compared the virulence of a non-mouse adapted EV-D68 strain (US/MO/14-18947, NR-49129) on different mouse strains, including AG129, A129 (interferon α/β receptor null), G129 (interferon γ receptor null), and 129S2/SvPasCrl (129 background strain). Male and female mice were infected intraperitoneally at ten days after birth at various doses. Doses and (samples sizes) for each strain were as follows: AG129: 10^3 pfu/mouse (n=9), 10^4 pfu/mouse (n=11), 10^5 pfu/mouse (n=10), 10^6 pfu/mouse (n=14); A129: 10^4 pfu/mouse (n=8), 10^5 pfu/mouse (n=6), 10^6 pfu/mouse (n=6); G129: 10^4 pfu/mouse (n=9), 10^5 pfu/mouse (n=10), 10^6 pfu/mouse (n=15); and 129S2: 10^4 pfu/mouse (n=12), 10^5 pfu/mouse (n=12), 10^6 pfu/mouse (n=19).We found that AG129 and A129 exhibited similar susceptibility to EV-D68, displaying clinical signs of limb paresis/paralysis, lethargy, and death. Notably, dyspneic breathing characterized by prominent abdominal breathing was observed in addition to previously reported clinical signs. The G129 and 129S2 strains also displayed susceptibility to EV-D68, although the severity of clinical signs was less pronounced compared to AG129 and A129. Histopathological analysis and immunohistochemistry confirmed EV-D68's tropism for skeletal muscle and spinal cord, with lesions identified in pelvic limb muscles, paravertebral muscles, and spinal cord, corresponding to the presence of EV-D68 immunoreactivity. Furthermore, lesions and EV-D68 immunoreactivity were observed in the diaphragm skeletal muscles. We did not observe lesions in the lung. These findings suggest that the observed dyspnea is attributable to the impact on the diaphragm rather than direct EV-D68 infection of the lungs. Overall, our study demonstrates that AG129 and A129 mouse models serve as reliable tools for investigating EV-D68-induced AFM and respiratory illness, facilitating future studies and the screening of potential antiviral candidates.

P406 Baseline Characterization of Cardiac Troponin I, Heart Rate, and Blood Pressure in Cynomolgus Macaques for Extrapolation to Human Cardiac Health and Drug Development

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Despite the increasing importance of cardiovascular assessment in non-human primates, comprehensive investigations on relevant biomarkers remain limited. Cardiac troponin I (cTnI) is a primary marker used to indicate cardiac injury in such assessments. This study aimed to establish baseline data for cTnI and evaluate its correlation with heart rate (HR) and blood pressure (BP) using a telemetry system under various conditions, such as restraint and training. Three groups of cynomolgus macaques (n=6 per group, three males and three females, Vietnamese origin) were assigned for cTnI measurements. Measurements were taken at baseline and multiple time points over a 14-day period using the AQT90 FLEX device. The average cTnI value during this period was <0.010 µg/L (n=22). Additionally, a separate group of four cynomolgus macaques was continuously monitored for HR and BP to compare correlations with cTnI, but no significant results were observed. Considering the increased heart disease risk and the evaluation of newly developed drugs, such as those targeting the transferrin receptor (TfR), it is crucial to establish reliable evaluation metrics, and cTnI remains a valuable marker for cardiovascular assessment in non-human primates. Although no correlation between HR, BP, and cTnI was found in this study, further refined experiments are warranted to reinforce its applicability as an assessment tool.

P407 Differentiation of Niraparib and Olaparib Brain Penetration in Healthy Rhesus Macaque Monkeys

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There remains an unmet need to provide effective treatment for patients with primary and metastatic brain tumors. Patients with brain metastases or primary brain tumors have poor prognosis and low 5-year survival rates; this is mainly due to a lack of drugs that can penetrate the blood-brain barrier (BBB). Synthetic lethality is an attractive mechanism for treating brain tumors after radiotherapy; however, there are no poly (ADP-ribose) polymerase inhibitors currently approved for central nervous system cancers. A recent study showed that niraparib reached and maintained pharmacologically relevant concentrations in the brain and glioblastoma tumor tissue, resulting in effective PARP inhibition in patients with newly diagnosed glioblastoma. We investigated the brain penetration of niraparib and olaparib in healthy monkeys to generate evidence of their ability to cross the BBB. Our objective was to evaluate the brain penetration and distribution of 2 poly (ADP-ribose) polymerase inhibitors (niraparib and olaparib) in a primate model of an intact BBB. Healthy male Rhesus macaque monkeys (n=2) were dosed daily via oral gavage for 5 days with either niraparib (6 mg/kg) or olaparib (10 mg/kg). Predose blood was collected daily; terminal blood, cerebrospinal fluid (CSF), and brain tissue were collected at necropsy and coronal brain sections were analyzed by matrix-assisted laser desorption/ionization imaging mass spectrometry (MALDI IMS) to quantitatively assess the tissue distribution of the dosed compounds. Blood, CSF, and bulk homogenates of brain tissue were analyzed by liquid chromatography-mass spectrometry (LC-MS) bioanalysis. Niraparib showed markedly higher brain penetration than olaparib in healthy Rhesus macaque monkeys, demonstrating enhanced ability to cross an intact BBB compared with Olaparib. Further studies are warranted to evaluate niraparib as a treatment for primary and metastatic brain tumors.

P408 Evaluation of *Yersinia pestis* Infection in an S2 Cell Culture Model

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Yersinia pestis is the causative agent of plague, which is primarily a flea-borne disease maintained in rodents and typically transmitted

to a mammalian host by flea bite. Despite well described host-pathogen interactions, there is limited knowledge of flea-pathogen interactions that may influence vector transmission of *Y. pestis* to the mammal host. Flea infection *in vivo* is technically challenging, but a Schneider 2 (S2) cell culture model has potential to characterize flea-pathogen interactions. The S2 cell is a macrophage-like immune cell derived from *Drosophila melanogaster*. Prior work in our lab suggests that *phoP* and *gmhA* mutant strains of *Y. pestis* are negatively affected by the flea immune system but can survive. In this study, we aimed to determine if *Y. pestis* can survive and replicate within S2 cells with comparisons between wild-type and mutants of a KIM6+ strain. To assess intracellular survival, we used a gentamicin protection assay. S2 cells were infected at a multiplicity of infection of 10 in triplicate and samples enumerated at 0-, 2-, 6-, and 24-hours post-infection. At 0-hours, reference colony-forming units (CFU) were counted to compare intracellular survival at later time points. To kill extracellular bacteria while minimizing cytotoxic effects, infected S2 cells were dosed with high concentration gentamicin (40 ug/ml) for 1.5 hours followed by a low concentration (8 ug/ml) for the duration of the assay. At time points, cells were lysed, serially diluted, and plated to count CFU. Wild-type KIM6+ showed an initial CFU decrease at 2 hours followed by a gradual CFU increase that met or exceeded 0-hour CFU by 24 hours. A *phoP* mutant showed a similar trend with reduced growth that did not exceed 0-hour CFU at 24 hours. This preliminary data suggests uptake of *Y. pestis* with the bacterium capable of survival and replication within the S2 cell over time. In summary, *Y. pestis* is phagocytized by S2 cells and appears to be capable of survival and replication in S2 cells with some differences in mutant bacteria. This suggests an *in vitro* S2 cell culture model has potential to elucidate flea-pathogen interactions with future work to evaluate *Y. pestis* mutants for transmission factors and evaluation of immune responses by S2 cells to infection.

P409 Novel Non-Invasive Combination Therapy Approach for Metastatic Cancer

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Currently, there are no curative therapies for metastatic cancers. Durable responses to therapy of metastatic cancer are enhanced when an anti-tumor immune response is activated. However, not all patients treated with immunotherapies demonstrate durable responses. Therefore, it is essential to develop novel combination therapy regimes that are less toxic and more effective. We suggest that it is possible to immunize patients against their own metastases by using Focused Ultrasound (FUS) therapy to augment tumor antigen release. FUS is an emerging non-invasive, non-toxic treatment modality for localized treatment of cancers. To synergize the stimulatory effect, FUS was combined with immune checkpoint inhibitors (ICIs: PD-1 or CTLA-4). Two different syngeneic mouse model systems, metastatic breast cancer (4T1 cells in Balb/c mice) and melanoma (B16 cells in C57BL6 mice), were used. FUS treatment was applied to a single subcutaneous or orthotopic tumor in the mammary gland to create stable and precisely controlled heating with some animals either receiving FUS or ICIs alone (either PD-1 or CTLA-4) or both (FUS+PD-1 or FUS+CTLA-4) or no treatment (n=5-11animals/group). All animals were euthanized either to collect samples throughout the experiment or when they reached humane endpoints (e.g., ulceration, size of 20mm, weight loss, etc.). MRI, flow cytometry, immunohistochemistry, and cytokine analysis of the tumors were carried out to characterize immunogenic cell death, tumor control, and immune responses generated by these treatments. FUS led to an increase in apoptosis (Caspase 3), cytokines (IL-6, IL-1a, IL-7, IL-9), immune cells (CD4, CD8), HSP70 with an increase in CD11b+ dendritic cells in tumors. The primary as well as secondary tumor volume decreased when FUS was combined with ICIs. Furthermore, lung metastases were greatly decreased. These data suggest that FUS leads to increased immune responses, immunogenic cell death as well as tumor control. In summary, a combination of local FUS with ICIs significantly decreases primary as well as secondary tumors, and metastases in syngeneic models. This paradigm shift has the potential to open treatment for metastatic disease.

P410 Influence of Microbial Richness and Post-weaning Gut Microbiota Transfer on DSS Colitis Disease Severity

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Differences in the gut microbiota (GM) of research mice can significantly impact model phenotypes. To study the impact of differing GMs on model phenotypes, transfer of selected GMs is often employed using techniques such as embryo transfer, cross-fostering, or co-housing. Previous studies in our lab demonstrated that co-housing C57BL/6 mice to transfer GM leads to greater dextran sodium sulfate (DSS)-induced weight loss one month later when compared to embryo transfer or cross-fostering methods ($p < 0.05$). Additionally, co-housing to transfer low-richness GM into recipients with higher richness GM is associated with more severe colitis and death when compared to GM transfer in the opposite direction ($p < 0.05$) in both acute and chronic DSS colitis. In this study, we sought to determine whether these differences in disease severity and survival were associated with microbial factors. To this end, we compared co-housing of C57BL/6J or C57BL/6N mice (6-8 mice/sex/substrain) with donor CD-1 mice as a method of transfer to gastric gavage of fecal material with the rationale that the latter would isolate the microbial component of this transfer effect. For the gavage group (6-8 mice/sex/substrain), following weaning fecal material from either high or low richness CD-1 GM donors was transferred to recipients with the reciprocal GM by gastric gavage once per week for 4 weeks while concurrently transferring dirty bedding three times per week. DSS was administered in water (2.5%) at 49 days of age for one week, and weight monitored daily for two weeks. In both co-housed and gavage groups, mice receiving a low richness GM showed significantly greater weight loss and mortality ($p < 0.001$), shorter colon lengths at necropsy ($p < 0.001$), and greater expression of several pro-inflammatory cytokines and chemokines when compared to mice receiving a high richness GM, confirming a role of the GM itself as opposed to non-microbial factors associated with co-housing. We speculate that, during donor GM exposure prior to DSS, low richness GMs allow greater donor microbe colonization and development of tolerance, while high richness GMs resist colonization, resulting in lack of tolerance to novel bacteria that is unveiled with DSS-associated intestinal damage. These findings are relevant in the context of model reproducibility, as well as fecal transplant therapy in human and veterinary medicine.

P411 Intramuscular Vaccination with the HSV-1(VC2) Live-Attenuated Vaccine Strain for Conferring Protection Against Viral Herpetic Keratitis in Rabbits

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Herpetic keratitis is one of the most common causes of infectious blindness, resulting from infection with herpes-simplex-virus type-1(HSV-1). This virus infects billions of people worldwide, lying dormant in the ganglia of nerves. During initial infection or a subsequent flare-up, the virus can infect the cornea and cause irreparable scarring. A previous study successfully prevented ocular

pathology from HSV-1 in mice who received an attenuated herpes vaccine, VC2. A vaccine trial was undertaken in New Zealand White rabbits to further study HSV-1 ocular pathology and the VC2 vaccine. An initial study investigated the virulence of HSV-1(17syn+) and HSV-1(McKrae)-GFP in rabbits infected intraocularly. All rabbits showed significant ocular disease; however, rabbits infected with HSV-1 (17syn+) died 7-8 days post-infection. Due to the lethality of HSV-1(17syn+), we used the HSV-1(McKrae) virus for a prophylactic vaccine study. Eighteen rabbits were segregated into three groups: a mock-vaccinated, heat-inactivated virus, and HSV-1(VC2) vaccinated animals. Two weeks after booster immunization, rabbits were inoculated intraocularly with HSV-1(McKrae). A board-certified veterinary ophthalmologist periodically examined all animals. Rabbits were sacrificed twenty-one days post-infection, and samples were collected to test for the virus. Clinical signs were not significantly different, but ocular swabs collected at 7-and 14-days post-infection revealed that rabbits vaccinated with either HSV-1(VC2) or the heat-inactivated HSV-1(VC2) failed to shed any virus while a significant number of viruses were observed in the mock-infected animals. Because these preliminary experiments have shown protection against clinical signs with a less virulent strain of virus, the next step will be a similar trial using the HSV-1(17syn+) strain to determine whether the vaccine can also provide protection from a more virulent strain with more serious clinical signs. The HSV-1(VC2) will also be tested for therapeutic applications, producing HSV-1 latency in rabbits and testing to determine whether the VC2 vaccine can prevent virus shedding from latently infected animals.

P412 Investigating Variations in Anti-CD28 Antibodies and Improving Clinical Scoring Practice for Cytokine Release Syndrome

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Anti-CD28 antibodies (aCD28 Ab) are often used in peripheral blood mononuclear cell (PBMC)-humanized NSG/MHC I/II double knock-out (DKO) mice as a positive control for Cytokine Release Syndrome (CRS). A clinical CRS scoring system is used to assess mice once daily on a 1-4 scale based on activity level and general clinical appearance, with scores of three and four being humane endpoints. However, recent studies showed an unexpected acceleration of symptoms in PBMC-humanized DKO mice injected intravenously with aCD28 Ab, leading to incomplete experimental data. We hypothesized this might be due to specific lot variations of aCD28 Ab or the high dose of 1.0 mg/kg used. To better identify mice nearing humane endpoints, we refined the CRS clinical scoring practice to include increased monitoring frequency. We compared three different lots of aCD28 Ab from two vendors in PBMC-humanized DKO mice. One hundred mice were irradiated at 100 Centi-gray (cGy) and injected with 15x10^6 PBMCs from two donors. On study day six, aCD28 Ab was intravenously administered at three different doses (1.0, 0.5, 0.25 mg/kg). Retro-orbital bleeds were performed at 6- and 72-hours post-dosing to evaluate cytokine profiles by Cytometric Bead Array. Bodyweights, clinical observations, and CRS scoring were performed once daily for the first seven days, then twice a week until the study ended. If mice reached a CRS score of two, CRS scoring was increased to twice daily. H&E-stained tissue sections were evaluated by light microscopy. The two aCD28 lots from vendor A showed similar effective outcomes, including decreased bodyweight changes, decreased survival time, increased cytokine induction, and histopathology findings of increased mononuclear cell infiltrates, whereas the lot from vendor B proved to be ineffective and, like PBS-treated control animals. Twice daily assessment of mice with a CRS score of two resulted in successful identification and terminal collection of 100% of the mice that reached humane endpoints. This study highlighted the necessity of considering vendor variability and increasing clinical assessment frequency when designing studies using aCD28.

P413 Long-term Isoflavones Administration Results in a Reproductive Disfunction

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Soy-based product consumption is gaining popularity in Europe since it is considered an alternative to animal proteins and presents a wide range of benefits, including protection against several hormone-related cancers and reduction of the risk of developing cardiovascular disease. These products contain isoflavones that are considered endocrine disruptors that cause deleterious effects on reproductive organs in males because their structure is similar to 17b estradiol. Therefore, the isoflavone consumption during a long period of time may cause changes at an endocrine level. This study aimed to determine the endocrine and reproductive effects of long-term exposure to isoflavones in adult male Wistar rats. Seventy-five 60 day old adult male Wistar rats were administered orally using a buttoned cannula with saline solution (control group), a low mixture of isoflavones (17 mg kg-1 day-1 genistein + 12 mg kg-1 day-1 daidzein) (low doses group), and a high mixture of isoflavones (170 mg kg-1 day-1 genistein + 120 mg kg-1 day-1 daidzein) (high doses group), every day over five months (20 weeks). Every month, five animals from each group were sacrificed by cervical dislocation after collecting blood from the dorsal aorta. Sperm quality parameters (sperm count, motility, viability, or membrane integrity) were performed, and serum and testicular levels of testosterone (T) were determined by enzyme immunoassay. The results revealed that low and high mixtures of isoflavones reduced significantly ($p<0.05$) the percentage of sperm motility and sperm count. In addition, serum and testicular testosterone levels in rats administered with low and high doses of isoflavones were decreased compared to the control group. In conclusion, isoflavones intake during a long period may cause deleterious effects on reproductive function.

P414 Metagenomic Analysis of Viral Sequences In Rats

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Historically, viral discovery was slow and only successful for easily cultured agents. NGS technology has enabled novel virus identification, particularly in the world of laboratory animal viruses. Inapparent viruses contribute to confounding and lack of reproducibility in scientific studies. Therefore, we collected fecal samples from 6 to 10 week old, conventionally raised, non-specific pathogen-free rats of unknown sex to investigate viral presence, diversity, and strain. Samples were pre-processed to maximize the concentration of intact viral particles while excluding host and bacterial cells. Total nucleic acid (DNA and RNA) was extracted using a magnetic bead-based extraction workflow. Nucleic acid was submitted to a commercial sequencing laboratory for library preparation and sequencing. Library preparation was performed per the manufacturer's recommendation for Nextera XT DNA Library Preparation Kit. The resulting library was quantified by qPCR and quality was evaluated by TapeStation. Equimolar pooling of libraries was performed and sequenced on an Illumina NovaSeq with a read length configuration of 150 PE for 40 M PE reads per sample. After sequencing, the raw data was trimmed and host reads were removed. Reads were classified using a k-mer based approach against a reference library containing viral, fungal, protozoal, and bacterial genomes. Unclassified reads were further evaluated by blastn or discontiguous megablast to identify related viruses. Information about singleton and contig classification were used to determine highly probable viral sequence. Identified viruses included known rodent pathogens in the families Parvoviridae and

Picornaviridae: rat bocavirus, rat parvovirus 1, boone cardiovirus, rat theilovirus and rosavirus B. Rat-associated porprismacovirus is a recently described smacovirus whose host was recently hypothesized to be archaea. In addition, quite a diversity of sequence was classified as virus of non-related species (human, rabbit, insects) in the families Picornaviridae, Picobirnaviridae and Coronaviridae. Further work will determine the prevalence of the viruses, the potential nucleic variation among strains found in different populations, and if present in laboratory rats, the potential impact on research and animal health.

P415 Mouse-Origin *Staphylococcus aureus* Results in More Severe Experimental Infection of the Preputial Gland than Human-Origin *Staphylococcus aureus*

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Staphylococcus aureus (*S. aureus*) is a gram-positive bacterium responsible for a variety of infections in many animal species. In laboratory mice, *S. aureus* is a significant pathogen resulting in conditions such as the preputial gland infection (PGI) and dermatitis. PGI is a naturally occurring disease in mice that is notoriously difficult for laboratory animal veterinarians to manage. An experimental PGI model is useful to evaluate this infection, including comparative studies involving *S. aureus* isolated from animals and humans. PGI is characterized by an influx of polymorphonuclear leukocytes (PMN) into the gland and eventually abscess formation. Activated PMNs produce myeloperoxidase (MPO), thus MPO can be used as a pro-inflammatory biomarker of PMN presence. We comparatively evaluated a *S. aureus* isolated from mice and from humans in our PGI model, hypothesizing mouse-origin *S. aureus* (M3) will result in more severe infection than the human-origin *S. aureus* (MW2) as assessed by body weight, glandular MPO activity, bacterial cell counts, tissue pathology, and intravital microscopy (IVM)-evaluated PMN infiltration of the gland. Specific Opportunistic Pathogen Free (SOPF) C57BL/6N (n=6) mice were used to evaluate weight loss, bacterial cell count, and tissue pathology at 6 days post infection. Catchup-IVM red mice (n=3) were used to evaluate the PGI using intravital microscopy at 6 days post-infection. Mice infected with M3 had more pronounced weight loss, higher MPO, higher histopathology score, and more extensive PMN infiltration of the preputial gland. No significant difference was observed in bacterial cell counts of gland tissue. Increased weight loss in M3-infected mice was attributed to more clinically severe infection. Elevated MPO activity suggested more pronounced preputial gland infiltration of PMN, also confirmed by histological evaluation and IVM. A broader understanding of the early inflammatory events in *S. aureus* PGI may assist veterinarians to better treat or prevent this infection in laboratory mice.

P416 Blood and Bone Marrow - Does it Contain Epithelial Cells?

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Epithelial cell adhesion molecule (EpCAM) is a surface marker commonly used to identify circulating epithelial cells found in blood and bone marrow (BBM) of patients with epithelial cancers. Although these circulating epithelial cells are thought to originate from the primary tumor before entering the bloodstream, normal donor controls also had low levels of these circulating epithelial cells in their BBM as well. We examined female and male normal murine BBM samples across various ages to determine if these EpCAM-expressing cells could be found in normal mice. The healthy control BBM both had EpCAM expression. We also used another epithelial marker, cytokeratin, to identify potential epithelial cells. EpCAM and cytokeratin expressing cells within these samples were investigated to further clarify the role of: epithelial stem cells, bone marrow-derived cells, and cytokeratin-expressing cells in tumor progression. The presence of EpCAM and cytokeratin in normal BBM of mice was demonstrated using traditional methods such as PCR, transgenic mice, flow cytometry, and immunofluorescence. However, these methods have been limited in their ability to understand the complexity of the heterogeneous population of epithelial-expressing bone marrow cells. To better understand the complex heterogeneous population, the use of single cell RNA sequencing (scRNA seq) analysis was used. scRNA seq provides a more detailed gene expression at a single cell level. Therefore, scRNA seq was focused on bone marrow samples analysis. Reference scRNA seq datasets, such as the Tabula Muris (mouse) and Tabula Sapiens (humans), allow for an unbiased approach to determine if these epithelial-expressing cells can be found in scRNA seq bone marrow datasets. Within the Tabula Muris and Tabula Sapiens datasets, EpCAM-expressing bone marrow cells and other epithelial-specific markers were identified. Heterogeneous expressions for stem cell markers were also seen in the EpCAM cells, confirming what was seen in previous flow cytometry results. Overall, this analysis of scRNA seq reference datasets has identified a potential population of epithelial stem progenitor cells that are heterogeneous in their expression of epithelial and stem cell-specific markers in normal mouse and human bone marrow.

P417 Comparison of Radiation-Induced Damage between Livers from Control and Chimeric Mice

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Assessment of human health risks associated with space radiation exposure is based largely on the knowledge gained from studies of animal models, mostly rodents, exposed to high-LET radiation on the ground. It has been recognized that translation of mouse model results to meaningful implications for human disease can be challenging. Considering limitations in utilizing non-human primates and clinical studies in humans, chimeric animals can potentially bridge the knowledge gap between rodents and humans. In this study, we used male chimeric mice (PXB mice) whose livers contain >90% human cells (n=32), compared against non-chimeric mice from the same genetic background (n=28). These mice were either exposed to gamma rays or 1 GeV/u iron ions at a whole-body dose of 0.5 Gray to investigate pathological changes in the chimeric livers. Staining of the liver tissues with H&E indicated that the human liver tissue in chimeric mice responded differently than the mouse liver tissue to gamma radiation and iron ion radiation on the cellular level, as evidenced by differences in inflammation and cellular damage seen on histopathology. Inflammation was measured with imaging analysis to quantify relative numbers of infiltrating immune cells to the liver and cellular damage was assessed qualitatively with assessment of changes to cellular morphology. This chimeric mouse model has great potential for use in transcriptomic and genomic studies to further investigate the molecular basis of these difference. This model also represents a viable option to improve translatability of animal models to human spaceflight health risks and hazards.

P418 Development of a Functional Observational Battery (FOB) Assay as a Screening Tool to Assess Acute Neurotoxicity of Antisense Oligonucleotides in Mice

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Although the use of antisense oligonucleotides (ASOs) as therapeutics has advanced, factors affecting their tolerability are not fully understood. While rare, instances of acute neurotoxicity following intracerebroventricular (ICV) injections of ASOs in mice have been documented. Effective preclinical assessment tools are crucial for early screening and generating data on sequence motifs and chemical modifications that impact tolerability. The Functional Observational Battery (FOB) Assay evaluates acute neurotoxicity of ASOs after ICV dosing in awake mice by assessing behaviors, physiology, and safety pharmacology. Mice were injected with 5µL of vehicle control or ASO via indwelling cannulas in the left lateral ventricle. Ten behaviors were scored on a scale of 0-2 at multiple time points, including 30 minutes, 1 hour, 2 hours, 4 hours, and 24 hours post-dose. The behaviors scored were tail pinch reaction, Straub tail, tail suspension, tremors, mobility, gait, respiration rate, posture, hindlimb clasping, and convulsions. With three mice per group, we observed a clear separation of ASOs based on their tolerability scores, categorized as Zero, Mild, Moderate, and Marked. Some ASOs were safely delivered, scoring zero and mild, with signs resolving within 4 to 24 hours. Other ASOs were poorly tolerated at early time points, leading to the humane euthanasia of the mice and indicating acute neurotoxic potential. All compounds showed a dose-dependent increase in score. Some ASOs with similar backbones but different sequences displayed different profiles. Taken together, this demonstrates the assay's sensitivity to dose- and sequence-specific toxicity. The FOB Assay successfully differentiates compounds in a dose and ASO sequence-specific manner, effectively identifying acutely toxic ASOs early in the screening process. The assay demonstrates high sensitivity and reproducibility, making it a valuable tool with the potential for eliminating poorly tolerated compounds and identifying toxic motifs before further development and efficacy testing.

P419 Differences in Relative Intensity Units of Plasma and Serum on Macaque SPF and COVID Immunoassays

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Serum and plasma can be used to test for various antibodies in the surveillance of nonhuman primate (NHP) populations on Colony Surveillance Assays (CSA). We wanted to understand the difference between testing plasma and serum. Both types of samples were assayed as pairs from the same NHPs, and a t-test was used on the resulting values to determine if there was any significant difference between the two. Testing was done using Panel A (N=120) and COVID (N=38) CSAs. We tested for B Virus, SRV 2, SRV 5, SIV, STLV lysate, and peptide using the Panel A assay and COVID spike protein 1, spike protein 2, and nucleocapsid using the COVID assay. Our tests determined that on the entire COVID panel and the Panel A's SRV 5 and B Virus tests, there was a nonsignificant difference between serum and plasma. However, in the Panel A's SIV, SRV 2, STLV lysate, and peptide tests, there was a statistical difference between the two sample types. With four out of nine tests indicating a significant difference between the two sample types, we can surmise that testing plasma is less accurate than serum and should be used only when there is no way to obtain serum. This is because the assays were designed to be run on serum, and any significant difference between the two may have a significant impact on the care of the NHP. The COVID CSA may be an exception since there was little difference between the two sample types.

P420 Role of Serotonin in the Nucleus of the Solitary Tract on Arterial Blood Pressure Regulation

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Hypertension affects nearly half of adults in the U.S. and can be a major risk factor for cardiovascular disease, including heart failure, heart attack, and stroke. Serotonin (5-Hydroxytriptamine; 5-HT) neurons project widely throughout cardiorespiratory regions. We previously showed that a loss of 5-HT in male rats led to hypertension, while females were unaffected. Here, we investigate the extent that 5-HT acts within the nucleus of the solitary tract (nTS) in the brainstem to maintain arterial blood pressure. We hypothesized that silencing nTS-projecting 5-HT neurons increases arterial blood pressure in adult male rats. The nTS of adult male LE-*Tph2*$^{tm1(cre)}$ rats (n=5) was surgically injected with a Cre-dependent, retrogradely transported adeno-associated virus expressing inhibitory Designer Receptors Exclusively Activated by Designer Drugs (Gi-DREADDs) to allow silencing of projecting 5-HT neurons. We also implanted femoral arterial and venous catheters for measuring blood pressure (MAP) and heart rate (HR) and for delivery of vehicle or Compound 21 (C21; 1 mg/kg) to activate the Gi-DREADD. Rats were given one week for recovery from surgery and acclimation to a plethysmography chamber prior to experimentation. Each rat was tested with both C21 and vehicle, with one week between experiments. On the experimental day, we recorded breathing, MAP, and HR for one hour, and then injected ~100ul of vehicle or C21 and monitored cardiorespiratory variables for another one hour. Gi-DREADD activation induced a marked increase in MAP (~30 mmHg; $p<0.001$) with no significant change in HR. The vehicle had no significant effect on either MAP or HR. As there was no change in HR, these data suggest that serotonin neurons projecting to the nTS have an inhibitory effect on the sympathetic drive to the vasculature. Future experiments using ganglionic blockade will test this possibility. Additional experiments will also include female rats. In summary, our preliminary data supports the hypothesis that 5-HT acts within the nTS to regulate arterial blood pressure in adult male rats, possibly through alterations in sympathetic vascular tone.

P421 Successful Targeted Delivery of an AAV Reporter via MRI-Guided Surgery to CNS Tissue: Feasibility, Tolerability, and Challenges

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Recent advances in clinical approaches to delivering targeted gene and cell therapies to the brain have necessitated *in vivo* safety studies. Neurodegeneration in the putamen as indicated in Parkinson's and Huntington's disease, or in the hippocampus, as indicated in Alzheimer's disease, requires precise treatment for gene replacement or disease reversibility. As an alternative to achieving central nervous system (CNS) exposure, administration into the cerebral spinal fluid results in a broader distribution of these therapies but often includes systemic exposure. By targeting current surgical approaches to disease-specific parenchymal structures, one can minimize broader distribution and focus the therapy on the intended area. In this work, we demonstrated the feasibility, tolerability, and challenges of direct targeting of parenchymal structures using magnetic resonance imaging (MRI)-guided Clearpoint catheter-based infusion in nonhuman Primates (NHPs). A 1.5T MRI scanner in conjunction with Brainsight software® was used to scan the brain and plan the bilateral trajectories to the putamen and hippocampus. Post-dose MRI confirmation using 2% Gadoteridol allowed for target confirmation. In addition, 2.6×10^{12} GC/dose site AAV-9-mCherry was used for biodistribution analysis at necropsy, 28 days post-dose. MRI scanning after infusion confirmed the correct dosing of the targeted structure. After surgery, animals were monitored for signs of neurological impairment, clinical abnormalities, and alterations in body weight. The surgical procedures were well tolerated, with no neurological or clinical impairments. Histological and biodistribution analyses were used to determine tissue response to the dosing procedure and indicated successful expression of mCherry transgene

in the target tissue. In general, mCherry expression was significantly greater in the target CNS tissue compared to the liver or dorsal root ganglia, which are typically locations of AAV-9 expression using alternative methods of CNS dosing. Together, these data offer a path forward when testing the delivery of cells or cell-specific gene therapies directly to the putamen or hippocampus and can provide a clinically relevant method for targeted brain delivery with accuracy in NHPs.

P422 Surgical Approach for Chronic Drug Delivery into the Hypoglossal (XII) Nucleus *via* Brainstem Cannula and Osmotic Minipump in Adult Rats

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Patients with motor neuron diseases (MNDs) experience loss of motor neurons (MNs) required for activities of daily living, often leading to swallowing and breathing dysfunction. Thus, there is a critical need for therapies aimed at preserving upper airway function. Upper airway dysfunction in MNDs is largely attributed to degeneration of XII lower MNs (LMNs) that innervate the genioglossus muscle causing extensive tongue weakness, suggesting a role for therapeutic exercise. Using our established model of targeted XII MN loss induced by intralingual injection of cholera toxin B conjugated to saporin (CTB-SAP), we have shown that tongue exercise mitigates XII axis deficits (*i.e.,* increases lick rate and force). However, the underlying mechanism of exercise-induced plasticity remains unknown. Preliminary IHC and western blot data suggest that XII brain-derived neurotrophic factor (BDNF) expression is increased in exercise-treated *vs*. sham exercise-treated CTB-SAP rats. Thus, we speculate that the source of tongue exercise-induced plasticity in CTB-SAP rats arises from a BDNF-dependent pathway to preserve upper airway function. To test this, we first need to develop an approach for chronic drug delivery targeting the XII nucleus. *We hypothesized this could be done by surgically implanting a cannula (attached via catheter to an osmotic minipump) into the XII nucleus of the brainstem.* Troubleshooting this approach involved determining the coordinates of the XII nucleus (N=15), the length of brainstem cannula necessary to reach the XII nucleus (N=6), and establishing the fixation method of the brainstem cannula to the skull (N=3) in anesthetized Sprague Dawley and Wistar rats. Successful implantation was confirmed by injecting dye into the XII nucleus. This approach to chronic drug delivery will prove crucial to determining the source of tongue-exercise-induced plasticity, as the next steps will involve adapting this approach to rats undergoing survival surgery. Future directions will involve targeting Tropomyosin receptor kinase B (TrkB; high affinity receptor for BDNF) by delivery of a TrkB receptor antagonist or agonist into the XII nucleus in control and CTB-SAP rats to observe whether this abolishes or enhances tongue exercise-induced therapeutic effects, respectively.

P423 The Role of RECK in Metabolic Alcohol-Associated Liver Disease (MetALD)

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Metabolic alcohol-associated liver disease (MetALD) is a subcategory of steatotic liver disease (SLD) that results from metabolic dysfunction and chronic alcohol consumption. We have previously reported that RECK (REversion Inducing Cysteine Rich Protein with Kazal Motifs), a membrane anchored glycoprotein, exerts hepatoprotective effects in metabolic dysfunction-associated liver disease (MASLD; previously known as nonalcoholic fatty liver disease, or NAFLD). This study explored RECK's role in MetALD. We hypothesized that knocking out RECK in hepatocytes would exacerbate hepatic pathology in response to an acute ethanol/high fat diet challenge. Both the hepatocyte-specific RECK knockout animals (RECKhep-/-), and littermate controls (RECKfl/fl) were challenged with an ethanol-containing liquid diet (n=7-9/group) following the NIAAA model of alcohol feeding (10 days + EtOH binge). Histology sections from RECKhep-/- animals displayed increased signs of fat accumulation (steatosis), inflammatory foci, and collagen deposition. qRT-PCR and Western blot analysis of whole liver tissue showed increased presence of proteins associated with inflammation as well as increased mRNA expression of genes associated with hepatic inflammation and fibrosis, including α-SMA ($p=0.108$), Col1a1 ($p=0.013$), TNF-α ($p=0.027$), IL-1β ($p=0.023$), and PDGFβ ($p=0.011$) in the livers of RECKhep-/- mice. These results support the hypothesis that hepatocyte-specific RECK knockout exacerbates hepatic pathology and confirms that RECK plays a similar role in the liver for both MetALD and MASLD, suggesting RECK's potential as a therapeutic target for MetALD patients. Work was supported by R01DK130243-01A1 (RSR, BC).

P424 A Histological Characterization of the Adaptive Immune Response Following Brain Injury

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It is increasingly recognized that injury to brain tissue can lead to complex neuroinflammatory responses that contribute both to ongoing pathology as well recovery processes. This includes rapid and dynamic microglial responses, as well as the infiltration of peripheral immune cells, including neutrophils. Comparatively little is understood about the adaptive immune response in brain tissue following injury, including the nature, temporal course, and associations with other pathologies. Here, we performed detailed histological characterization of the adaptive immune response following a porcine model of controlled cortical impact (CCI) both acutely (30 minutes, n=2 and 72 hours, n=3) and in the chronic phase (6 months, n=3). Immunohistochemistry, specific for B-cells and T-Cells was performed on coronal plane whole brain formalin fixed paraffin embedded tissue at the level of the basal ganglia at the head of caudate nucleus and the posterior hippocampus at the level of the posterior commissure. In addition, immunoenzymatic double labeling was performed on serial sections to examine for associations with blood-brain barrier permeability or axonal degeneration. We demonstrate evidence of early (72h) infiltration of T-cells in association with focal cortical contusion. Interestingly, there was significant T-cells observed in the peri-contusional tissue and underlying white matter at 6 months post-CCI. These data suggest T-cells may play a significant role in the pathophysiological response to injury in both the acute and chronic phases. Further work to understand the phenotypes and mechanistic role of T-cells post-injury will be important to examine.

P425 Inflammatory Comorbidities in the Heart of the HIV-1 Transgenic Rat

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Antiretroviral therapy (ART) has increased life span and improved the quality of life of the HIV infected. One reason for the increase in longevity is the improvement of immune function, which results in the minimization of opportunistic infections. Interestingly, HIV noninfectious comorbidities (NICMs) have emerged as a medical challenge. The pathologic mechanisms are unclear; however, it is believed that the production of toxic HIV gene products promotes chronic inflammation. As a result, several organs have been affected. The heart is one such organ that displays NICMs such as inflammatory foci, myocyte death and dropout, and fibrotic changes. These changes result in increased mortality due to cardiac pathology despite ART treatment. Further research is needed to investigate the role of HIV gene products in the production of this pathology. To explore the role of HIV gene products in cardiac pathology, we used the HIV-1 transgenic (TG) rat model. This model has been shown to produce measurable amounts of GP-120, TAT, and NEF. It is hypothesized that in the aged HIV-1 TG rats (greater than one year), the toxic effects of HIV transgenes will be noticeable by altered histologic changes. To identify that these HIV gene products exist, we selected a group of aged HIV-1 TG, and control rats and hearts were collected. A gross inspection of the hearts was made before embedding them in paraffin for microscopic analysis. The stains used for analysis were H&E, Trichrome, and immunocytochemical labels for GP-120, TAT, and NEF. Histologic analysis confirmed that pathology similar to those found in HIV-infected patients was present. HIV-1 TG rat hearts displayed multifocal sights of inflammation as demonstrated by H&E stains. Moreover, areas of myocyte death and fibrotic replacement were observed with the use of trichrome stain. The inflammatory cells proved to be positive for HIV-1 transgenes GP-120, TAT, and NEF by means of immunocytochemistry. No such findings were observed in the control animals. These observations suggest that this model replicated in part the histopathologic changes found in the associated cardiac comorbidities. In summary, we have established that there is evidence of the presence of HIV viral proteins in the HIV-1 TG rat heart. By studying these occurrences in the HIV-1 TG rat, we can develop treatments to mitigate these conditions. Identifying mechanisms in an experimental model can help our understanding of NICMs.

P426 Non-Invasive Electroencephalogram (EEG) Seizure Detection in Swine

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Seizures induced by chemicals (such as the nerve agent soman, used here) remain a significant threat to our civilian and military personnel, and improved models for seizure analysis and treatment are desired. Although rodents and primates have historically been used for such studies, miniature swine (such as the Göttingen minipig) are increasing in popularity because they are more readily available and affordable than non-human primates and also closely resemble human anatomy and physiology (including a gyrencephalic brain and larger body mass). Implanted telemetric recording of EEG signals through skull screw electrodes is a reliable technology for seizure recording but requires expensive transmitters be placed through invasive surgical methods. The use of non-invasive EEG recording via scalp electrodes (as is common in human EEG recording) precludes the need for surgery lengthy surgical recovery (often two weeks or more), enhancing throughput and reducing infection risks inherent to surgical procedures. Here, using 12 and 19 minipigs per group, we elaborate and compare two different non-invasive EEG systems and discuss the pros and cons of each. Detailed baseline, seizure onset, status epilepticus, and post-seizure periods were analyzed to assess signal strength, quality, movement artifact, and general usability. Göttingen minipigs tolerated the scalp electrodes, and both methods provided satisfactory EEG recording to assess seizure onset, severity, and cessation. Non-invasive EEG proved comparable to a standard implanted telemetric method (n=7 pigs with both implanted and non-invasive EEG). Fast Fourier Transformation was used to examine the combined power spectrum (0-25 Hz), and the correlation of seizure onset and duration was further used to compare the systems. It is our hope that non-invasive approaches such as those detailed here could be adopted more widely because they are less expensive, less invasive, and more humane.

P427 Qualification of a Veterinary Glucometer for Use in Yucatan Miniature Swine

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Diabetes is a global health issue with rising cases every year. Animal models, such as chemically induced diabetic miniature swine (MS), are critical to investigating improved treatment options. A glucometer is essential in the daily monitoring of blood glucose (BG) to ensure adequate health and insulin dosing in diabetic MS. Due to the discontinuation of the glucometer used historically at our institution, we sought to identify a new model for use. We hypothesized a veterinary glucometer would provide adequate results for monitoring BG in diabetic MS. Twelve male Yucatan MS (n= 6 alloxan-induced diabetic, n=6 non-diabetic) were withheld their AM feed and insulin administration, and 3 ml of whole blood was collected from each pig. Blood was analyzed in duplicate using two canine glucometers (the historical model and the new model). The remaining blood was processed, and the serum was analyzed with a chemistry analyzer. Acceptance criteria were based on ISO performance standards used for human glucometers. The average BG for non-diabetic MS using the chemistry analyzer was 68.5 mg/dL (SD: 10.6) and 269 mg/dL for diabetic MS (SD: 39.7). For MS with BG values less than 100 mg/dL (non-diabetic MS), the average difference in BG using the historic model was 5.7 mg/dL (SD = 3.6) and the average difference using the new model was 5.3 mg/dL (SD = 2.7). All values fell within the ISO standards (less than +/-15 mg/dL difference between the glucometer and the chemistry analyzer). For MS with BG values greater than 100 mg/dL (diabetic MS), the percentage difference between the glucometers and the chemistry analyzer was generated. The average percentage difference in BG using the historic model was 9.5% (SD = 4.9), and the average difference using the new model was 2.6% (SD = 2.3). All values generated by the new model fell within ISO standards (+/- 15%). However, 2 of the values from the historic model fell outside of the acceptance criteria. The results of this study confirm the new model of glucometer meets ISO standards for glucometer performance. The new model performed more accurately than the historic model and provides confidence that the new model is adequate for performing point-of-care BG measurements in MS to support clinical decisions and guide insulin dose adjustments.

P428 Once Weekly Tail Vein Intravenous Infusion Study in CD-1®IGS Mice Using Simplified Materials and Techniques

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Requests for chronic (up to 26 weeks) intravenous (IV) infusion studies in mice are increasing. IV Infusion in mice is challenging - traditional methods include use of through-the-needle (TTN) tail vein catheters or surgically implanted catheters. Scientists utilizing TTN catheters describe warming the tail, use of equipment to illuminate vessels, anesthesia, or a combination to facilitate placement. TTN catheter placement is complex, and since studies are infrequent, training is difficult to maintain. Experience with jugular catheter-implanted mice showed 20% catheter dislodgement or lost patency at eight weeks. A 26g ½" over-the-needle (OTN) pediatric catheter became commercially available, and a pilot study was conducted

to assess the viability of using these temporarily placed catheters in appropriately restrained, unanesthetized mice. Twenty M/F mice were placed into two groups of 10M/10F and dosed with 0.9% sterile saline once weekly for 13 weeks. Doses were administered with calibrated syringe pumps at injection rates of 50 mL/kg/hr and 25 mL/kg/hr for 30 and 60 minutes, respectively. At 13 weeks, 95% of the animals were still able to be dosed, so the study was extended to 26 weeks. Study endpoints included assessment of mortality, clinical observations, and body weights. There were two unscheduled deaths that were considered potentially procedure-related; there were no effects on clinical observations or body weights. The endpoint for removal from the study was the inability to place the catheter for two consecutive weeks. Groups were terminated early once the n/sex reached 7, and both groups reached that endpoint by week 21. Dosing-related microscopic changes at the infusion site included degeneration/necrosis of the tail vein, skeletal muscle degeneration, and ulceration. Based on the results of this study, tail vein infusion dosing is viable for use in studies with weekly or less frequent dosing for up to 13 consecutive doses. The simplified equipment allowed technicians trained to place OTN catheters in other species to easily transition their skills/techniques to mice.

P429 Hypothermia as a Sole Euthanasia Method for Neonatal Mice

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Per the AVMA Guideline of Euthanasia, there is no data supporting the use of hypothermia as a solitary method of euthanasia for neonatal mice. However, a secondary physical method of euthanasia, such as decapitation, could be time-consuming and mentally fatiguing for care staff, particularly if many animals need to be euthanized simultaneously. In this study, we tested hypothermia as a sole form of euthanasia in neonatal mice (Day 0-2 and Day 8-10 of age) in various group sizes (1, 3, 6, and 10) using an ice-water bath (4 to 5°C), a refrigerator (4 to 7°C), and a freezer (-22 to -10°C) with time points based on a pilot study. To prevent direct contact to cold surfaces, neonates were contained in floating plastic dishes for the ice-water bath and in unsealed plastic bags atop one layer of paper towels for the refrigerator and freezer. We collected data on time until anesthesia, euthanasia, and recovery (if not euthanized). The temperatures of the devices and mice were also collected via temperature probe and infrared thermometer, respectively. Generalized linear models were utilized to predict what factors influenced successful euthanasia and at what body temperature a pup was unlikely to recover. Only the freezer consistently induced anesthesia in 5-10 minutes and euthanasia in 25-30 minutes for single and grouped Day 0-2 neonates and single Day 8-10 neonates. Histopathology of neonates collected at the time of anesthetic induction showed no evidence of lesions for any device, indicating a lack of trauma and potential discomfort during the euthanasia process. Through the use of modeling, we determined that pups of the age range 0-2 days and 8-10 days had a 1% chance of survival if they reached the temperature of 5.531°C and 7.620°C, respectively, regardless of the hypothermia device. Conclusively, the use of a freezer for hypothermia-induced humane euthanasia of neonatal mice satisfies the evaluation factors stated by the Panel of Euthanasia: it rapidly induces unconsciousness and death, is reliable and appropriate for the selected signalment, poses little occupational hazard and environmental concerns, and has the potential of alleviating emotional fatigue.

P430 Comparison of an Environmental Sampling Method to Traditional Direct Sampling for PCR Detection of Rodent Pathogens During Quarantine

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An effective and reliable screening method for rodent pathogens is an indispensable component of an institution's rodent health surveillance program to support animal health and prevent detrimental effects on research. Therefore, a vigilant rodent quarantine program is essential to prevent introduction of excluded pathogens. Most rodent quarantine programs use direct samples from live animals for pathogen PCR testing. Collection of direct samples may elicit undue stress to the animal and be time-consuming depending on the number of animals to be sampled. In this study, we compared our standard direct animal sampling method to soiled bedding sampling with a proprietary contact media provided by a vendor for the detection of rodent pathogens in mice quarantined at our institution. The direct animal sampling included the collection of fecal pellet, fur swab, and oral swab from each mouse in quarantine. Our aim was to determine if contact media sampling during quarantine prompts an operable alternative to traditional direct animal sampling. The study included imported mice from various institutions from October 2023 to May 2024. Direct animal samples and exposed contact media were submitted to a commercial laboratory for PCR analyses. The total number of positive agent assay detections by direct PCR sampling was 91 in contrast to 106 with contact media sampling. These results suggest that contact media sampling offers equivalent or improved detection of rodent pathogens as was identified within this study. We also observed that holding contact media samples for 4 to 5 months prior to testing may have degraded and limited the detection of MNV and Astrovirus -1 (RNA viruses). The average number of mice received per shipment varied from 2 to 21. The total time spent collecting direct samples was approximately 50 minutes compared to 25 minutes for contact media sampling. In summary, contact media sampling in quarantine is a viable option to direct animal sampling for detecting rodent pathogens, and can be performed in significantly less time with reduced animal handling.

P431 Pathogen Surveillance of Wild and Feral Rodents Trapped Proximate to Lab Animal Facilities

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Rodents used in biomedical research are maintained as specific pathogen-free (SPF) by employing biosecurity measures to eliminate and exclude adventitious infectious agents known to confound research. To mitigate the risk that wild and feral rodents pose to SPF rodent colonies, it is essential to have a rigorous pest control program that includes maintaining intact facility barriers to prevent the entrance of wild rodents and a program for trapping wild and feral rodents. This study summarizes the results of pathogen screening of wild and feral rodents captured at Charles River facilities in North America and Europe between the years 2013 and 2023. A total of 374 rodent samples, comprising 331 mice and 43 rats, were screened for viruses, bacteria, and parasites using the traditional diagnostic methodologies of microbial culture, serology, and direct exam, as well as PCR. The most frequently detected viruses were murine astrovirus-2 (13.5%), mouse adenovirus (13.1%), mouse cytomegalovirus (12.5%), rodent parvovirus (11.2%), murine chapphamaparvovirus-1 (7.1%), and rodent coronavirus (6.4%). With a frequency of 65.9% positive, *Helicobacter* were the most common bacteria; followed by *Rodentibacter* (42.8%), *Staphylococcus xylosus* (8.3%), *Klebsiella oxytoca* (6.7%), *Campylobacter* (5.5%), S. *aureus* (5.5%), and *K. pneumoniae* (4.9%). The most common parasites were mites (46%), GI protozoa (18.6%), pinworms (9.6%), and *Demodex* (7.5%). These results corroborate that wild and feral rodents may serve as a source of adventitious infection for SPF colonies, thus underscoring the importance of pest control as part of a comprehensive biosecurity program for laboratory animal facilities.

P432 A Cost-Benefit Analysis of the Effect of Different Cage Sizes and Enrichment on Laboratory Rat Welfare

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Lab animal welfare influences the ethics, quality, and translatability of animal-based research. For rats, current laboratory housing standards may limit the performance of natural behaviors, result in abnormal behaviors, create negative affective states, and compromise physical health. A large body of research shows that increasing cage size and providing enrichment improves rats' welfare and ability to be representative models. Cost is described as a primary barrier to refining lab animal housing, so we aimed to identify the most cost-effective housing changes for improving rat welfare. We pair-housed 72 female Long-Evans rats (Rattus norvegicus) in different cage sizes and levels of enrichment (3 x 3 design). We used opaque, enclosed hides, wooden blocks, and cardboard tubes filled with shredded paper as enrichment to facilitate natural behaviors. After four weeks of living in their assigned condition, we evaluated the rats' welfare by: 1) coding recordings of their home cage behavior to assess behavioral diversity (Shannon Diversity Index) and one aspect of emotional well-being (remodeling frequency), 2) using an elevated plus maze to assess a second aspect of emotional well-being (percent open-arm time), and 3) measuring coat condition, body condition, and BMI to assess physical health. Dividing the cost of each housing condition by the effect size of the observed welfare improvements, we found that the most cost-effective way to improve rat welfare was to house rats in the largest of our cage sizes (3019 cm^2). Rats living in larger cages for four weeks displayed more behavioral diversity and better emotional well-being than rats housed in standard caging but did not have improved physical health. A major contributor to the cost-effectiveness of the large cage was that it was cheaper per square inch. We could not find a commercial lab cage larger than our medium cage, so we modified a commercial food storage bin of the same material to fit a standard rat caging lid, resulting in a lower cost. Rats neither interacted with nor escaped from this bin. This study begins to address financial barriers to improving welfare by suggesting a cost-effective housing refinement and laying the foundation for future analyses incorporating a broader range of costs and benefits.

P433 Evaluation of Chlorine Dioxide Room Levels with and without a ClO_2 Clean Air Scrubber

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Chlorine dioxide is a chemical sterilant used commonly in germ-free and gnotobotic animal facilities around the world. This chemical is hazardous and requires precautions when used. Personnel safeguards are, in part, due to vapors off-gassing from the solution. In this study, we assessed utilizing a ClO_2 Clean Air Scrubber to lower the level of chlorine dioxide gas in a gnotobiotic housing room. This is an engineering control, which is considered more effective than utilizing PPE, such as chemical respirators, by The National Institute for Occupational Safety and Health (NIOSH) hierarchy of controls. We hypothesized that running a ClO_2 Clean Air Scrubber would allow personnel to use a chlorine dioxide dunk tank system for up to 1.5 hours a day while remaining below the OSHA permissible exposure limit (PEL) of 0.1ppm (0.3mg/m^3) over an 8-hour work shift. To assess this, chlorine dioxide levels were evaluated with an ATi PortaSens III gas detector at three locations within the same room every 10 minutes over a 1.5-hour time period with and without using a ClO_2 Clean Air Scrubber. The time-weighted average (TWA) was 0.094ppm (2.82 mg/m^3) without the ClO_2 Clean Air Scrubber and 0.036ppm (0.11 mg/m^3) with the ClO_2 Clean Air Scrubber over 1.5 hours. While the TWA for both options was below the OSHA permissible exposure limit, there was a 39% reduction in chlorine dioxide in the room using the ClO_2 Clean Air Scrubber. In conclusion, a ClO_2 Clean Air Scrubber is a valuable engineering control to reduce chlorine dioxide gas levels and may be preferred over PPE in germ-free and gnotobiotic facilities.

P434 Impact of Water Flow Initiation Timepoint in Larval Zebrafish and Rotifer Polyculture on Fish Survival, Growth, and Sex Differentiation

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Zebrafish are increasingly recognized as a powerful model organism, uniquely suited to a wide range of studies with relevancy in drug development and genetic disease modeling. Despite their popularity, husbandry standards vary significantly between institutions. Feed and water quality standards for larval zebrafish significantly impact growth rate and sexual differentiation. Many institutions raise larval zebrafish in rotifer polyculture tanks with daily administration of supplemental rotifer solution. To evaluate the impact of water quality and food availability on larval welfare, we assessed survival, size, and sex differentiation in zebrafish provided daily rotifer solution with water flow introduced at 5-, 10-, or 15-days post-fertilization (dpf). We compared these outcomes with larvae provided daily dry feed with water flow introduced at 5dpf. AB (wildtype) zebrafish were randomly assigned to one of four larval care groups: (1) rotifer solution with water flow started at 5dpf, (2) rotifer solution with water flow started at 10dpf, (3) rotifer solution with water flow started at 15dpf, and (4) dry feed with water flow started at 5dpf. All fish were housed at 30 fish per tank from 5-15dpf. After 15dpf, all fish were housed in 2.8L tanks under identical care parameters. Survival was assessed at 30 and 90dpf. Sex and fork length were assessed at 90dpf. Fish reared in rotifer polyculture tanks with flow started at 10dpf and dry diet tanks had significantly higher 30dpf survival rates but were significantly smaller at 90dpf than those in other groups. 90dpf survival and sex differentiation did not differ significantly between groups. Our results suggest rotifer and dry diets perform similarly, but that earlier introduction of continuous water flow in rotifer polyculture may be associated with improved water quality but reduced food availability and growth rate.

P435 Applications of Continuous Location and Temperature Monitoring in Spaceflight and Analogue Research with Mouse Models

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Many animal models in spaceflight and spaceflight analogue research are used to study the health effects and hazards of space travel. Rodent models, specifically mouse models, are very commonly used in this research and mice dominate the animal-based research work on the International Space Station (ISS). Animal welfare and health is a priority with all animals used in spaceflight research, but it is held in especially high priority for mice flown to the ISS. Due to the extreme time, funds, and labor needed to complete spaceflight research with animal models, it is also a high priority to obtain as much data and scientific information from each animal as possible. Because of these two priorities, remote monitoring capabilities provided by microchip-sensing equipment have been pursued as a tool for ground and flight-based spaceflight research involving mice. Here, we describe the potential and preliminary findings of incorporation of a home cage monitoring system that continuously tracks the movement, location, and temperature of each mouse in the cage via microchips. The system, a UID product, uses a plate with a grid of radio-frequency identification (RFID) scanners that rests below the cage and sends the temperature, cage/resource

location, and movement activity to a linked controller equipped with data analysis software. In this pilot study, microchipped male (n=5) and female (n=5) mice were group-housed (2-3 mice per cage) and monitored for a two-week period. Data was acquired from the tracking plates and used to create a set of metadata for testing machine learning analysis (in this study, Random Forest was used). Though the sample size was not large enough to determine significant differences in the parameters (movement, location, temperature) between sexes of mice, we conclude that this approach has potential for use in an experimental setting evaluating multiple treatment and/or disease conditions. For our research purposes, this approach can not only provide higher resolution health and behavioral data but also dramatically improve animal welfare with non-invasive measurements that do not disrupt the animals' sleep cycles or activities, which will be particularly useful for spaceflight studies. Healthy mice were used for this study, which established standard resource zones and temperature/movement parameters. These standard parameters can be used to compare responses to flight hardware, test diets, and other spaceflight-related items that may be incorporated into flight habitats or transporters.

P436 Assessment of Consumption of a Newly Formulated Hydration Gel in Mice and Rats

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Hydration gel has been used by our facility both as supplemental hydration for at risk animals as well as in our commercial production shipping operations as a source of water during transit for many years. A newer formulation was developed by the manufacturer using an alternative food-grade hydrocolloid intended to improve supply chain availability. This study was designed to evaluate the effect and palatability of the newer formulation on both mice and rats. Weaned mice and rats were used. Three cages of five mice per strain (C57BL/6NCrl, Crl:NU(NCr)-Foxn1^{nu}, Crl:CD1(ICR)) and three cages of four rats per strain (LEW/Crl, Crl:CD(SD), Crl:NIH-Foxn1^{rnu}) were given normal chow ad lib and each cage was given either control (older formulation) or test (alternative hydrocolloid). Body weights, hydration status, and clinical assessment were completed daily for 14 days. Consumption of both gel products was recorded daily and replaced when <25% of the gel remained. Overall, no animals exhibited clinical signs of illness or dehydration, and body weights increased over time. The average intake of the control hydration gel per strain per animal for mice was 5.5 ± 0.8g, and for rats 58.7 ± 10.1g. The average intake of the test gel/strain/animal for mice was 7.1 ± 1.6g, and for rats 60.4 ± 6.5g. The average intake/cage between Crl:CD1(ICR) control and test groups was significantly different ($P<0.05$); however, there was no significant difference between average intake/cage in either control or test groups for all other strains. Thus, there was little difference between intake/animal between control or test formulation of the hydration gel, and no clinical concerns or body weight loss was noted at any time point.

P437 Use of 2D Scannable, Barcode Ear Tags for Identification of Rats

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Precise identification of animals is vital to the collection of accurate data and analysis. Traditional visual identification methods, such as metal ear tags or ear punches/notches, present several challenges, including tag loss and tissue regrowth as animals age. A newer method using 2D scannable barcode ear tags made of a light eight, biologically inert polymer addresses these issues by reducing tissue reactions that can lead to fall-out. These tags are also available in various colors for easy visual identification and can be scanned with a light scanner, eliminating misinterpretation associated with ear punches/notches. The ear tag fits similarly to a piercing where a cylindrical wedge attached to the barcode is fastened to a separate piece behind the ear with the use of an applicator. Although currently marketed for use in mice, the objective of this pilot study was to assess the feasibility of these tags in rats focusing on retention, appropriate fit to account for a thicker pinna, as well as tolerance to the tag. Rat carcasses were used initially to assess two sizes of the same tag, standard (larger) and mini, and if they could be used in the pinna of rats over 80 days of age. The results showed no application issues, and the tags provided adequate spacing to accommodate for the thicker pinna of rats compared to mice. For the main study, 46 rats on a Wistar Han background from 89-354 d of age were tagged and singly housed for the remainder of the study. 21 animals were given the standard tag, and 25 animals were given the mini tag. Animals were observed daily for two weeks for signs of pain, irritation or inflammation, discharge, self-mutilation, or injury, as well as tag fallout. Observations lasted 2-5 minutes per rat without disturbing the cage, followed by a physical exam. The results indicated that 33% of the standard tags and 8% of the mini tags were lost however, the means were not significantly different ($P>0.05$). Of the animals where tags were lost, mild red scabs were noted at the site of application. Overall, the results of the study suggest that short-term use of the tags in rats older than 80 days does not impact the overall health of the animal and that tag size did not affect the rate of fallout. Further studies should be conducted to assess the long-term use of the tags, fall-out rates, and at various age groups.

P438 Evaluation of Stress Related to Transportation of Surgical Modified New Zealand White Rabbits

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Transportation of laboratory animals from production to study sites is required to support biomedical research. This transport may raise concerns about the potential transport-related stress of animals. We aimed to evaluate the level of stress experienced by surgically modified rabbits related to transit by measuring stress hormones in the blood. We investigated the potential stress response due to the transportation of rabbits in an environmentally controlled dedicated truck by measuring blood corticosterone (CORT) levels. Eight male and seven female 3.0 to 4.0 kg, New Zealand White rabbits [Crl:KBL(NZW)] were used in the study. The rabbits were implanted with either a jugular or femoral vein catheter connected to transcutaneous buttons placed in the scapular area and had a smooth recovery. Animals were monitored for ten days and then shipped to the study site in an environmentally controlled dedicated truck. The total journey took about 36 hours (about one and a half days). At the surgery and study sites, rabbits were housed in individual cages and maintained 16 to 22 °C with a relative humidity of 30%-70% and a 12:12 hour light:dark cycle. Feed and water were provided ad libitum. Body weights, detailed physicals, and catheter patency checks were performed regularly. Blood samples were collected via transcutaneous button at the surgery site before shipping, on the day of arrival at the destination study site, and day2, day7 day14 after arrival. The blood CORT was measured using the ELISA method. The animals arrived at the study site in good condition and remained clinically healthy throughout the study. The average blood CORT was 2.2±1.1 ng/mL (0.9 to 4.1 ng/mL) in males and 2.5±1.0 ng/mL (1.0 to 3.9 ng/mL) in females before shipping. On the day of arrival at the study site, blood CORT was 3.7±0.6 ng/mL (2.9 to 4.5 ng/mL) in males and 3.1±0.4 ng/mL (2.8 to 3.8 ng/mL) in females. The CORT levels for day2, day7 and day14 after arrival were 3.5±0.6, 4.8±0.8 and 4.6±0.6 ng/mL for males, 3.0±0.7, 3.6±1.2 and 4.0±1.1 ng/mL for males, similarly to day1 and after transportation. The slight increase in CORT level post transportation is not significant and stable for 2 weeks. This study indicates the stress level as measured by blood CORT due to transportation in rabbits is negligible.

P439 Rhythm and Snooze: Assessing the Impact of Gabapentin on ECG Parameters and Sedation in New Zealand White Rabbits

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Oral gabapentin has been routinely used as an effective sedative agent in a variety of species, and previous data have suggested that a dose of 25 mg/kg can decrease reactivity in rabbits without overt physiologic impact. Although this presents a promising refinement for preclinical use, there is insufficient data establishing a practical benefit in common data collection procedures or the impact on cardiac-specific indices relevant to drug development. In this study, we evaluated the effect of a single dose of gabapentin (GABA, 25 mg/kg PO) in 6-month male New Zealand white rabbits (n = 6) on ECG parameters routinely assessed in drug safety, in comparison to conscious (CTRL) conditions and sedation with ketamine/dexmedetomidine (K/D, 10/0.05 mg/kg SQ). Lead II ECG collection was performed in sternal recumbency under gentle manual restraint following acclimation in a quiet room and at peak predicted sedation (GABA: +2 hours, K/D: +20 minutes). Sedation scoring was performed concurrently, with fecal/urine output and water/food intake measured for the following two days. Under CTRL conditions, rabbits demonstrated normal ECG indices (HR: 197±16 bpm, PR: 60±9 ms, P-duration: 45±5 ms, P-amplitude: 0.045±0.01 mV, QRS: 72±15 ms, QT: 181±15 ms). GABA resulted in no significant changes in any interval or amplitude, while K/D significantly decreased HR and P-amplitude (HR: -17±10%, P-amplitude: -34±22% vs. CTRL). Sedation scores and ins/outs were marginally affected in GABA rabbits, while K/D consistently resulted in full sedation with a significant reduction in food intake through Day 1 (-24±21% vs. CTRL). All changes in K/D animals, including insignificant trends in decreased water intake and fecal output, were resolved by Day 2 post-sedation. Taken together, these results support the previous body of work on the safety of gabapentin in rabbits with limited systemic consequence in comparison to ketamine/dexmedetomidine. While this study additionally identified no significant perturbations in ECG parameters, gabapentin administration in this context failed to elicit a favorable behavioral influence. Therefore, at a dose of 25 mg/kg PO, gabapentin is well-tolerated in rabbits but does not appear to provide a notable impact on tractability during non-aversive procedures.

P440 The Analgesic Effect of Two Different Extended-Release Meloxicam Formulations in Rats

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Meloxicam is a nonsteroidal anti-inflammatory drug (NSAID) drug frequently administered once a day to control mild to moderate pain in rodents. Extended-release meloxicam offers a refinement of less frequent dosing and an extended therapeutic window as compared to the standard meloxicam formulation. The aim of this study was to compare the analgesic efficacy of two different extended-release meloxicam formulations to a standard meloxicam formulation for an incisional pain model in rats. Adult Long Evans rats (n=32) were randomly assigned into one of four treatment groups (n=8 per group): 1) Saline (Saline, 0.9% NaCl, 5 mL/kg, SC; once), 2) Meloxicam (Melox; 2 mg/kg, SC, SID); 3) Meloxicam extended-release polymer (Melox-ER, 4 mg/kg, SC, once); or 4) Meloxicam extended-release suspension (Melox-XR; 4 mg/kg, SC, once). While under isoflurane anesthesia, a 1-cm longitudinal skin incision was made on the plantar hind paw five minutes after drug administration. Mechanical and thermal hypersensitivity assessments were performed one day prior to surgery (D-1), 4 hours after surgery (D0) and three consecutive days following surgery (D1, D2, and D3). Mechanical (D0-D2) and thermal (D0-D3) hypersensitivity was observed in the saline group. Compared to the saline group, Melox attenuated mechanical hypersensitivity on D0 and D1, Melox-ER did not attenuate mechanical hypersensitivity at any timepoint, and Melox-XR attenuated mechanical hypersensitivity only on D2. When comparing thermal hypersensitivity to the saline group, Melox did not provide attenuation at any timepoint, Melox-ER provided attenuation on D0 and D3, and Melox-XR provided attenuation on D0 and D1. No abnormal clinical signs were noted, but injection site reactions were noted in the Melox-ER and Melox-XR groups. Results indicated that Melox-ER at 4 mg/kg provides varied attenuation of thermal hypersensitivity, and Melox-XR at 4 mg/kg provides attenuation of thermal hypersensitivity for up to 48 hours. We recommend using Meloxicam-ER at 4 mg/kg every 24 hours or Meloxicam-XR at 4 mg/kg every 48 hours for minor incisional pain in rats.

P441 Effects of a Peripheral Alpha2-Adrenergic Antagonist, Vatinoxan, on Adult Wistar Rats Sedated with Subcutaneous Medetomidine and Midazolam

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Vatinoxan, an alpha2-adrenoceptor antagonist that poorly penetrates the blood-brain barrier, is used in veterinary medicine to attenuate the cardiovascular effects of medetomidine. As the combination has not been previously studied in rats, we evaluated the effects of medetomidine (0.25 mg/kg) and midazolam (2 mg/kg; MM) with or without vatinoxan (5 mg/kg; MMV) in a randomized cross-over design using ten adult male Wistar rats. We hypothesized that MMV would shorten the onset of sedation and provide reliable sedation with less hypertension and bradycardia compared with MM. Drugs were administered subcutaneously in the neck. Rats were maintained in a temperature-controlled chamber and provided with flow-by oxygen. The time to loss of the righting reflex was measured. Oscillometric mean arterial pressure (MAP) was monitored with a tail cuff for 55 minutes after injection. Pulse rate (PR) and oxygen saturation were monitored via pulse oximeter and recorded until 55 minutes after injection. All rats were administered atipamezole (1.25 mg/kg) and flumazenil (0.2 mg/kg) at the end of each trial. Compared to MM, time to loss of the righting reflex was significantly reduced ($p=0.050$), PR was maintained significantly higher ($p<0.05$), and MAP was significantly lower with concomitant vatinoxan ($p<0.05$) without causing hypotension (<60 mmHg). In conclusion, subcutaneous vatinoxan, when administered with medetomidine and midazolam, significantly shortened the onset of sedation and mitigated hypertension and bradycardia, showing potential for a safer and more efficient sedation of adult male Wistar rats.

P442 Pharmacokinetics of Subcutaneous Buprenorphine or Meloxicam Administration in the Jamaican Fruit Bat

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The Jamaican fruit bat (*Artibeus jamaicensis*) is a valuable model for the study of infectious diseases due to their size and unique virus-host interactions. Given their important role and increasing use in research, they may frequently undergo procedures that require analgesia. Knowledge of analgesics in this species is lacking.

This study aimed to determine the pharmacokinetics of a single subcutaneous buprenorphine or meloxicam injection to guide future analgesia studies. Buprenorphine (0.5 mg/kg) was administered subcutaneously, and blood was collected at 1, 2, 4, 8, 12, and 24 hours post-injection. At each timepoint, 0.3mL of blood was collected non-terminally from their cephalic veins (n=3). Three bats were euthanized for intracardiac sample collection to provide baseline values. Meloxicam (5 mg/kg) was administered subcutaneously, and bats were euthanized for intracardiac blood collection at 0.5, 1, 2, 4, 8, 12, 36, 48 hours post-injection. Buprenorphine had a maximum plasma concentration one hour after injection, with a maximum concentration of 22.6 (0.52) ng/mL, a terminal half-life of 1.83 h, and a total exposure (area under the curve) of 94.9 (8.8) h*ng/mL. The plasma concentration of buprenorphine was below the purported therapeutic plasma level in rats of 1.0 ng/mL between 8-12 h after administration. Meloxicam had a maximum plasma concentration at 0.5 h after injection, with a maximum concentration of 29.1 (1.3) µg/mL, a terminal half-life of 3.9 h, and a total exposure of 136.5 h*µg /mL. The plasma concentration of meloxicam was below the purported therapeutic plasma level in dogs of 390 ng/mL 12-24 h after administration. This data indicates that buprenorphine at 0.5 mg/kg will provide analgesia for 8-12 hours, and meloxicam at 5 mg/kg will provide analgesia for 12-24 h, although confirmation with efficacy studies are still needed.

P443 Comparative Safety and Efficacy of Extended-Release Buprenorphine Formulations for Mouse Reproductive Surgeries under Tribromoethanol

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Extended-release buprenorphine formulations are commonly used to control post-operative pain in rodents with minimal handling-related stress. An FDA-indexed formulation is now available which has been demonstrated safe and effective with ketamine-xylazine and isoflurane anesthesia; however, safe use in combination with tribromoethanol, a non-pharmaceutical grade anesthetic sometimes favored for short, high-volume procedures, has not been reported. In this study, we compared the safety and efficacy of the FDA-indexed formulation at the labeled dose (3.25 mg/kg) to the compounded extended-release buprenorphine formulation used by the centralized Transgenic Service at our institution at the manufacturer-recommended dose (1 mg/kg) in CD-1 mice under tribromoethanol anesthesia. A pilot (n= 5 females per drug) was initially conducted with anesthetic and analgesic in the absence of surgical manipulation, after which the formulations were compared in embryo transfer and vasectomy surgeries (n= 10 males or females per drug). Relative efficacy was assessed at 6, 24, 48, and 72 hours after surgery using a cageside ethogram, frequency of rearing behavior, and weight change. No differences were seen between analgesic treatment groups. Safety was evaluated by measuring intraoperative respiratory rate and recovery time, incidence of analgesic injection site reactions, and, for embryo transfers, pregnancy success, litter size, and pup weight. Ulceration was only observed at the injection site of mice receiving compounded drug. However, this difference was not statistically significant. Pup weight at weaning was significantly lower in the FDA-indexed group (mean 17.1 g vs 18.2 g, p = 0.03). No differences were observed in other metrics. These results indicate that FDA-indexed extended-release buprenorphine has a similar safety and efficacy profile to the widely-used compounded formulation but may have subtle effects on offspring that should be further characterized.

P444 Analgesic Efficacy of Oral Carprofen Treated Gel and Tablets for an Incisional Pain Model in NSG Mice

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Stress in research animals affects their welfare and alters physiological responses, potentially impacting research outcomes. Laboratory rodents often experience stress from repeated parenteral drug administrations due to handling and needle punctures. In this study, we compared the efficacy of oral carprofen tablets and carprofen-treated nutritional gels versus injectable carprofen for alleviation of postoperative mechanical and thermal hypersensitivity in NSG mice. Male and female mice (n = 59) were randomly assigned to one of five groups: 1) Placebo (Placebo tablets or NutraGel, daily); 2) carprofen tablets (CarpTabs, 2 mg, daily); 3) carprofen injection (Carp25SC; 25mg/kg, SC daily), low-dose carprofen treated gels (CarpGel-low at 0.11mg/mL of gel, daily); and high-dose carprofen treated gels (CarpGel-high at 0.22mg/mL of gel, daily). Mechanical and thermal hypersensitivity were assessed before surgery on D-1 and at D0 (4 h), D1, and D2 afterwards. Plasma carprofen concentration (n = 56) was assessed over 1-4 days. Daily clinical observations, fecal occult blood testing, and gross necropsies were performed. Results showed that mechanical and thermal hypersensitivity persisted in the placebo group throughout the study (D0-D2). CarpTabs and Carp25SC groups effectively attenuated mechanical hypersensitivity compared to the baseline (D0-D2). Compared to the baseline, CarpGel-low attenuated mechanical hypersensitivity on D1 and CarpGel-high attenuated mechanical hypersensitivity on D2. All carprofen treatment groups, except Carp5Gel-high at D0, attenuated mechanical hypersensitivity compared to placebo. Thermal hypersensitivity persisted in all carprofen treatment groups compared to baseline values. However, compared to the placebo, CarpTabs, Carp25SC and CarpGel-low provided attenuation of thermal hypersensitivity. Plasma carprofen concentrations didn't significantly differ among oral formulations. Fecal occult blood testing was positive only in the CarpGel-high group, one of six mice at one and two days after administration. These findings support using, orally administered CarpTabs and CarpGel-low as an alternative analgesic to Carp25SC for incisional pain in NSG mice.

P445 Evaluation of Novel Total Intravenous Anesthesia (TIVA) by Guaifenesin/Propofol in Mice *(Mus musculus)*

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General mouse anesthesia may occasionally require employing atypical agents, as the customary agents (e.g., isoflurane, ketamine/xylazine) may cause research interference. Here, we assessed the effectiveness of a total intravenous anesthesia (TIVA) combination of a muscle relaxant, guaifenesin (5% GG), and an injectable anesthetic, propofol (P), to maintain general anesthesia in male FVB 129 F1 mice (n=3) for 60 minutes (min). Following isoflurane anesthetic induction, a tail vein catheter was placed. An intravenous loading dose of GG, 1.0 ml/kg, followed by 2.0 ml/kg/h, along with P at 150 mg/kg/h. Animals were provided 100% O2 via a mask throughout TIVA. Anesthetic parameters (heart rate, respiratory rate, %SPO2, and body temperature) were recorded every 10 min for 60 min. The TIVA was discontinued at 60 min, and mice were observed for complications (apnea, prolonged recovery), moved to a recovery cage, monitored for time to ambulate, and returned to their home cage. The GG-P combination successfully maintained general anesthesia for 60 min with no significant changes in anesthetic monitoring parameters. After GG-P cessation, mice ambulated within 5 min and returned to a home cage within 15 min without complications. This TIVA technique using the GG (0.5-2 ml/kg/h) and P (80-150 mg/kg/h) combination supplemented with oxygen provided effective mouse general anesthesia for 60 min. This is the first evaluation of GG and P combination for general mouse anesthesia.

P446 Effect of Isoflurane Anesthesia on Hematologic Parameters of Swine (*Sus scrofa domestica*)

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Swine are commonly utilized in biomedical research as surgical models or in other experiments requiring the use of anesthesia. Isoflurane is a common inhalant anesthetic used in swine, which has been shown to alter hematologic parameters in other species. However, the effects of isoflurane on hematologic parameters of swine have not been defined. In this study, we examined the effect of isoflurane anesthesia on hematologic parameters in 27 Yorkshire/Landrace hybrid domestic swine over time. Swine were sedated with intramuscular injection of either tiletamine-zolazepam or a combination of ketamine-acepromazine-hydromorphone, induced with 3-5% Isoflurane, and maintained with 1-3% Isoflurane. Venous whole blood was collected for hematologic analysis at baseline after sedation, then at 30 and 60 minutes after starting inhaled isoflurane. Initial results show significant decreases in hematocrit, RBC count, and hemoglobin at each time point after isoflurane compared to baseline. Mean hematocrit had decreased by five percentage points after 60 minutes of isoflurane administration. Additionally, reticulocytes were not detected in any animal at the baseline blood draw but were present in 12 of 27 swine after 60 minutes of isoflurane anesthesia. These results indicate that isoflurane anesthesia alters certain hematologic parameters in swine, and care should be taken to avoid misinterpretation of complete blood counts drawn from anesthetized swine.

P447 An Investigation of Breathing Systems with and without Disinfection Post-Procedure in Swine

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Disinfecting and drying of anesthesia rebreathing circuits after each use is time-consuming and often results in tubing and rebreathing bags wearing out more rapidly. Our standard practice has been to clean all tubing and rebreathing bags with dilute 2% chlorhexidine solution and then let them dry after each use. Literature for both humans and small animals suggests that disinfecting is not necessary and that allowing anesthetic tubing to dry between uses is sufficient; however, the necessity for disinfecting after use in swine is unknown. During anesthesia, swine produce a significant amount of humidity, resulting in saturation of bacterial/viral filters and condensation in the tubing. We hypothesized that due to the high amount of moisture, anesthetic tubing used in swine procedures, regardless of breed, will require disinfection between patients. To determine the bacterial load in the tubing after anesthetic events lasting at least 1hr in both domestic (n=6) and Yucatan (n=9) swine, swabs were collected from the proximal end of f-circuits and the endotracheal tube at the conclusion of the anesthetic event. The tubing was swabbed again after either being cleaned using dilute 2% chlorhexidine solution and then dried or just allowed to dry. The swabs were sent out for aerobic and anaerobic culture and bacterial identification. After anesthesia, the endotracheal tubes showed abundant bacterial growth and the tubing showed light to no growth. Contrary to our hypothesis, there was no bacterial growth present in the tubing regardless of if it was disinfected or not. Our preliminary data, collected from 15 anesthetic events, indicates that the standard practice of disinfecting anesthesia tubing after use is not necessary in swine. The results of this study will have both a cost and labor saving effect on our program without negative impact on animal health.

P448 Determining a Sedative Dose of Dexmedetomidine in the African Green Monkey

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There is a knowledge gap regarding dexmedetomidine-induced sedation in non-human primates. Dexmedetomidine is an appealing candidate for short-term procedures because it causes minimal respiratory depression and produces rapidly reversible sedation. This project sought to determine a protocol for short-term sedation of African Green monkeys using intravenous infusion of dexmedetomidine. Ten African Green monkeys were placed in a restraint chair using pole and collar techniques, and then an intravenous catheter was placed in the saphenous vein. Dexmedetomidine was infused at a fixed rate via a syringe pump, and the monkey was monitored for sedation via loss of posture, loss of hand grip, and changes in heart rate and blood pressure. The rate and duration of dexmedetomidine infusion were adjusted between monkeys to identify a protocol that produced ~15 minutes of hand grip loss while maintaining mean arterial pressure ≥45 mmHg. An infusion of 10 µg/kg of dexmedetomidine at 40 µg/kg per hour met this criteria. Loss of posture was not observed as frequently as other signs of sedation, such as markedly reduced muscle tone, loss of grip, yawning, and brief periods of eye closure. Six of ten monkeys received dexmedetomidine at a rate of 40 µg/kg per hour, which led to the loss of hand grip response at 9.3 ± 2.1 minutes (mean ± SD) after initiation of infusion. The three animals that received a total dose of 10 µg/kg lost hand grip for 14.3 ± 0.6 minutes. All ten monkeys were able to return to their home cages without incident after a brief period of recovery in the chair. Dexmedetomidine has a strong safety profile and a broad therapeutic index, but the main side effects of bradycardia and hypotension are potentially concerning. Each animal in this study that received the optimized dexmedetomidine dosing protocol maintained mean arterial pressure in a safe range, with minimum mean arterial pressure measurements ranging from 73-77 mmHg. This illustrates that African Green monkey can be successfully sedated with dexmedetomidine with minimal detrimental cardiac effects.

P449 Tiletamine-Zolazepam Total Intravenous Anesthesia (TIVA) for Imaging in Sheep

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Total intravenous anesthesia (TIVA) is an alternative to inhalant anesthesia (IA) when IA is unavailable or contraindicated. This study investigated the anesthetic efficacy of tiletamine-zolazepam (TZ) through continuous rate infusion in sheep undergoing a 120-minute non-invasive imaging procedure. We hypothesized the TZ continuous rate infusion would provide effective general anesthesia for imaging. Six male Dorset sheep were sedated with 4-6 mg/kg TZ intramuscularly, intubated, and maintained on 5-15 mg/kg/hr TZ intravenous continuous rate infusion. Measured anesthetic parameters included heart rate, oxygen saturation (%SpO2), end-tidal carbon dioxide ($ETCO_2$), body temperature, and direct arterial blood pressure (systolic, diastolic, and mean); blood gas analysis was performed during anesthesia. Time to extubation and standing (recovery) were measured. Other clinical observations (thrashing, activity, vocalization, and general appearance) were also assessed throughout recovery. Heart rate, %SpO2, $ETCO_2$, body temperature, and direct arterial blood pressure were stable

throughout imaging anesthesia. Time to extubation and standing (recovery) were 24.5 ± 3.7 and 31 ± 5.5 min, respectively. No abnormal clinical observations were noted. This data suggests that Tiletamine-Zolazepam TIVA provides effective general anesthesia for up to 120 minutes of non-invasive imaging.

P450 A Comparison of Morning and Afternoon Vaginal Smear Sampling for Estrous Cycle Monitoring in Sprague Dawley® and Wistar Han® Rats

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Determination of the phases of estrous cycle in experimental rat studies is important for reproductive function studies and is required by different regulatory authorities. Vaginal smear/cytology is a widely used and accepted technique for estrous cycle phase determination. Vaginal smears are usually collected in the morning (e.g., between 8 to 11 am at our facility). However, sample collections in the early morning could lead to a missed proestrus stage and could be challenging when multiple studies are conducted at the same time in the same test facility. To minimize missed proestrus stage and to provide timing flexibility of sample collection, we evaluated the vaginal smear samples collected in the morning and afternoon in Sprague Dawley® and Wistar Han® rats. Ten female rats per strain were assigned to morning (08:00 to 08:45) or afternoon (14:00 to 14:45) vaginal smear collection groups. Vaginal smear samples were collected daily for two weeks using vaginal lavage, and slides were read fresh. Slides were allowed to dry and retained for reevaluation. Our results demonstrated that the mean cycle length and the number of cycles are similar between morning and afternoon groups for both strains, fewer missed proestrus stages were noted in the afternoon groups compared to the morning groups, and reevaluation data showed that afternoon sampling provided more accurate initial estrous cycle phase determination compared to morning sampling.

P451 Impact of Aging and the Estrous Cycle on Anxiety Responses in SHR and WKY Rats

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SHR rats are a model for Attention Deficit Hyperactivity Disorder (ADHD), while WKY rats are a model to study endogenous depression. Both strains are extensively employed in research on these conditions, making the evaluation of their baseline anxiety levels using the elevated plus maze (EPM) essential in neuroscience and pharmacology research. This study aimed to quantify anxiety levels in SHR and WKY rats (6 and 12 months of age) during different phases of the estrous cycle using the EPM. Twelve female SHR/NCrl rats and seven female WKY/NCrl rats, with estrous cycles lasting 4-5 days, were included in the study. The rats were grouped by age and strain, and their behavior was assessed in the EPM, comparing the time and distance traveled in the open and closed arms using specialized software. Statistical analysis (one-way ANOVA and the Kruskal-Wallis test), revealed significant differences in exploration times in the open and closed arms of the EPM between the proestrus, estrus, and diestrus phases in both SHR and WKY rats. SHR rats exhibited lower anxiety, indicated by higher time and distance values in the open arms compared to WKY rats, and these differences persisted with age. In contrast, WKY rats showed increased anxiety with age. These findings provide valuable insights into the mechanisms of anxiety and aging in these animal models, informing the development of pharmacological treatments for anxiety associated with perimenopause in 12-month-old rats.

P452 Avoiding Prenatal Toxicity of Tamoxifen Exposure in Pregnant Mice

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Lymphatic networks in mammalian lungs are essential for the transition to air breathing at birth. Lymphatic endothelial cells (LECs) within these networks facilitate myriad vital functions. However, the precise origin of lung LECs during the crucial early postnatal period remains unclear. In other organs, such as the heart, myeloid cells contribute to lymphangiogenesis. To investigate the possible contribution of the myeloid lineage in early postnatal lung lymphangiogenesis, we utilized a myeloid Cre reporter mouse in the first week of postnatal life. We showed that lung LECs are labelled with >75% efficiency when exposed to tamoxifen via intraperitoneal (IP) injection on postnatal day (PND) 1 and 2 (n=18, six independent experiments). One explanation for our findings is a novel myeloid origin for neonatal lung LECs. To resolve this issue, we next used a lymphatic Cre reporter mouse to label canonical prenatal venous-derived origin LECs. Our experimental design could determine whether the prenatal population of LECs is replaced after birth by a myeloid progenitor. This required administering pregnant mice tamoxifen which is usually a potent embryocidal chemical. To overcome its toxicity and obtain healthy pups, we administered 3 mg of tamoxifen plus 3 mg of progesterone dissolved together in corn oil via IP injection on E13.5. Preliminarily, we have been able to obtain healthy, live pups after cesarean section on P19.5 with >85% efficiency of lineage labeling observed at PND1-2 (n=4, two independent experiments). This study design has the potential to uncover novel perinatal aspects of development in the mouse and can help resolve pre- and postnatal cell lineages that contribute to organogenesis. The midgestational tamoxifen plus progesterone regimen we have utilized may be useful for other investigations requiring prenatal labeling of cells or tissues of interest, with the goal for obtaining live pups to study postnatally.

P453 Effect of Insert Size on Knock-In Rate in Mice with CRISPR/Cas9 and AAV Vectors

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Our laboratory has been working on introducing genetic modification technology to construct mouse model libraries for rare/intractable diseases in humans (https://animal.nibiohn.go.jp/research/e_rare-disease-model-library.html) for studying the molecular mechanisms of genes in such diseases. As part of these efforts, we re-evaluated a knock-in mouse production method using a combination of a CRISPR/Cas9 system and AAV vector. In the present study, we investigated the effect of insert size on the knock-in rate in mice. We used three AAV vectors containing: #1) the CAG promoter, the EGFP cDNA, and the SV40 polyA signal (~2.7 kb); #2) the splicing acceptor, the EGFP cDNA, and the SV40 polyA signal (~1 kb); and #3) EGFP cDNA only (~0.7 kb). The first two vectors targeted Rosa26, while the last vector targeted Muc1. All vectors also contained a homology arm of 700 bp each on the left and right sides. Cas9 protein and guide RNA corresponding to each target were introduced by electroporation into C57BL/6N pronuclear stage embryos obtained by in vitro fertilization. The embryos were then co-cultured with ~10^{10} GC/mL of AAV vectors overnight. The next

day, the 2-cell embryos were transferred to uterine foster mothers. The resulting pups were evaluated for successful knock-in by fluorescence or PCR. From 120, 108, and 150 2-cell stage embryos, 17 (14%), 30 (28%), and 29 (19%) pups were born, and five (29%), 20 (67%), and 19 (66%) of the pups have knock-in alleles for vectors #1, #2, and #3, respectively. Although AAV vectors are effective and can knock in longer strands than ssODNs, shorter strands are still more efficient, so the length of each vector component must be considered for efficient knock-in.

P454 Confirmation of Germ-Free Health Status in Embryo Transfer Rederived Mouse Models

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Studying diseases involving the microbiome in conventional housing is challenging due to limited control of microbial exposure. Gnotobiology minimizes these challenges by establishing and maintaining germ-free (GF) animals where microbes are defined and controlled, thereby clarifying the role of microbe-immune interactions in normal physiology and diseases. In this study, we utilized embryo transfer rederivations to generate genetically modified GF anexic animals to facilitate cancer and microbiome research. GF Swiss Webster mice are maintained in isolators for transgenic production. Recipient females are hormone-primed to synchronize estrus and paired with vasectomized males to induce pseudopregnancy. Donor mice are superovulated and naturally mated to a male of the same strain allowing for 20-50 embryos to be harvested from the donor uterus. Twelve embryos are implanted into each uterine horn of the recipient female. Two ET trials were performed. In one trial, two of four recipients yielded eight pups, four of each sex. In the second trial, one of four recipients yielded seven pups, two females and five males. This ET rederivation presents two critical points for contamination risk. First, highly trained personnel must follow strict protocols. Second, GF recipient females exit an isolator, are transported to a class II BSC with an integrated surgical microscope where donor embryos are implanted, and, upon recovery, are returned to a new isolator. Monitoring GF colonies microbiologically is vital for maintaining GF status. The isolators are set up and monitored to ensure sterility. Isolators are confirmed free of microbes by sampling feces, drinking water, food, and swabs of the isolator interior into a composite sample. Feces are gram stained, while the composite sample is evaluated with 16s PCR for all bacteria, plated on blood agar, thioglycolate broth, and fungal media, which is incubated aerobically and anaerobically to confirm the absence of microbes. The isolators containing the offspring generated from the ET rederivation trials were confirmed GF with no aerobes, anaerobes, or fungi present, no bacteria on fecal gram stain, and 16s PCR negative.

P455 Genetic and Phenotypic Comparison of C57BL/6-Tg(TRAMP)8247Ng/J (B6 TRAMP) Live vs Cryopreserved Repository Mice

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Mouse repositories have several options for managing inventory, including live colonies and cryopreservation of sperm or embryos. While maintaining live mice may provide faster access to animals, factors to consider include space, number of animals, husbandry costs, potential losses due to disease outbreaks, breeding issues, and genetic and phenotypic drift. Cryopreservation is considered a colony management tool that assuages most concerns of maintaining live colonies, although there are still considerations for strain feasibility, recovery costs, and recovery time. The C57BL/6-Tg(TRAMP)8247Ng/J (known as B6 TRAMP) mouse strain is a widely used model of human prostate cancer. In a facility that maintained both a live colony and cryopreserved embryos of B6 TRAMP mice, a concern arose regarding possible genetic and phenotypic drift in the live colony. To determine if there was copy number variation, transgene copy numbers were assessed by ddPCR and were confirmed to be stable across the colony. To investigate if there was a change in prostatic lesions, two cohorts of male mice from the live repository and the cryopreserved repository were aged for 30 (live n = 15, cryo n = 23), 40 (live n = 16, cryo n = 16), or 50 (live n = 14, cryo n = 18) weeks. At study or humane endpoints, mice were euthanized, and prostate lobes were collected. Each of the four prostatic lobes was evaluated on hematoxylin and eosin-stained sections and scored based on a previously published histologic grading scheme. There were no statistical differences by Mann-Whitney test in prostate tumor scores of each lobe between live and cryopreserved repository mice at each time point. In conclusion, the genetic quality control program and colony management protocols of the facility resulted in similar genetic and phenotypic characteristics between live and cryopreserved B6 TRAMP mice.

P456 Using ARMS-PCR to Enhance Genotyping Efficiency in Cas9 Genome Edited Mice

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Genetically modified (GM) mice are important in studying gene functions and disease mechanisms. With the advancement of the powerful CRISPR-Cas9 technique, editing the genome for precise modifications at specific DNA locations has become much easier. Typical alterations mediated by CRISPR-Cas9 include the introduction of insertions or deletions (indels) and point mutations that modify specific amino acids within proteins. Therefore, implementing a stringent and efficient genotyping method would help validate the accuracy of these genetic modifications, particularly when managing large cohorts of GM mice during breeding programs. Commonly, researchers incorporate restriction enzyme recognition sequences to facilitate genotyping, offering a viable alternative to the direct sequencing of PCR amplicons. However, these methods are time-consuming, technically demanding, and high-cost. They may also suffer from instability issues related to the efficiency of the restriction enzymes. Recently, Amplification Refractory Mutation System PCR (ARMS-PCR) has been applied for detecting single-nucleotide mutations or indel mutations. ARMS-PCR uses specially designed primers whose 3′ ends complement the mutant or wild-type template. To increase the specificity, deliberate mismatches are introduced at the second base of the primer, ensuring that amplification is restricted to perfectly complementary target mutations. The genomic DNA was prepared by biopsy of the toes or ear tags from the GM mice. Our data encompasses over ten mutations in eight genes, including *Ins1*, *Mmut*, *Slc5a3*, *Il31ra*, *Apoe*, *Osmr*, *Prkdc*, and *H2ab1*. The ARMS-PCR data show our designed primer can robustly discriminate among wild-type, heterozygous, and homozygous mutations in a PCR reaction. This technique leverages allele-specific primers to enhance the precision and efficiency of mutation detection, streamlining the genotyping workflow. In conclusion, this study has optimized the genotyping efficiency for Cas9 genome-edited mice. Using the ARMS-PCR method significantly enhanced the speed (reducing time spent by 50%-90%), reduced the contamination possibility and the technical demand (PCR amplification only with no need to analyze further), and reduced the cost (savings of 50%-75%) of genotyping procedures.

P457 Establishing a Multi-Strain Influenza PCR Protocol

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Animal models play a critical role in influenza virus research, which in turn helps mitigate the vast public health and economic impacts associated with its morbidity and mortality. Recent developments have led to the implementation of oral swabs as a method of ferret viral load collection in influenza studies as opposed to nasal wash. Swabs provide increased standards of animal welfare and more efficacious serial sampling with less technician time. Consequentially, more samples can be collected from animals in a given study, necessitating the use of efficient assays to obtain reliable viral titer data. Focus forming plaque assays are inherently time-consuming due to the required incubation period and scale poorly, with each additional sample requiring a corresponding plate for dilutions. Oral swab and nasal wash samples were collected from three different cohorts of male ferrets (N = 16, 24, 24) at timepoints 2, 4, and 7 days post-influenza infection (dpi). Samples were analyzed through both plaque assay and RT-PCR. Viral load was reliably detected through RT-PCR at dpi2, dpi4, and dpi7. In contrast, plaque assay was less sensitive at later timepoints. Additionally, oral swab samples did not differ from nasal wash in frequency of copy detection. Utilization of RT-PCR as a universal assay for influenza A virus detection provides a faster, less resource-intensive alternative compared to traditional methods.

P458 Novel Rodent Models of Baker-Gordon Syndrome

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Baker-Gordon syndrome is a rare neurodevelopmental disorder characterized by mutations in the synaptotagmin-1 (*SYT1*) gene. This gene codes for the protein synaptotagmin-1, which is a synaptic vesicle transmembrane protein responsible for binding calcium to facilitate exocytosis and neurotransmitter release. Currently, there is no treatment for Baker-Gordon syndrome, and little is known about the factors driving variable symptoms in patients. To better understand how these mutations are causing disease, we used various approaches to develop three distinct mouse models. The first approach used the CRISPR-Cas9 system to incorporate a D365E (corresponding to human D366E) missense mutation into the endogenous mouse *Syt1* gene. The resultant pups died within one to two days of age, and DNA sequencing revealed these pups to be heterozygous for the D365E mutation. The second approach utilized CRISPR-Cas9 to insert a DNA template, including the CAG promotor and Cre-dependent *Syt1* D365E mutant cDNA, into the mouse *Rosa26* safe harbor locus. This model was crossed with a ubiquitously expressing CMV-Cre model to induce mutant *Syt1* D365E expression. Proper insertion of the desired Cre-dependent allele was confirmed with DNA sequencing, and phenotypic analysis was performed on offspring with the induced mutant Syt1 D365E expression. The third approach utilized an adeno-associated virus (AAV) vector to express the desired mutant *Syt1* D365E allele in tissues and specific cells of interest. The recombinant AAV vector contains a ubiquitous CAG promotor and Cre-dependent *Syt1* D365E mutant cDNA tagged with the fluorescent protein EGFP. This vector was retro-orbitally injected into 3-day-old CMV-Cre neonatal mice with limited success as determined by EGFP immunohistochemistry (IHC). From these results, we can conclude that CRISPR-Cas9 genome editing has produced a viable mouse model with the desired *Syt1* D365E mutation. This model will be utilized to further characterize the phenotypes, investigate which cell types are driving these phenotypes, and evaluate potential therapeutics for Baker-Gordon syndrome.

Platform Sessions

PS1 Academic Animal Resource Centers' Financial Health Check

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Benchmarking one's academic Animal Resource Center (ARC) program against one's peers is an inevitable activity to reassure oneself of the financial sustainability of one's program. Typically, such comparisons stick to per diem rates, which have relatively consistent expense composition between species and over the years, and animal care cost recovery. However, the reality is that the evaluation of one's program's financial health needs to include the animal program's total cost, revenue stability, and resource purchasing power. Trend data and data specific to the 2023 Yale Animal Resource Cost and Benchmarking survey© shed some light on the larger financial picture. Between 2011 and 2023, only 20-30% of participants included >90% of allowable vivarium space in their F&A rate, contributing to why 42 – 54% report that their indirect funds are insufficient to cover program expenses. In the 2023 survey, 41% of participants reported needing $1–5 M to cover expenses that did not involve animals and were not included in the indirect rate. In the 2019 survey, the institutional budget covered 50% of these expenses, with the rest distributed between the ARC, School, and Department budgets. In the 2023 survey, the institutional contribution decreased to 21%, with a smaller percentage contributed by the other sources. This correlates with an increase in the number of participating programs with negative cash flows >$2.5 M. The situation is compounded by a) non-federal awards being an increasing source of direct award dollars supporting live animal research, awards that frequently pay then do not pay the institution's total direct rate, b) the declining purchasing power of a dollar, tracked by the BRDPI (NIH dollar purchasing power) and HEPI (higher education dollar purchasing power), and c) the increasing CPI (inflation rate a major factor in salary increases), all negatively impacting the ARC's bottom line at a time when investigators' ability to pay higher per diems is declining. Thus, the combination of industry and public economic trends and your internal data enables a perspective for understanding the overall health of your program.

PS2 Animal Per Diem Rate Calculations

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Accurate calculation of animal per diem rates is vital to the financial operations of an animal research facility. A per diem rate is the daily cost to care for an animal or cage of animals. The Cost Analysis and Rate Setting Manual for Animal Facilities (CARS) recommends that costs are analyzed *annually* and cost-based per diem rates are adjusted accordingly. Despite this best practice, many facilities do not perform an annual analysis of their per diem rates, leading to a lack of understanding of the total costs for each species, an under-recovery of costs, and the charging of non-cost-based per diem rates. To address this, we implemented an annual review and calculation of animal per diem rates using a customizable Excel model in compliance with the NIH CARS manual. A key component of the animal per diem calculation is the Time and Motion study. A Time and Motion study is an exercise to accurately capture the amount of time it takes to perform various healthcare, husbandry, and service-related tasks by Animal Facility Personnel. During a 2–4-week period, personnel track their activities in 15-minute increments, providing critical insights into time allocation with each species. Since salary and wages normally constitute 70-80% of total operating expenses at an animal facility, understanding personnel time

allocation is essential to understanding the actual costs to support each species. We have found that Time and Motion studies are the most accurate way to determine the time and effort associated with different species. By incorporating results from the Time and Motion study and fiscal year operating expenses, the customizable per diem model can determine the true costs associated with each species. Calculating annual per diem rates in our model helps decrease the gap between the rates charged versus the actual costs associated with time and materials per animal. The benefits of this process include compliance with the NIH CARS manual guidelines, transparent cost insights for each species, analysis of needed annual subsidy, inflation-adjusted per diem rates, and a clear understanding of total operating expenses at your facility.

PS3 On-line SOP Access via Smart Phones

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Access to Standard Operating Procedures (SOPs) and other relevant documents, such as policies and forms, are critical for work standardization in the vivarium. Our lab animal care program, like many others, used to keep SOPs as printed documents in a heavy binder. That required managers, technicians, trainers, and trainees to check the binder or request a printed copy of the SOP for training or review, an inefficient and unreliable arrangement. We then considered one-page SOPs posted at each location where the corresponding work was performed for immediate access to proper instructions and guidance. However, that, in turn, would require reprinting and reposting whenever an SOP was modified or issued. At the same time, we created an internal e-library of SOPs, policies, and forms on a departmental website for better central management. However, few technicians knew about the e-library or did not have the technology or knowledge to use it regularly. Shortly after, all animal technicians were issued workplace-restricted smartphones for better communication with facility managers and veterinary services. Because of the availability of this technology in every technician's hand and with the assistance of Boston University IT, we developed a smartphone app to access our entire SOP library from anywhere and at any time so technicians and others can view the SOP and its photos or videos whenever needed. An "On-line SOP Access" SOP was created to standardize the process and is included in our onboarding checklist and training program description for new employees. Two-factor authentication was included to prevent unauthorized access. Twenty-four employees responded to a survey and said 20 used it, 22 said it's easy to use, with two people using it "always," 3 "never," and 20 "sometimes."

PS4 Digital SOPs: Accessed with Ease!!

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Facilities frequently require staff to be proficient in completing work-related tasks according to standard operating procedures (SOPs) with the objective of maintaining consistency and compliance. The conventional approach, which relies on in-person meetings and manual compliance tracking, not only delays the effective date of SOP training but also poses challenges for staff who are unable to attend the initial training sessions. This traditional reliance leads to inefficiency and logistical issues, particularly when updates occur and individuals with varied staff schedules have the same training requirements. To overcome these challenges, the "Vivarium SOP App" was developed. This sophisticated app is designed to automate the SOP review and training process, integrating seamlessly with a company-wide online learning management system. It enables the facilitation of initial face-to-face training sessions and the automatic assignment of follow-up online modules to absentees, providing direct access to SOPs and incorporating quizzes to assess comprehension. A key feature of the Vivarium SOP App is the capability for managers to monitor the training status of employees, easily identifying any pending training sessions through the effortless upload of modified SOP documents. The implementation of the Vivarium SOP App has markedly reduced the administrative overhead associated with SOP training management. The app's tracking and notification system has improved compliance rates and ensured the timely completion of reviews. The online module quizzes have provided valuable insights into staff engagement and understanding of the SOP content. Enhanced by the app's functionality for real-time managerial oversight and the simplified SOP document upload process, the efficiency of the training process has significantly increased. The Vivarium SOP App has effectively transformed the SOP review and training landscape, addressing previous inefficiencies and providing a comprehensive digital solution. Its features, including managerial oversight and easy SOP document management, highlight the app's potential to enhance SOP training and compliance monitoring significantly.

PS5 Use of a Digital Platform for Task Management Within an Animal Facility

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With the increasing adoption of LEAN practices and the desire for more efficient time management within animal facility operations, our department evaluated a digital software platform to reduce the reliance on paper forms, streamline task assignments and reassignments, and strengthen compliance oversight with regard to facility parameters and scheduling. We chose a software platform that was initially developed for the restaurant industry to assist with food safety compliance and employee accountability, and its design is adaptable to the laboratory animal facility environment. Over the period of 1 year, we worked to develop a series of lists to replace the many forms (e.g., room log sheets, eye wash station forms, autoclave log sheets, etc.) that are used throughout our five animal facilities. We worked closely with our team in the evaluation of the platform as it was developed. We went live with the platform in January 2024. The platform has offered several advantages over the use of paper forms, including 1) technician tasks can easily be assigned and modified, and tasks can be reassigned to a different technician in cases of vacation or sick leave, 2) videos and photos can be uploaded to the platform to be used for training purposes or task verification, 3) reports can be generated to examine trends and task compliance, and 4) email alerts, or alarms through the platform itself, are generated when a set parameter (e.g., room temperature) is out of range. Overall, the platform has been well received by our husbandry and cage wash personnel and allows technicians and supervisors to utilize their work time better. While task completion can be easily studied through the platform, the adoption of this software does not negate the need to verify task completion in person by visiting rooms.

PS6 A New Era – Leveraging XR, Voice Assistance and Automation to Benefit Research Within the BioTech Industry

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Scientists performing in vivo work within vivariums have historically been left to their own devices for many years. Oftentimes, outdated technologies such as pen and paper, mundane data entry, and even memorization have been used to document and log information throughout the experimental process. This results in numerous data quality concerns, information loss, and ample distractions from the science being performed. We've initiated an effort to transform the way these scientists are working within the vivariums by implementing new technologies that can help them become more efficient, decrease potential errors, reduce variability, increase their focus on the experiment, improve

cross-functional collaboration, and better leverage information to drive the development of new therapeutics. We have worked on implementing a variety of new technologies within research, such as:- RapID Lab ear tags and UID Radio Frequency ID chips to enable the scanning of animals instead of the manual way of identifying research animals, ensuring traceability throughout the experiment lifecycle.- Voice enables scientific LabFlows to capture and digitize common experimental measurements and metadata when performing in vivo work.- HoloLens to improve the collaboration between colleagues as well as troubleshooting of laboratory instruments- PowerApps to quickly develop point-and-click prototypes that can be tested and used in lab settings. Our presentation will focus on our approach to implementing these technologies, the value delivered within research, and the challenges faced along the way.

PS7 Employee Burnout: Recognizing and Addressing Burnout in Yourself (and your Staff!)

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Most people, at some point in their careers, have experienced job burnout. Burnout is a syndrome resulting from workplace stress that has not been successfully managed. Many people experiencing burnout have feelings of energy depletion or exhaustion, increased mental distance from one's job, feelings of negativism or cynicism related to one's job, and reduced professional efficacy. It can be caused by many internal and external factors, but recognizing it and coming up with a plan to address it is essential. Burnout can happen to anyone, no matter how long they have been in their position, but it is especially common with employees dealing with the emotional impact of animal research. This talk will discuss how to recognize burnout and educate attendees on the hallmark signs and causes of the syndrome. Strategies for dealing with personal burnout will be discussed, including action plans to address workplace stressors and look to the future. Strategies for self-care and support will be discussed as a critical part of learning to manage overall stress levels and address or prevent burnout. The second critical piece of the presentation will discuss how to recognize and address burnout within your work team. Advice will be shared, and tips will be provided for managers on how to spot signs of trouble early and help their employees work through and deal with burnout. Overall, this will help to educate attendees on the difference between stress and burnout and hopefully give them some tools to address it within themselves and their work teams.

PS8 Is the 5-Day Work Week Outdated? Our Husbandry's Transition to 4-10s

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The COVID pandemic was and continues to be a major disruptor in the labor market. Employees are voluntarily leaving their jobs at a 25% higher rate compared to pre-pandemic levels. The laboratory animal field was not immune to these trends. During fiscal year 2022-2023, we had up to a 50% attrition rate among our hourly husbandry staff. Management struggled to balance their administrative duties and ensure critical daily tasks were complete, thus experiencing a higher rate of burnout. Management had to look for drastic non-monetary changes to help retain and attract new talent. The change from working 8 hours 5 days a week to 4 days 10 hours (4-10s) a week was proposed. Following Section 4 of California Wage Laws, we partnered with our Human Resources department to hold private informational and voting sessions for the husbandry staff to vote to adopt or not adopt the 4-10s. The resolution was adopted by a 76% majority vote. The 4-10s were implemented before the start of fiscal year 2023–2024. One year later, we wanted to assess the success of the 4-10s. Anonymous surveys were implemented for three different groups: husbandry staff who transitioned from a 5-day work week to the 4-10s (n=13), husbandry staff that started with the 4-10s (n=13), and the staff who are not on the 4-10s schedule (n=21). Overall, 82% of respondents either agreed or strongly agreed that the 4-10s change was a positive change, with the remaining 18% feeling neutral. Among the two husbandry groups, 93% (15/16) would not want to change back to the original 5-days-a-week schedule. Common feedback included: 1) enjoying more time off, 2) the commute was worse, and 3) workdays are more exhausting. Lastly, the attrition rate for fiscal year 2023-2024 dropped to 20%. These results affirmed that the schedule change netted positive results. Our experiences can help inspire other programs to think of alternative ways to attract and retain talent.

PS9 A Novel Approach to Mitigating *Corynebacterium bovis* Infection in Immunocompromised Mice

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Corynebacterium bovis (Cb) causes skin disease in immunodeficient mice. This study explored a novel approach to prevent Cb-associated skin disease that relies on the theory of competitive exclusion in which two bacterial species depending on the same environment and resources cannot equally coexist. A non-pathogenic Cb isolate (#24596) and a non-pathogenic component of the skin microbiota, *Corynebacterium amycolatum* (Ca; # 23001642), were utilized as "vaccines" to determine if they could protect against challenges with a pathogenic Cb isolate (#7894) in nude and NSG mice. NSG (N = 3F) and nude (N = 6; 3M, 3F) mice were inoculated topically with 1 X 10^8 colony-forming units (CFUs) of either Cb #24596, Ca #23001642 or a sterile media control 1 (NSG) or 2 (nude) week(s) prior to being challenged with either 10^8 or 10^4 CFUs, respectively of isolate #7894. Colonization was confirmed via aerobic culture of skin swabs. The animals' skin was assessed at least thrice weekly and scored 0 – 5 based on lesion severity. Nude mice colonized with #24596 remained disease-free for 14 days. Skin lesions were seen in nude mice that received Ca. All NSG mice developed clinical disease beginning on day 19 with no difference in disease course or severity between vaccinated and unvaccinated groups, with each group demonstrating a mean peak score of 5 by day 23. Mice were euthanized 4- (nude) or 6-weeks (NSG) post-challenge, macroscopic changes were documented, and full-thickness skin biopsies were obtained and scored 0 – 4 based on the presence and severity of hyperkeratosis, acanthosis, inflammation, and bacterial colonies. All mice displayed varying degrees of acanthosis and orthokeratosis with intracorneal bacterial colonies. These findings suggest that clinical disease can be prevented in nude mice using this vaccine strategy, whereas highly immunodeficient NSG remains susceptible.

PS10 Excessive Cytokine and IgE Production in Murine Hem1 Immunodeficiency Contributes to Allergic Airway Disease in a House Dust Mite Model

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Inborn errors of immunity (IEI) are a group of genetic diseases in humans that can present as increased susceptibility to infections, autoimmunity, hyperinflammation, allergic disease, and occasionally malignancy. A recently identified gene linked to IEI, *NCKAP1L,* encodes for Hematopoietic Protein-1 (HEM1), an adaptor protein that is critical for normal actin polymerization.

Clinical investigation and immunophenotyping of HEM1 deficient children and murine models have documented similar characteristic features of IEIs, including recurrent infections and autoimmunity. However, the cellular mechanisms behind an increased prevalence of allergic airway disease (asthma) observed in HEM1-deficient children have not been further evaluated. In this study, we evaluated the development of asthma and primary immune cell populations and cytokines driving airway and lung pathology in various Hem1 deficient mouse models. We hypothesized that Hem1 deficient T cells were driving asthma pathogenesis by releasing proinflammatory cytokines. Constitutive *Hem1* deficient mice (*Hem1*$^{-/-}$), conditional T cell-specific *Hem1* deficient mice (*CD4CreHem1*$^{fl/fl}$), and age-matched control mice (10-25 week old males, n=5-8/group) received saline or house dust mite via the oropharyngeal route on days 0 and 14 for sensitization and days 26, 27, and 28 for challenge. On day 29, lungs, bronchoalveolar lavage fluid (BALF), and mediastinal lymph nodes were collected for spectral flow cytometric analysis to identify representations of different myeloid and lymphoid cell populations. The serum and supernatant of the BALF were analyzed for cytokine and IgE levels via multiplex immunoassay. Lungs were formalin-fixed for histopathologic evaluation using H&E, Masson's Trichrome, and PAS staining. Our results indicate that in the face of allergic airway disease, *Hem1* deficient mice had decreased total numbers of myeloid and lymphoid immune cells in the airways, lung parenchyma, and lymph nodes. However, Hem1 deficiency resulted in increased levels of asthma-related proinflammatory cytokines (IL-4, IL-5, IL-13, and IL-17) and IgE in the BALF, resulting in asthma-related lung pathology. These results suggest that dysregulated cytokine and IgE production may contribute to asthma in Hem1 deficient humans and mice.

PS11 A Busulfan Conditioning for NSG Mice

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Allogeneic hematopoietic cell transplantations (alloHCT) are potentially curative treatments available for patients suffering from leukemia. The success of these procedures is dependent upon myeloablative conditioning regimens that can destroy the bone marrow and cancer cells in preparation for bone marrow transplant. Total body irradiation (TBI) is one of the most used methods of conditioning. Busulfan conditioning has been increasingly used in the clinic to replace irradiation-induced toxicities. Our laboratory has successfully used TBI to engraft hematopoietic stem cells (HSC) into immunodeficient mouse strains such as NSG (NOD-scid-gamma). However, engraftment of genetically modified HSCs into NSG mice has been detected at reduced levels, even using a large number of cells. To increase the engraftment capabilities of these HSCs, we investigated the use of busulfan to replace TBI as a myeloablative method. We performed bone marrow transplants on ten male and ten female NSG mice following intraperitoneal busulfan treatments at a selected dose of 17 mg/kg for four consecutive days. We provided autologous bone marrow support to enhance the health of mice post-busulfan treatment. Half of the mice divided evenly by sex in a cohort were given autologous bone marrow 24h post-dose. Blood was collected retro-orbitally each week up to 33 days after busulfan treatment and analyzed by hematological analysis. Mice that received autologous bone marrow showed hematopoietic reconstitution and 100% survival. Mice without autologous bone marrow had progressive weight loss with 20% survival. Mice without bone marrow grafts had progressive decreases in CBC counts with greater than 60% fewer erythrocytes, 40% fewer neutrophils, 60% fewer leukocytes, and 80% fewer platelets. These data demonstrate that busulfan can effectively be used in immunodeficient mice as a method to improve hematopoietic reconstitution and possibly a substitute for radiation myeloablation in NSG mice.

PS12 Assessing the Efficacy of Topical Fluralaner for the Treatment of *Demodex musculi* Infestations in NSG mice

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Demodex musculi (Dm) is a microscopic, prostigmatid mite that inhabits the pilosebaceous unit of mice. It is the most frequently diagnosed mite in laboratory colonies and infests up to 13% of mice shipped from non-commercial vendors. Dm infestation (DmI) causes immunomodulation and, in immunodeficient mice, debilitating clinical signs that may impact the reproducibility of certain studies. Current treatment options are limited, and many infested mice are ultimately euthanized. Fluralaner, an isoxazoline-class ectoparasiticide, has been used successfully to eliminate Demodex infestations in other species. However, its use in mice is not well-documented. This study aimed to explore the potential use of topical fluralaner for the treatment of DmI in NOD.Cg-*Prkdc*^{scid}*Il2rg*^{tm1Wjl}/SzJ (NSG) mice. Twenty-seven female NSG mice were infested with Dm and allocated into cages of three to four animals each. Cages were randomly assigned to one of two fluralaner treatment groups: 100 mg/kg or 250 mg/kg. Starting at day 0 (D0), all animals in each cage were weighed and received the respective dose of topical fluralaner over the dorsal interscapular area. This was repeated every two weeks for six weeks (four treatments total). Every month after D0, mice received deep skin scrapes and fur plucks for direct microscopy and pelt swabs for quantitative polymerase chain reaction (qPCR). At four months, a terminal necropsy was performed, and tissues were collected for histopathologic analysis. All diagnostic modalities indicated that no cage in either treatment group was successfully cleared of mites. However, qPCR results suggested a significant reduction in mite burden following treatments. After treatments were stopped, the mite burden remained similar between time points and then increased. There were no significant differences in mite burden between treatment groups at any time point. Additional research is needed to determine if higher and/or more frequent topical fluralaner doses will improve efficacy when treating DmI.

PS13 The Impact of Host Genetics/Microbiome on the Severity of *Corynebacterium*-Associated Hyperkeratosis in Outbred Athymic Nude Mice

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Corynebacterium bovis (Cb), the etiology of hyperkeratotic dermatitis (HD) in nude mice, may significantly impact research outcomes. Little is known about the differences in the course and severity of Cb-associated disease in different stocks of outbred athymic nude mice. Three genetic stocks of nude mice (designated A, B, and C), 1 of which was obtained from 2 geographically separate colonies with distinct microbiomes (A1 and A2), were inoculated topically with 1 x 10^8 CFUs of a pathogenic Cb field isolate (#7894; n = 6). Clinical signs were assessed daily and scored 0 – 5 based on lesion severity. Mice were euthanized at either 14 (A1, A2, C, and B) or 28 (B) days post-inoculation (dpi); macroscopic changes documented, six full-thickness skin biopsies per mouse were obtained and histologically scored 0 – 4 based on the presence and severity of hyperkeratosis, acanthosis, inflammation, and bacterial colonies. No mice in group A1 developed clinical disease; 1 of 6 in group B developed mild HD (mean peak score [MPS] – 0.3) at 14 dpi; and

all mice in group C and A2 developed significant clinical signs (MPS – 3) at five dpi which resolved by 11 dpi. Despite differences in clinical presentation, all mice had hyperkeratosis and/or acanthosis with associated bacterial colonies of varying severity. Mouse stocks A1 and B, which had minimal or no clinical signs, were colonized with *Corynebacterium amycolatum* (Ca). In contrast, stocks C and A2 were not colonized with Ca, raising the possibility that Ca and/or other components of the skin microbiome may prevent clinical signs and mitigate pathologic severity. These findings suggest host genetics and/or the skin microbiome can markedly influence the presentation of HD in nude mice.

PS14 Effects of Long-Term Carprofen Administration in C57BL/6J Mice (*Mus musculus*)

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Osteoarthritis is the leading cause of disability in the United States and affects approximately half of adults over the age of 65. Many osteoarthritis patients take non-steroidal anti-inflammatory drugs (NSAIDs) on a long-term basis, often concurrently with proton pump inhibitors (PPIs), such as omeprazole, to prevent gastric ulceration. Mice (*Mus musculus*) are a commonly utilized animal model of osteoarthritis; however, there is little data regarding the long-term administration of NSAIDs or co-administration of PPIs and NSAIDs in mice. This study sought to determine if the administration of carprofen, a commonly used veterinary NSAID, has adverse effects when administered for 22 days and if co-administration of omeprazole reduces the incidence of adverse effects. Four groups of C57BL/6J male (n=5 /group) and female (n=5/group) mice were administered carprofen 10 mg/kg and omeprazole 8.2 mg/kg, carprofen 10 mg/kg, omeprazole 8.2 mg/kg, or control suspension once daily by oral gavage for 22 days. All mice were euthanized, and complete blood count (CBC), serum chemistry, fecal occult blood, and pyloric histopathology and gastritis scoring were conducted. One-way ANOVA or Kruskal-Wallis was conducted for normally and non-normally distributed CBC and chemistry data, respectively. Kruskal-Wallis was used for gastritis scores. Post-hoc analysis was conducted using Dunn's multiple comparisons test. All animals remained clinically healthy for the duration of the study. Fecal occult blood tests were negative for all animals. Compared to the control group, albumin was significantly higher in the carprofen group. Neutrophil and platelet counts were significantly lower in the carprofen and omeprazole groups ($p<0.05$ for all comparisons). No animals had pyloric mucosal ulceration, and gastritis scores were not significantly different between groups ($p>0.05$). The results show that carprofen and omeprazole may be safely administered to C57BL/6J mice for 22 days and may be a useful animal model for long-term NSAID and PPI administration in humans with osteoarthritis.

PS15 Acidic pH in Drinking Water Induces Enamel Erosion in Mice

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The use of hydrochloric acid treatment of drinking water in many academic research colonies and commercial vendors prompted us to investigate its effect on tooth enamel health in mice. Acidified drinking water may be problematic because drinks with a low pH, such as fruit juices and soft drinks, have been demonstrated to cause demineralization of tooth enamel in humans and rodents. This study explored the hypothesis that acidified drinking water at the recommended range of 2.5-3.0 pH can lead to enamel erosion and compromised tooth integrity in mice. Specifically, we sought to quantify the effects of pH 2.5 or pH 3.0 drinking water exposure on molar enamel and bone mineral density over one and three months. 8-week-old male and female C57BL/6NCrl mice (n=42) were provided purified drinking water of pH 7.0, or hydrochloric acid-acidified purified drinking water of pH 2.5 or 3.0 for 1 or 3 months. Methylene blue was used to quantify enamel erosion of the molar teeth, while dual-energy x-ray absorptiometry was used to quantify the bone mineral density of the molar teeth. After one month of drinking water exposure, we did not observe a statistically significant difference between the groups in enamel erosion or bone mineral density. However, after three months, we observed a significant difference in enamel erosion for the pH 2.5 group compared to the other groups, suggesting a potentially destructive process. There were no differences in bone mineral density between groups at any time point. These findings indicate that acidified drinking water of pH 2.5 may have deleterious effects on the enamel integrity of molar teeth in mice; however, drinking water of pH 3.0 appears safe for tooth enamel in mice during a short-term exposure of 3 months. Based on our findings, we recommend that research facilities avoid the use of acidified drinking water at pH 2.5 to prevent tooth enamel loss. As this study only had a 1-3 month exposure period, further study is needed to determine the effects of longer-term use of acidified drinking water at pH 3.0 in mice.

PS16 Evaluation of Oral Gabapentin Administration Methods in Mice

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Gabapentin is a commercially available, FDA-approved medication that can be used as an analgesic primarily to treat neuropathic pain but also surgical and inflammatory pain. The goal of this study is to evaluate the efficacy of three low-stress, convenient methods of administering gabapentin orally to mice compared to the gold standard of oral gavage. Gabapentin was delivered to adult, male, and female C57BL/6J mice via oral gavage or passively via medicated feed, flavored gel, or flavored drinking water (collectively, consumables) for up to five days; a negative control group was provided standard feed and water. Gabapentin was supplied at a daily dose of 150 mg/kg per day or administered via oral gavage twice daily at 75 mg/kg (150 mg/kg total dose per day). Blood was collected at six hours, eighteen hours, one day, two days, and five days after the initial presentation of consumables or after the first dose of oral gavage (n = 4 mice per group per sex). Serum gabapentin levels were determined by liquid chromatography-mass spectrometry and compared to the previously reported rodent gabapentin therapeutic range of 1.4 – 16.7 µg/mL. We found that the consumables were palatable and readily consumed, as shown by increases in mean body weight over five days for all passive consumption groups. No treatment group had serum gabapentin concentrations within the target therapeutic range for all time points measured. The oral gavage group reached serum levels at the highest concentrations during the study. All passive consumption groups reached minimum target concentrations for at least one measured time point. This study demonstrates the feasibility of passively administering gabapentin to mice via medicated feed, water, and flavored gel. However, dose increases may be required to achieve adequate serum gabapentin concentrations at all time points.

PS17 Examining Inflammatory Signaling in Astrocytes as a Key Modulator of the Neurovirulence of Western Equine Encephalitis Virus in a Novel Mouse Model of Parkinson's Disease

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Parkinson's disease is the second-most common neurodegenerative disease after Alzheimer's disease, constituting over 10 million ongoing cases in humans worldwide. The pathological hallmark of the disease and cause of subsequent motor symptoms is the loss of dopaminergic neurons and aggregated alpha-synuclein in the substantia nigra pars compacta of the midbrain. Neuroinvasive infections with mosquito-borne alphaviruses, such as the Western equine encephalitis virus, induce post-encephalitic parkinsonism with neurologic sequelae that closely resemble Parkinson's disease due to persistent neuroinflammation. Although the standard for these studies has been intranasal inoculation with Western equine encephalitis virus, footpad injection more closely resembles natural mosquito infection. It activates the same brain regions as Parkinson's disease by entering the central nervous system through circumventricular organs where the blood-brain barrier is naturally absent. In addition, we hypothesize that footpad injection will not require adjunct immunotherapy as seen in previous mouse studies using intranasal inoculation. 50 C57Bl/6 mice (24M, 26F) aged 3-10 months were inoculated with recombinant Western equine encephalitis virus expressing firefly luciferase at 0.05, 0.1, 0.2, 5, 10, and 20 x 10^5 PFU via footpad injection. A grip test and pole test were used to measure grip strength and motor coordination, respectively, on days 0, 7, 14, 21, and 28. An in-vivo imaging system (IVIS) was used to evaluate viral uptake in the footpad and brain after subcutaneous injection with 150mg/kg luciferin. Brains were collected on half the mice on day 14 and half on day 28 and processed routinely with formalin fixation, paraffin embedding, and 10um transverse coronal sections. Stains for tyrosine hydroxylase using DAPI and cyan revealed neuronal loss and reduced gliosis restricted to the substantia nigra pars compacta, which increased consecutively for each viral group. Behavioral testing also revealed deficits in the two higher viral load groups. However, there was no need for adjunct immunotherapy. Future studies utilize this mouse model to investigate potential therapeutic strategies for viral parkinsonism.

PS18 3D Printing and the 3Rs. Using Conventional 3D Printing to Refine Lab Animal Procedures and Surgical Training

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3D printing has revolutionized biomedical research. While bio-printing reshapes a future with a significant Replacement of animal models, the impact that conventional 3D printing platforms can have in the Reduction and Refinement of animal procedures in the present is still greatly overlooked by the lab animal community. 3D printing is already commonly available in many academic and research institutions, but overestimations of the complexity of the 3D design processes and the cost of the printing equipment seem to be holding back the development and implementation of 3D tools and solutions across lab animal programs. Current consumer-level 3D printing technology offers an affordable, quick, and accessible platform with endless applications in our field. With little to no experience in 3D design or printing processes, this technology can be used in the development of utterly customizable tools to refine a wide range of lab animal procedures. This communication will demonstrate how an academic laboratory animal care program has successfully implemented a program-wide approach that offers a growing number of tailored 3D printed solutions that contribute to refining and optimizing a variety of lab animal procedures and training tools. The presentation will cover the basic concepts of the equipment, materials, and software needed, showcasing a variety of published and unpublished examples, including case studies, of how 3D printing is being used in a wide variety of LAS applications.

PS19 Face and Content Validation Study of a 3D Printed Mouse Model for Surgical Training: Multicenter Validation Methodology and Preliminary Results

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A novel, hyperrealistic, 3D-printed rodent surgical simulator was developed with the aim of providing a reliable animal-free rodent surgical training platform. After months of successful internal use within our rodent surgery training program, a multicenter international validation study was launched. The study involved six academic and research institutions from Europe and the United States, aiming to objectively evaluate the content (suitability as a teaching tool) and face (realism and appropriateness) validity of the training model. The participant centers developed a systematic approach to harmonize the testing and data collection across all participant institutions to provide objective, reliable results. A set number of experienced (3) and inexperienced (6) rodent surgeons received the same standardized orientation information and performed the same standardized tasks (for a pre-established number of iterations) using identical tools and supplies. Upon the completion of the tasks, the participants filled out a user feedback survey. Standardized pictures (after the completion of each task) and pre-determined scoring criteria were used to evaluate the performance of each participant in a double-blind fashion across all institutions. This oral communication will emphasize the importance of conducting an adequate validation assessment of training systems and methodologies as a way to ensure optimal training and competency assessment. It will detail the validation approach and study design used and will present the pre-print preliminary results of the study. The authors believe these collaborative efforts between lab animal professionals across different institutions and territories, aimed not only at validating training tools but also at harmonizing training approaches, are key initiatives further to benefit research and animal welfare across the board.

PS20 How to Fix a Ratty Attitude: Improving Handleability Using Enhanced Enrichment of Aged Male Sprague-Dawley Rats

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Rats are social animals and are traditionally housed with at least one other individual. The Guide recommends that research rats be socially housed, beginning as juveniles or in bonded groups. However, older rats are not thought to bond well with conspecifics, especially if they have been single-housed. In our facility, twenty 6-month-old Sprague Dawley rats were procured for long-term studies. Rats were older, retired from breeding, and accustomed to individual housing at the vendor. Individual housing was required for the duration of their surgical studies. Upon arrival, rats were difficult to handle, making study and husbandry activities challenging. Rats were housed in conventional caging with sani-chip bedding, chew toys, and a hide. While this environment was adequate for short studies or socially housed animals, our team wanted to provide additional environmental enrichment. The aim was to provide additional mental stimulation and improve temperament for cage change, examinations, and administration of medications. Their aversion to handling did not initially allow

human interaction as an enrichment option and created stress during study and husbandry activities. Treats were offered to mitigate these concerns; however, the rats did not show much improvement with the treats alone and instead gained weight. In a new attempt to address the concerns in enrichment, handling, and weight gain, a program was developed to allow the rats out for exercise and play. Individual time out on a table with a few toys quickly progressed to group playtime with a variety of enrichment options: nesting boxes, mazes, tunnels, wading pools, hides, chews, and more. During the 10-20 minute sessions, rats explored the environment and sampled the enrichment options. As sessions progressed, interaction with a conspecific was introduced in the enrichment space. They often cuddled together and demonstrated chittering and hopping behaviors. Within 7-10 sessions, rats became easy to handle and were routinely observed interacting with the enrichment space. All single-housed male rats participated in supervised social enrichment with no instances of incompatibility. An enriched exercise space can improve handleability and provide social enrichment for single-housed, aged male rats.

PS21 Refining Rabbit Management Programs through Stakeholder Focus Groups

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Rabbits are an important species in scientific research, often chosen as a non-rodent second species for developmental toxicology studies due to availability, practicality of management, and ease of breeding. As work with dogs and primates becomes more restricted, rabbits will become more valuable as a research species, requiring special attention to overall behavior management, procedural refinements, recognition of pain and distress, euthanasia procedures, and welfare assessment. To address these topics, and in recognition of the 2023 Lunar Year of the Rabbit, a group of subject matter experts formed the Rabbit Welfare Working Group to develop a list of recommendations for best practices for working with rabbits in a research setting. Topic-specific subcommittees met virtually from 2023–2024, and a virtual Rabbit 3Rs Workshop was held in 2024 to discuss rabbit 3Rs topics further. The Rabbit Welfare Working Group developed 12 recommendations based on discussion and consensus building. The recommendations were then discussed with corporate leadership across business units to achieve support. The recommendations will be used as a tool to monitor sites over time and will be used to develop a rabbit welfare assessment tool to allow for an objective review of rabbit management programs and help sites identify priorities for improvement.

PS22 An Overview of Behavior Analysis Introduced into a Rhesus Macaque Medical Research Care Program

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Behavioral analysis, most commonly employed with learners diagnosed with Autism Spectrum Disorder, allows us to understand the functions of behavior and support behaviors the learner deems significant. When incorporated into a laboratory animal care program with rhesus macaques (Macaca mulatta), we noted reduced frustration behaviors during daily husbandry, veterinary, enrichment, and research tasks. Specifically, we explored teaching strategies such as errorless learning, token systems, picture exchange communication systems, and antecedent arrangements under the Applied Behavior Analysis (ABA) umbrella to improve the experience of primates residing and learning at Neuralink. Therefore, we will share an overview of how Applied Behavior Analysis strategies were incorporated into the everyday operations of primate care in medical research to improve animal well-being and task performance results. Metrics evaluated include the number of trials per minute, level of engagement, and the presence of frustration-related behaviors. Preliminary results in three male rhesus macaques serving as their own control suggest that when using Applied Behavior Analysis teaching strategies such as a token system (delayed reinforcement by exchanging tokens for a higher-value reward), animals engaged for longer periods (mean = 78 min vs. 61 min, 24% increase) and performed fewer frustration-related behaviors (mean frequency = 0.1 vs. 56, 199% decrease). However, the number of trials per minute was not significantly different between conditions (mean = 49 tpm for both), suggesting token systems improve animal experience and maintain data quality. Subsequently, we observed the incorporation of applied behavior analysis into a neuroscience laboratory, which significantly improved the overall well-being of rhesus macaques and their trainers working in medical research.

PS23 Errorless Learning in the Laboratory: Teaching Rhesus Macaques in Brain-Computer Interfacing Studies

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In the laboratory environment, rhesus macaques (Macaca mulatta) are utilized for functionality neuroscience studies, which often require animals to learn complex cognitive tasks. Animals are typically taught utilizing a trial-and-error method, originally detailed by Edward Lee Thorndike, in which problem-solving is achieved through repeated, varied attempts until success is reached. However, because frustration has been found to be associated with negative welfare outcomes (Ziegler Hill and Shackelford 2017), errorless learning, a teaching method in which learners are provided prompts and cues to maximize success, is instead often implemented in clinical settings to prevent incorrect responses. In more recent publications, errorless learning has been shown to significantly lower frustration-related responses in dogs learning a stimulus discrimination task (Handley et al., 2020). It was hypothesized that by implementing an errorless learning teaching strategy for macaques in a neuroscience lab to acquire mastery of behavioral tasks, animals would learn with higher levels of engagement and would display significantly fewer frustration-related behaviors. To evaluate this, one cohort (n=4) of macaques was taught a simple stimulus-response task utilizing an errorless learning framework tailored to the individual learner. A second cohort (n=4) used a software criterion-led model in which animals learned the game in stages and progressed through approximations upon obtaining mastery at the previous stage. The third cohort (n=4) was exposed to the final task criteria through trial and error. We found that, on average, macaques displayed fewer frustration-related behaviors and had higher levels of engagement in errorless learning conditions. Based on this pilot work, we anticipate that additional animals who learn with errorless learning will engage longer and become proficient at complex cognitive tasks more quickly than those who learn by way of trial and error. It is also expected that animals learning errorlessly will have reduced frustration and, therefore, may require less restraint and restriction, which will improve overall welfare.

PS24 Refining the Monitoring of Weight in Rhesus Macaques (*Macaca mulatta*): Use of Percentile Growth Curves

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Weight loss is a key metric in animal health and welfare. In laboratory research, certain levels of weight loss (15% and 20%) are commonly used as humane endpoints. However, weight loss

is usually a calculation that relies on the last recorded weight and fails to capture whether an animal is growing as expected. Juvenile and adolescent animals should still be growing and increasing in weight. In human health, percentile growth curves for weight and other measurements are used to identify whether a child is growing as expected. We propose that percentile growth curves are a more refined way of monitoring growth in rhesus macaques and take into account healthy growth. We used 15 years of breeding colony weight records to construct percentile growth curves for male and female rhesus macaques (Macaca mulatta; 8291 weights in total from 830 macaques, taken between 2008 and 2023). We used the GAMLSS package in R to fit Lamda-Mu-Sigma models to the weights (separate models for males and females). This model allows both the plotting of an individual animal's weight across time on the centile growth curves and given an animal's weight and age to calculate a Z-score. We demonstrate with case studies how percentiles and Z-scores can be used to capture both weight loss and failure to grow and how events such as procedures and injuries may impact growth. This is now being used on a routine basis in the macaque breeding colony to identify animals with potential weight issues.

PS25 Neurologic Abnormalities in an Immunosuppressed Cynomolgus Macaque

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A 10-year-old, 8.0 kg intact male cynomolgus macaque (*Macaca fascicularis)* presented with acute onset behavioral abnormalities and anisocoria. The primate underwent a unilateral allograft lung transplant two months prior, a biopsy of the transplanted organ 20 days prior, and irradiation and bone marrow transplantation one week prior to presentation. He was receiving an immunosuppressive regimen consisting of aCD154, ABT199, mTOR-NP, and cyclosporine. He was receiving preventative antibiotics for severe leukopenia. On cage-side examination, he exhibited mild epistaxis, nasal bridge swelling, aggressive fits of scratching, and anisocoria. Differential diagnoses included infectious encephalitis, stroke, and neoplasia. He was sedated for diagnostic testing. A CBC revealed severe leukopenia and thrombocytopenia with marked toxic change in the neutrophils; a nasal swab was positive for MRSA; radiographs showed a left-sided (graft-sided) effusion; and a cytomegalovirus (CMV) titer was >400,000 copies/mL. The primate was started on DHPG (10mg/kg IM q24h) and cidofovir (5mg/kg IV q7d) as antiviral therapies in addition to broad-spectrum antibiotics (cefepime and vancomycin). The neurologic and nasal symptoms subsided. Approximately ten days later, the primate presented with punctate pustules and open wounds along the extremities. Differential diagnoses included septic emboli, drug reactions, and self-inflicted trauma. On work-up, a CBC revealed a WBC of 10K cells/uL, and radiographs showed a persistent left-sided effusion. Samples were collected for a CMV titer, blood culture, and pustule culture. The primate declined and was euthanized two days later, at which time further testing showed worsening leukocytosis. Blood, thoracic effusion, and pustule cultures were positive for MRSA, confirming a disseminated MRSA bacteremia. The CMV titer was 1,581,706 copies/mL. Histology of the brain showed lymphoplasmacytic infiltrates suggestive of resolution of previous CMV encephalitis. Treatment of CMV encephalitis in severely immunosuppressed macaques with antiviral therapies (DHPG, cidofovir) is effective but risks further immunosuppression that creates vulnerability to other infectious complications.

PS26 Acute Inappetence and Lethargy in a Rhesus Macaque

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A 28-year-old male rhesus macaque (*Macaca mulatta*) acutely presented with inappetence and lethargy. An initial physical exam revealed a thin body condition with generalized muscle atrophy, marked lumbar kyphosis, bilateral stifle crepitus, with reduced range of motion, and joint capsular swelling. Multiple round, firm masses were palpated within the caudal abdomen. Radiographs displayed gaseous intestinal contents, with no obvious mass effect, and severe lumbar spondylosis deformans. A complete blood count revealed mild anemia. Significant serum chemistry results included severe azotemia, marked hypophosphatemia, and marked hypercalcemia. A urinalysis showed moderate-marked hematuria, rare calcium oxalate crystals, and normal specific gravity. Chronic kidney disease was prioritized as the top differential, and initial treatment consisted of intravenous and subcutaneous fluids, analgesics, hematinics, and anti-emetics. A recheck examination performed three days later revealed a quiet heart murmur (Grade II/VI), in addition to the previous findings. Pronounced renal abnormalities were found on abdominal ultrasound, including a kidney with irregular margins, multifocal cystic structures, and reduced corticomedullary distinction. A repeat serum chemistry showed persistent azotemia, hypophosphatemia, and hypercalcemia. Due to poor prognosis, the animal was euthanized and submitted for necropsy. Numerous abnormalities were found on gross necropsy, including colonic diverticula, multifocal cysts within the liver and kidneys, and marked enlargement of the right adrenal gland. Histopathology and immunohistochemistry confirmed the expansion of the zona fasciculata within the right adrenal gland. The kidney parenchyma bilaterally displayed large cystic structures, glomerulosclerosis, tubular degeneration, interstitial fibrosis, and necrosis. The final diagnoses for this animal include adrenal-dependent hyperadrenocorticism and presumptive polycystic kidney disease. Polycystic kidney disease is the most reported developmental renal anomaly in NHPs, particularly for slender lorises (*Loris lydekkerianus*). Hyperadrenocorticism is rare in both human and nonhuman primates. Both conditions likely contributed to the profound calcium and phosphate disturbances noted in the serum chemistry.

PS27 A Raft Above the Ocular Deep: A Focal Lens Lesion With a Mysterious Undertow in a Rabbit

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An 18-month-old female New Zealand White rabbit (*Oryctolagus cuniculus*) presented for a central opacity in the right eye. Twenty days prior, the rabbit received bilateral imaging-guided subretinal injections to model human choroidal neovascularization. Ophthalmologic examination identified a well-demarcated, smooth, pale-tan irregularity at the level of the lens, which obstructed the view of the fundus. Intraocular pressure (IOP), Schirmer's tear test, and corneal fluorescein staining were within normal limits. Epiphora, blepharospasm, and anterior aqueous flare or debris were not noted, and examination of the left eye was unremarkable. Differential diagnoses for the opacity included cataract (lens degeneration), uveitis +/- posterior hypopyon (lens rupture, lens luxation), infection (bacterial vs. E. cuniculi), metabolic (xanthoma), or neoplasia (lymphoma vs. amelanotic melanoma). Over the next two weeks, the opacity increased in surface area, IOP decreased, and epiphora was noted. Suspected uveitis was then responsive to a short course of Neomycin-Polymyxin-Dexamethasone. Throughout the remainder

of the study (seven months), the opacity persisted unchanged, IOP dropped twice but responded well to medical management, and the animal remained comfortable. Histology confirmed clinical suspicion of lens degeneration with cataract formation as the source of the persistent opacity. Unexpectedly, the opaque lens had hidden a posterior growth of histologically normal mature bone complete with well-differentiated marrow, prompting an additional diagnosis of intraocular heterotopic bone. While rabbits are often a favorable model for ocular study, the relatively large size of their lens makes intraocular injection technically challenging- potentially predisposing rabbits to lens trauma, even when guided by imaging. Subsequent intermittent leakage of lens fibers leads to chronic inflammation, which often necessitates surgical intervention. While penetrating injuries and chronic inflammation are recognized as risk factors for the development of heterotopic bone in multiple species, this case represents a unique circumstance with aseptic penetration. This report signifies potential atypical consequences to otherwise common experimental (and clinical) procedures.

PS28 Abdominal Mass in an Audiogenic Rabbit

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A 2.5-year-old female audiogenic (EIII/J) rabbit presented with decreased appetite and decreased fecal output. The rabbit was a retired breeder and not experimentally manipulated. The initial physical exam was within normal limits except for mild dehydration and a mild decrease in body condition score. A standard protocol of ad libitum nutritional support and 40-60 mL of subcutaneous, isotonic fluids once daily until correction of dehydration was initiated. Subsequent examinations revealed the development of polyuria, polydipsia, and a mass in the cranial right abdominal quadrant. Diagnostics included dipstick urinalysis, ultrasound imaging of the abdomen, and serology. Top differentials included renal obstruction, Encephalitozoon cuniculi, and neoplasia. Urinalysis showed a decreased specific gravity of 1.011 and 3+ proteinuria with no other abnormalities. Ultrasound imaging revealed bilaterally enlarged kidneys with an irregular shape and a subjective increase of hypoechoic areas, suggesting loss of normal renal architecture. The rabbit was humanely euthanized due to a poor prognosis; a necropsy was performed. Gross necropsy showed bilaterally severely enlarged kidneys with an irregular contour, pale tan discoloration, and hemorrhages extending from the corticomedullary junction to the cortical surface. Histopathology revealed that the majority of the renal parenchyma had been effaced by neoplastic lymphocytes, as well as multiple infarcts and severe necrosis. These neoplastic lymphocytes also underlying the retina of each eye were present. Serological screening for common infectious agents was negative. Both serology and PCR of kidney tissue yielded negative results for E. cuniculi. The final diagnosis was renal and retinal lymphoma. While there have been several reports of spontaneous lymphoma in the kidneys and cornea as a result of a genetic mutation, this is the first report of presumed metastasis to the retina in laboratory rabbits of any strain or stock. Further breeding of this colony has continued, with close attention paid to littermates of the impacted doe to prevent the perpetuation of an inherited predisposition.

PS29 Bloat in an Ovine Heart Valve Replacement Model

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A 9-month-old, 53 kg female Suffolk sheep presented for bloat 18 hours after a heart valve replacement surgery involving a thoracotomy, cardiopulmonary bypass, and carotid artery cutdown. Other than a reaction to protamine, given to reverse heparin, consisting of an arrhythmia, hypotension, and bradycardia that necessitated discontinuing protamine and administering epinephrine 0.2mcg/kg IV, surgery and recovery were uneventful. Activated clotting times were prolonged but improved immediately postoperatively. The next morning, the sheep was quiet and had an open mouth, breathing with pale mucous membranes and a firm, bloated abdomen. The main differentials for bloat are primary ruminal tympany due to diet or secondary ruminal tympany caused by esophageal obstruction, esophageal compression, or nerve dysfunction. A stomach tube was placed, and a large volume of gas was released to relieve the bloat. By midmorning, the sheep had developed an intermittent productive cough with bouts of regurgitation. The abdomen remained soft but appeared mildly distended. A soft submandibular swelling was noted. The top differentials for this submandibular swelling were edema secondary to parasites or hemorrhage and subsequent clot formation secondary to disruption of the carotid artery closure. Esophageal compression from this swelling was the suspected cause of bloat. A fecal float was performed to look for *Haemonchus contortus*, but no parasitic eggs were seen. A packed cell volume/total solids revealed a low PCV, which can be expected after heart valve replacement. Diuretic, antimicrobial, and anti-inflammatory therapies were initiated. However, abdominal distension continued to progress, regurgitation became more frequent, and the submandibular swelling increased. The investigators elected for humane euthanasia. Necropsy confirmed hemorrhage at the site of the carotid artery cut down, compressing the esophagus. No complications were noted with the valve. In the event of incomplete heparin reversal, it is important to consider the effect that a rapid increase in blood pressure from crash drugs may have on vascular closures. In ruminant models utilizing cervical vascular cutdowns, it is also important to consider the normal physiology of eructation and how pathology at the cutdown site may lead to free gas bloat.

PS30 The Case of the Down Alpaca

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A 10-year-old alpaca gelding with a history of zinc-responsive dermatosis presented acutely obtunded in the field. On physical exam, he had a head droop to the right, was quadriplegic and quadratic, and had a slow pupillary light reflex bilaterally. He was tachypneic and open-mouth breathing. With a thunderstorm overnight, the top differentials involved trauma to the cervical vertebrae, cervical musculature, or nervous system. Cervical radiographs revealed no evidence of fractures or subluxation. Blood was collected for complete blood count and serum biochemistry, revealing dehydration and inflammation. Cytology and culture of cerebral spinal fluid revealed a mild increase in neutrophils and a mixed cell pleocytosis, indicating acute neurovascular inflammation without detecting infectious agents. The patient was placed on intensive supportive care, including oxygen, IV fluids, gastroprotectants, anti-inflammatories, and steroids to reduce CNS inflammation and pain. He was also treated with antibiotics, parasiticide, thiamine, and mannitol to cover a variety of possible etiologies for acute neurological deficits. However, his condition declined in the hospital over the following three days, and he was euthanized. Serum IgM Antibody ELISA returned positive for West Nile Virus. Although previous serologic studies approximate 80% of cases of West Nile Virus in camelids to be mild or asymptomatic, there is a risk of developing severe clinical disease and neurologic symptoms that can easily become fatal despite intensive nursing care. In these rare, severe cases, treatment is generally unrewarding unless clinical disease is observed early, the animal has been previously exposed to the virus, and intense nursing care is implemented. Mosquito control and vaccination with an equine-labeled product are strategies to mitigate disease, although the efficacy of vaccination compared to natural immunity is unknown.

PS31 Acute Vomiting and Labored Breathing in a Yucatan Miniature Pig

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A male, castrated Yucatan miniature pig (*Sus scrofa domesticus*) underwent experimental right hind limb orthopedic surgery and recovered uneventfully. At the time of surgery, 5mg/kg of a long-acting ceftiofur antibiotic was administered IM. A week later, a large swelling with erythema was noted on the right lateral neck with no other associated clinical signs. Another dose of the antibiotic was administered; however, within minutes after injection, the pig displayed signs of vomiting, lethargy, and labored breathing. Bloodwork indicated leukopenia, thrombocytopenia, hyperkalemia, hypochloremia, elevated creatine kinase, and dehydration. Despite treatment and supportive care, the pig declined within 12 hours and was euthanized. On necropsy, the neck mass extended into the underlying musculature with multifocal to coalescing areas of abscessation and diffuse edema. Multifocal to coalescing areas of hemorrhagic gastritis and one focal area of gastric mucosal ulceration were noted in the stomach. The lungs were diffusely mottled purple to dark red and oozed yellow foam on the cut section. Histopathology results were consistent with an extensive localized infection, hemorrhagic gastritis, and acute respiratory distress syndrome (ARDS). The neck lesion was suspected to be an injection site infection from the initial surgical procedure. Differential diagnoses for the gastric lesions were infection and stress. ARDS was likely independent of the neck and gastric lesions. The differential diagnoses for the immediate clinical signs post-antibiotic injection and ARDS were peracute drug reaction, anaphylaxis, and drug contamination. Further testing of the antibiotic ruled out bacterial contamination; therefore, anaphylaxis is the suspected cause of clinical decline in this patient. Long-acting ceftiofur antibiotics are commonly used in swine medicine. While warnings of allergic reactions are described on the drug labeling for humans handling the medication, reports of drug reactions in swine are not widely reported.

PS32 Neck Mass in Northern Tree Shrew (*Tupaia belangeri*)

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One eight-year-old adult male, singly housed northern tree shrew, presented with a slow onset of a range of clinical signs within a 6-month period, including patchy haircoat, alopecia, weight loss, and hyperactivity. Additionally, an approximately 2 mm in diameter, raised, subcutaneous, skin-colored mass was visualized on the midline of the ventral neck. Top differentials included abscess, hyperthyroidism, granuloma, and neoplasia (lymphoma, lipoma, adenocarcinoma). Diagnostics included point-of-care blood glucose monitoring, which was within normal limits, and serial weight checks. A full diagnostic workup was offered to the lab, but the request was declined due to the animal's advanced age. This animal was humanely euthanized due to declining body condition and acute onset of decreased activity. A necropsy was performed, with the most notable finding consisting of a 7 x 7 x 3 mm soft mass localized to the right thyroid gland, which was approximately ten times the size of the left thyroid gland. Histopathological examination of this tissue revealed 90% effacement and replacement of normal right thyroid tissue with a malignant epithelial neoplasm. Additionally, though the left thyroid gland appeared grossly atrophied, histopathology revealed 50% replacement with the same epithelial neoplasm. Both thyroid glands revealed neoplastic cell infiltration into the capsule and vascular invasion, which confirmed the diagnosis of follicular-compact thyroid carcinoma without evidence of metastasis. This is the first report of a spontaneously occurring thyroid carcinoma in the northern tree shrew.

PS33 Subcutaneous Flank Mass in an African Spiny Mouse (*Acomys cahirinus*)

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A 7-month-old female African spiny mouse (*Acomys cahirinus*) presented with a large swelling overlying the left hindlimb, extending from the flank to the hip. The animal was quiet and exhibiting a left hindlimb limp. It was anesthetized with inhaled isoflurane for physical examination. This revealed a 1 x 1 x 1 cm soft, lobulated, subcutaneous mass, which contained serosanguinous, malodorous fluid upon fine needle aspiration (FNA) of the lesion. No abnormalities were noted on orthopedic examination. Differential diagnoses included subcutaneous abscessation and neoplasia. The mass was lanced and flushed with a mixture of sterile saline and povidone-iodine. The wound was left open to drain and heal by the second intention. Systemic antibiotic therapy was initiated with amoxicillin water (~0.25 mg/mL) for seven days, and carprofen (5 mg/kg SC) was administered as needed for pain relief. The culture of the mass did not yield any bacterial growth. Cytology of the FNA sample revealed cohesive aggregates of pleomorphic, neoplastic epithelial cells. The mass recurred within three weeks, although slightly smaller with a firm consistency. The animal's quiet demeanor and gait abnormalities had resolved, so the mass was monitored. Two months after the initial presentation, the mass markedly increased in size over a 7-day period, and the spiny mouse displayed tachypnea and a left hindlimb limp. It was subsequently euthanized and necropsied. A gross necropsy revealed a pink, lobulated, subcutaneous mass with a necrotic center measuring 2.5 x 1.5 x 1 cm. Histology of the mass confirmed a diagnosis of mammary gland carcinoma, which was locally invasive with no evidence of distant metastasis. Although mammary carcinomas are well-documented in laboratory mice (*Mus musculus*), to our knowledge, mammary tumors have not previously been reported in African spiny mice. They should be considered as a differential diagnosis for masses found on the ventral or sides of female spiny mice.

PS34 A Comparative Evaluation of Animal Bedding Suitability for Radioligand Research

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Ensuring the safety and health of both research animals and researchers is a crucial part of any research involving radiation hazards. One significant possible source of radiation exposure for both mice and technicians is cage bedding, which can become irradiated through exposure to radioactive excretions after intravenous injection with radioligand therapies. Researchers may be exposed to radioactive particles in suspension when cage bedding is agitated during routine handling and sample collection, while animals may ingest radioactive bedding and exhibit abnormal gastrointestinal readings during biodistribution studies. In order to determine if multiple bedding types are suitable for radioactive work, a study of several key variables for animal and researcher health was conducted in two different beddings. Female athymic nude mice were subcutaneously injected with cancer cells and randomly placed into cages with either uniform virgin paper pulp cellulose or 1/8-inch corncob bedding. Mice were monitored for differences in overall well-being, including food consumption, nesting behaviors, cage ammonia levels, ocular health, and tumor necrosis. Once tumors reached a volume of 300mm^3 LWW, mice from both cage types were injected intravenously with ^{212}Pb therapy, and then a cage change was simulated 4 and 24 hours post-injection.

Radioactivity readings were collected from the area around the cage, the handler's face and chest, and the cage bedding and filters. A biodistribution study was also conducted to observe any differences in radiation levels in the gastrointestinal tract at 4 and 24 hours post-injection. Preliminary results indicated no statistically significant differences across these measurements between the two bedding types. The results of this study indicate that both types of bedding are equally suited to radioligand work. This conclusion will be beneficial for ensuring that the safety and health of both researchers and animals are maximized. Additional research to replicate and further support these conclusions is ongoing.

PS35 Are Room-Level Temperature and Humidity Readings Truly Reflective of What Mice Experience Inside a Microisolator Cage?

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Standard to most animal facilities is the process of recording room temperature and humidity in animal housing rooms. Great effort is made to standardize the temperature and humidity of the room within set parameters. However, it is unclear if the temperature and humidity of the room are an accurate reflection of the conditions inside rodent microisolator cages. We used a newly developed battery-operated wireless sensor system to measure and transmit temperature and humidity to an accessible cloud interface from inside occupied rodent microisolator cages. Using the system, we determined that environmental conditions inside an occupied cage differ from room-level conditions. In general, there is a clear diurnal intra-cage cycle of both temperature and humidity. Both increase during the night/active phase. The in-cage and room humidity are roughly higher at night and lower during the day, generally mirroring typical outdoor conditions. However, humidity intra-cage is increased above room level at night when the mice are active. This humidity pattern is not replicated in an unoccupied microisolator cage. Similarly, the occupied in-cage temperature is higher than room temperatures, even more so at night. Potential minor fluctuations in room temperature also seem to be blunted when animals occupy the cage but not in an empty cage. It is important to understand that the in-cage environment of enclosed caging systems, such as mouse microisolators, differs from room-level conditions, which may be important for experimental outcomes in certain studies. Using an in-cage monitoring system to document environmental conditions is the first step in determining if there is an experimental impact.

PS36 Exhaust Dust Samples from the Prefilter Location of Individually Ventilated Cage Racks Provide High Diagnostic Sensitivity for Mouse Pathogens

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Real-time PCR testing of dust from the return air systems of open airflow IVC rack systems provides a significant refinement in rodent colony health monitoring by delivering improved pathogen detection and replacing sentinel rodents for this purpose, consistent with the 3Rs. Several studies have reported results from dust samples collected from the plenum ports, airstream, rack exhaust prefilter, and other areas where exhaust dust collects. Devices used to collect exhaust dust samples include flocked swabs, sticky swabs, gauze, filters, or collection media specifically designed for this purpose. Both the location on the rack and the material used to collect the exhaust dust can impact pathogen detection. In this study, we evaluated real-time PCR pathogen detection of samples collected from two different types of open airflow IVC racks housing naturally infected mice on corncob bedding over a 3-month period using the manufacturers' recommended collection devices (n=1 or 2) as well as a high binding capacity matrix placed in front of the rack exhaust air prefilter (n=4 or 3). In IVC rack A, 14 viral, bacterial, or parasitic pathogens were detected using the manufacturer's device, whereas 19 pathogens were detected in the exhaust prefilter dust sample. Of the pathogens detected in both samples, the exhaust prefilter sample contained 4- to 71-fold more copies per PCR reaction. In IVC rack B, six viral, bacterial, or parasitic pathogens were detected using the manufacturer's device, whereas 15 pathogens were detected using the exhaust prefilter sample. Of the pathogens detected in both samples, the exhaust prefilter sample contained 22- to 96-fold more copies per PCR reaction. These data demonstrate that exhaust dust samples collected from the prefilter of open airflow IVC racks provide high diagnostic sensitivity for mouse pathogen detection.

PS37 Impact of Bedding Type on Pathogen Detection with Sentinel-Free Agitated Soiled Bedding (SFSB) Rodent Health Monitoring

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Reduction of animal numbers in research, a cornerstone of the 3R's principles, can be directly applied to animal health monitoring. Several studies have recently demonstrated the efficacy of agitated Sentinel-Free Soiled Bedding (SFSB) cages in monitoring rodent colony health versus traditional sentinel animal monitoring strategies. Materials such as swabs, Remay paper, filters, or other collection media directly placed in SFSB cages have been evaluated for use with this testing strategy. However, most studies have utilized corn-cob bedding and have not focused on the influence of bedding type on SFSB monitoring. In this study, we evaluated the utility of additional bedding types (multiple paper-based beddings, aspen chips, and combination beddings) for use with SFSB monitoring strategies. We performed six experiments where high binding capacity matrices (n=5) were placed in soiled bedding collected from naturally infected donor mice (n=5 per group) at a 2-week cage change interval. Donor mice were tested via fecal and fur swab PCR on arrival. After exposure to two weeks of accumulated soiled bedding, matrices were tested by PCR for rodent viral, bacterial, and parasitic infectious agents. We compared prevalence in each animal group to pathogen recovery on exposed matrices. Direct comparison between bedding types was not applicable as each experiment was individually performed. We found that all expected viral, bacterial, and parasitic infectious agents were consistently detected by SFSB replicates regardless of the bedding type used. These data demonstrate that SFSB provides a reliable method to monitor colony health regardless of the bedding type used. While SFSB has been previously proven to be more sensitive than soiled bedding sentinels, the majority of published studies were performed on corncob bedding. These novel findings provide support for the utilization of SFSB-based rodent health monitoring on a wider variety of bedding types.

PS38 Automated Dispensing Cabinets, Advanced Technology Solutions for Managing Controlled Substances

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Diversion of controlled substances within a veterinary or laboratory research setting may lead to serious impacts on animal welfare, health risks or harm to individuals diverting controlled substances, and liability risks for practitioners, researchers, and the institution. The development of a controlled substance management program that complies with all federal, state, and local laws is paramount for public safety and should utilize a collaborative or interdisciplinary approach with the development of guidelines, policies, training, and monitoring to enhance accountability and discourage diversion. Furthermore, a controlled substance management and diversion program should include assessment and monitoring of risk-based areas such as procurement, storage, security, dispensing, administration, record keeping, waste, and disposal. In these efforts,

automatic dispensing cabinets are useful technology solutions for managing controlled substance inventory, automating records, documenting waste, tracking chain of custody, and systematic auditing. As part of a controlled substance management program, we implemented the use of automated dispensing cabinets to manage the usage and storage of controlled substances for surgical, anesthesia, and clinical services. Cabinet formulary and dispensing settings were re-configured for veterinary and research applications, including species-specific patients. Clinical data categories were assigned to capture individual patient identifiers, vial numbers, and removal criteria (single or group, clinical research, training, or surgery). Removal criteria are restricted to specific drugs or concentrations, areas, species, individual, or clinical roles. Waste and disposal functions are handled by a dedicated controlled substance management team with integrated discrepancy criteria to document inventory or waste variance. Timely reports, including removal or access history, are now available remotely and are used for tracking usage trends, inventory, access, and expiration dates, resulting in a >50% reduction in labor and wages that were previously needed with a lock box to maintain documentation. A formal analysis will be included. Due to their vast capabilities and customization, automated dispensing cabinets provide an effective customizable technology solution and are a valuable component for managing controlled substances and minimizing diversion.

PS39 Cage Grid Cleanliness Evaluation – Extending Mouse Grid and Lid Change Intervals from Four to Eight Weeks

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We have evaluated the possibility of extending the mouse grid and lid change interval from 4 to 8 weeks. By extending the interval, the AST Got (Animal Sciences and Technologies, Gothenburg) department will be able to shift hours spent on grid change to animal husbandry and welfare work, providing more study support to TAs and increasing our safety study load. Expenses and environmental impact will be reduced due to less washing and autoclaving as well as the heavy physical workload for staff. Less frequent changes will potentially reduce stress for the animals, leading to better science. The study was conducted over ten weeks in collaboration with an external company, sampling a total of 125 cages comprised of either IVC or static cages with group or single-housed males or group-housed females. Bacterial growth was assessed using TPC pressure plates, and ATP levels were measured with a luminometer to evaluate general cleanliness. Relevant cut-off values have been set for each assessment after extensive literature reviews. Statistical evaluation shows no biologically relevant variation between weeks 2 and 8, and results fall far under the thresholds, so the conclusion is that the change of grids and lids can be made every 8th week instead of every 4th week.

PS40 Practical Considerations for Managing Colonies of Aged Mice: Lessons From Four Strains

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The presentation will highlight practical considerations and observations from an ongoing effort to maintain large colonies of male and female inbred C57BL/6J (B6) and genetically diverse HET3 mice through the age of 90 weeks, compared with pilot studies involving BALB/cJ and BALB/cByJ. We will first describe the housing, enrichment, and other husbandry conditions, then define the list of tolerated and non-tolerated conditions for mice in the aging colonies. We have found relatively low attrition rates under these conditions. Since 2020, average weekly loss rates for B6 in various age ranges were: 0.7% of females & <0.2% of males aged 25-51 weeks; 0.8% of females & 0.3% of males aged 52-75 weeks; and 0.9% of females & 0.6% of males aged > 76 weeks. Average weekly loss rates for HET3 were: 0.1% of females & 0.6% of males aged 25-51 weeks; 0.1% of females & 0.7% of males aged 52-75 weeks; 0.4% of females & 0.7% of males aged >76 weeks. The top causes of attrition from the B6 colonies were dermatitis, mortality, and eye abnormalities, while aggression (particularly in males) and mortality accounted for most BALB and HET3 losses. The dermatitis rate was lower for B6 females housed in non-ventilated caging, but the B6 male mortality rate was higher in this cage type. Rectal prolapse (a common condition reported in aged B6 mice) was rarely documented; this could be a function of the high health status of the rooms. Attrition rates were not appreciably different when males or females from B6 or either BALB substrain were housed at a reduced density. We will describe how the appearance changes with age, including striking variations in body weight/size and changes in a fur coat. Comparative natural history studies found strain-specific differences in aging-related phenotypes, including the abundance of T cell populations. For example, among CD4 T cells in the spleens of male mice, naive cells declined between 8 and 78 weeks from 82 ± 3% to 36 ± 9% in B6 versus 81 ± 3% to 65 ± 10% in HET3; BALB/cByJ cells declined at an intermediate rate. We will conclude with suggestions for housing and stress reduction in aged colonies.

PS41 Changes to Traffic Pattern Standard Operation Procedure Improve Operational Efficiency while Maintaining Animal Health and Research Reproducibility

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Maintaining health status in rodent facilities is paramount for protecting animal health and research reproducibility. At our institution, the traffic pattern (TP) standard operating procedure (SOP) for rodents historically mandates a full shower with uniform change when moving from high to low-risk rooms (from high to low-risk: ABSL-2, specific pathogen-free [SPF], mouse norovirus [MNV] negative). However, this TP SOP decreases operational efficiency for veterinary staff and animal care staff, who must plan their day to perform activities in TP in order to avoid showers. To balance operational efficiency while ensuring animal health, our facility implemented a two-part change to the rodent TP SOP. 1) When going against the TP, a change in PPE is sufficient in many cases. With the advent of laminar flow hoods and proper micro-isolator technique, the potential of pathogen spread in rodent colonies is minimal when PPE is changed when working with colonies of different health status. 2) MNV negative rooms are offered on an as-needed basis for researchers who request it, instead of offered by default, allowing more rooms to be SPF. MNV is increasingly considered endemic in research institutions, does not clinically impact immunocompetent mice, and has minimal impact on most research aims. Under this new TP SOP, room-level health status was maintained. No MNV was detected in MNV-negative rooms, and no additional pathogens of interest were detected in SPF rooms during two rounds of subsequent quarterly sentinel testing. For veterinary technicians, the average time to see cases was moved to earlier in the day (12:39p after the new TP SOP was implemented, 1:15p previously, p= 0.01), allowing for more flexibility in the afternoon to perform other duties. We interpret that veterinary technicians were willing to see new cases and recheck earlier in the day because it was less burdensome to move against the traffic pattern. For animal care staff, this new TP SOP allowed for flexibility of duties (e.g., cage change in the SPF room in the morning while being able to unpack MNV-negative animals in the afternoon). Finally, the new TP SOP improved general space utilization; researchers with previously separate MNV negative and SPF colonies were able to combine their colonies into a single room. Changes to the TP SOP improved operational efficiency for veterinary staff, animal care staff, and researchers while maintaining animal health and research reproducibility.

PS42 Advancing Decontamination in Animal Life Sciences: Insights from an Avian Research Center Study

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The quest for safer and more efficient decontamination methods within animal life science laboratories necessitates a shift from traditional, high-risk chemicals towards innovative solutions. This presentation examines one facility's path towards safer decontamination by challenging the efficacy and safety of high-consequence chemical decontaminants, such as formaldehyde and other agents known for harmful effects, compared to low-concentration hydrogen peroxide (HHP™) technology. Motivated to mitigate risks associated with conventional chemicals, which may lead to significant health hazards and environmental burdens, the facility questioned: Could a decontamination system achieve sporicidal efficacy without compromising safety for the staff or the research subject? To answer this, the facility tested HHP™ decontamination's ability to effectively decontaminate poultry eggs, pivotal for research and vaccine production, without impairing viability or hatchability. This study investigated the facility's traditional decontamination method of formaldehyde fogging and compared it to multiple treatments using HHP™ decontamination on cycles with 29-30 eggs each. Post-treatment, researchers incubated the eggs and later observed the eggs, examined them for viability using candling methods, and determined egg survivability measured by hatch rate. The microbial efficacy of each treatment was also validated via biological indicators, with consistent results ≥ 6-log sporicidal reduction even in the presence of bioburden. The study not only confirmed the absence of adverse effects on egg integrity (with a viability rate of 93%) but also demonstrated a superior average hatch rate (85%) compared to formaldehyde, proving a safer method of sporicidal decontamination was possible. Achieving high levels of efficacy without harming delicate specimens such as eggs suggests that the new method may be a superior choice for other sensitive animal research applications. This breakthrough is especially relevant for biosafety officers, facility managers, and directors of operations, presenting a compelling case for a viable solution through new technology, heralding a new era of safety and efficiency in decontamination practices.

PS43 Characterization of Myeloid Cell Hyperactivation Syndrome and Overall Survival in Humanized Super Immunodeficient NOG-EXL and NSG-SGM3 Mice

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Super immunodeficient mice such as the NOG-EXL and NSG-SGM3 combine severe immunodeficiency with transgenic expression of human myeloid stimulatory cytokines. This results in enhanced human immune cell reconstitution and improved expansion of myeloid cell populations upon humanization with CD34+ hematopoietic stem cells (HSCs). However, the application of these humanized immune system models can be complicated by adverse events. Humanized NSG-SGM3 (huNSG-SGM3) mice develop a lethal macrophage activation syndrome (MAS) and mast cell hyperplasia that limit their use in long-term studies. To what extent humanized NOG-EXL (huNOG-EXL) mice suffer from the same conditions has been unclear. In this study, we aimed to compare the effects of HSC engraftment in these two mouse strains in an orthotopic patient-derived glioblastoma organoid xenograft model. NSG-SGM3 mice (n=10) humanized in-house were compared to NOG-EXL mice (n=10) humanized in-house and to commercially available huNOG-EXL mice (n=12). Mice were euthanized at humane or study endpoints, and a complete pathological assessment was performed. A semiquantitative multiparametric clinicopathological scoring system was developed to characterize the myeloid proliferative disorder. Humanized NOG-EXL mice survived longer (to experimental endpoint) than huNSG-SGM3 mice (22 vs 16 weeks post humanization), with significantly less severe MAS and lack of mastocytic proliferation. Overall, huNOG-EXL mice had less severe lesions characterized by human myeloid cell activation, with limited tissue distribution, compared to huNSG-SGM3 mice. Major findings included mast cell infiltration of the pancreas and liver (huNSG-SGM3 only) and increased eosinophilopoiesis and histiocytic infiltration of the spleen (both strains). Engraftment of human lymphocytes, assessed by immunohistochemistry, was similar in the two strains. The longer survival and decreased MAS severity in huNOG-EXL mice enabled their use in a xenograft transplantation study. In summary, humanized NOG-EXL mice develop a milder MAS and do not develop mast cell hyperplasia relative to humanized NSG-SGM3 mice. In conclusion, the NOG-EXL model may be better suited than the NSG-SGM3 model for immuno-oncology studies requiring long-term survival post-humanization.

PS44 Development of Adeno Associated Virus (AAV) Neutralizing Antibody (NAb) Detection Assays for AAV5 and AAV6 Serotypes

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Adeno-associated viral vectors have become the cornerstone of gene therapy studies. Exposure to the most utilized viral serotypes, including AAV2, AAV8, and AAV9, is common in humans and non-human primates. This leads to immunity through the production of neutralizing antibodies (NAbs) against the viral particles, limiting their utility in certain contexts. As a result, alternative AAV serotypes are being considered. We sought to develop cell-based AAV Nab detection assays for serotypes reported to have lower neutralizing antibody prevalence, including AAV5 and AAV6. Utilizing a luminescent reporter inside AAV serotype-specific capsids, the transduction of the viral vector was measured at a range of viral titers to identify the optimal multiplicity of infection (MOI). Optimized assays were qualified through testing by two technicians over three days to assess the diagnostic sensitivity, specificity, and reproducibility of these assays to detect the presence of NAbs against AAV. Specificity and sensitivity were greater than 98% in these assays, and results were reproducible across six replicates. Selectivity was also determined by performing the assay in the presence of purified AAV NAbs against other AAV serotypes. Cross-reactivity was observed between AAV6 viral vector and AAV1 NAbs, as has been described in the literature, but not NAbs against other serotypes tested (AAV2, AAV4, AAV5, AAV8, and AAV9). AAV5 showed no cross-reactivity with NAbs against other serotypes up to concentrations of 500 ng/mL. Finally, four known positive samples were serially diluted from 1/10 to 1/5120 to assess reproducibility in the limit of detection of the assay when performed by separate technicians on different days. The detection limits for all samples tested in the AAV5 and AAV6 assays were found to be within one two-fold serial dilution across all trials. These assays offer a specific and sensitive method for routine pre-screening of AAV5 and AAV6 NAbs, allowing for informed candidate selection for studies. Furthermore, summarized results of our future screening will greatly expand data on the specific prevalence of these antibodies in animals from different source colonies, countries of origin, and species, minimizing the number of animals needed for pre-screening going forward.

PS45 Development of Novel Diagnostic qPCRs to Detect and Prevent Spread of Infectious Diarrhea in NSG Mice

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In late 2020, our mouse barrier facility experienced an outbreak of diarrhea and acute deaths of females during mid-lactation. Affected mouse strains were immunodeficient, carrying either the SCID (Prkdcscid) or RAGnull mutations and the IL2rgnull mutation, predominantly in NSG mice. The diarrhea was transmissible to naïve NSG mice by gavage of intestinal homogenates from diarrheic mice or by co-housing, indicating a contagious agent, and was designated "infectious diarrhea." Conventional diagnostics failed to identify the etiology. Given the critical role of NSG strains in biomedical research, this diarrhea outbreak has been a particular challenge for our researchers. An in-depth metagenomic analysis of DNA samples from diarrheic and control fecal samples identified *Clostridium cuniculi* and an enterotoxin as candidate factors underlying the diarrheal outbreak. We hypothesized that the presence of *C. cuniculi* and its enterotoxin in fecal samples can serve as biomarkers for infectious diarrhea. qPCR diagnostic assays using primers designed for *C. cuniculi* and its enterotoxin were generated to test this hypothesis. We analyzed 40 fecal samples from NSG or NSG-related mice with clinical signs of infectious diarrhea, 100 fecal samples from healthy NSG mice, and 20 fecal samples from NSG or NSG-related mice with diarrhea due to other known causes. *C. cuniculi* and its enterotoxin were identified only in the feces of mice with infectious diarrhea. Healthy NSG mice, NSG mice with diarrhea due to other causes, or non-NSG strains with diarrhea were negative by qPCR. All positive samples contained both *C. cuniculi* and its enterotoxin DNA. Healthy NSG mice co-housed with NSG mice with infectious diarrhea or housed on their soiled bedding resulted in the experimental mice expressing *C. cuniculi* and enterotoxin DNA markers within 24 hrs, sometimes with clinical signs of diarrhea. The study suggests that the infectious diarrhea is mediated, at least in part, by the transmission of *C. cuniculi* and its enterotoxin. The novel qPCR assays for *C. cuniculi* and its enterotoxin are effective diagnostic tools for the detection and prevention of the spread of infectious diarrhea in NSG mice.

PS46 Development of Serological Methods for Detection of *Trypanosoma cruzi* Exposure

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Trypanosoma cruzi is a protozoan parasite that is the causative agent of Chagas disease. *T. cruzi* is transmitted by members of the Triatominae subfamily, commonly referred to as kissing bugs. Historically, the range of species most associated with the transmission of *T. cruzi* was predominantly Central and South America; however, globalization and the warming climate have led to an expansion of their range as far north as Pennsylvania. As a result, colonies of non-human primates throughout the US are at risk of infection. While PCR-based methods of detection can be highly effective at diagnosing *T. cruzi* infection in the acute phase, these assays are less sensitive when the infection enters the chronic phase. In these instances, serological detection is preferred, so we sought to develop a robust serological detection method for routine diagnosis of *T. cruzi* infection in NHPs. We developed and assessed the sensitivity and specificity of both native and recombinant antigens across serological platforms, including Enzyme-Linked Immunosorbent Assays (ELISAs), Multiplexed Immunofluorescent Assays (MFIAs), and Indirect Fluorescent Antibody Assays (IFAs). We showed that, while highly sensitive, serological tests based on recombinant antigens alone lacked specificity and yielded false positive results (1% of >5,000 samples), necessitating confirmation by secondary methods. When coupled with assays utilizing lysates from the native *T. cruzi* parasites, this specificity improved markedly by more than 50%. Utilizing results from >22,000 samples tested by our serological methods, we measured the prevalence of *T. cruzi* infection in NHP colonies to be 5.89% as compared to 0.23% by PCR-based techniques (6,500 samples), consistent with limitations in PCR detection of the chronic phase of infection. NHP samples consisted of periodic routine health monitoring and quarantine screening following import. Most commonly, whole blood collected in EDTA tubes was submitted for PCR testing, while serological testing utilized serum or whole blood absorbed into a microsampling device. This data confirms that diagnosis of *T. cruzi* infection by MFIA should include a whole protozoan lysate antigen; otherwise, results should be confirmed by a secondary serological method such as ELISA and/or IFA.

PS47 Effects of Valsalva Maneuver and Trendelenburg Positioning on Success Rate for Ultrasound Guided Jugular Vein Access in Pigs

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Minimally invasive techniques for jugular vein (JV) catheter placement are challenging in pigs. We hypothesized that the Valsalva maneuver (V) and Trendelenburg positioning (T) increase jugular vein diameter in pigs and that the Valsalva maneuver combined with Trendelenburg positioning (VT) improves success rates for ultrasound-guided JV access regardless of operator experience. In eight healthy Yorkshire-cross pigs, maximum JV cross-sectional diameters were recorded using four methodologies: neutral position (0° inclination, no breath-hold) (N), V, T, or VT. Eight operators, blinded to the study objective, attempted ultrasound-guided introducer needle placement using two methodologies: VT or N. Order of side and vein were randomized, and outcome measures were recorded for VT and N (time to initial puncture, number of attempts, success (yes/no)). Operators completed a survey regarding experience level and evaluation of placement methodologies. Data was assessed using linear and generalized mixed effects models with post-hoc analyses. The external JV (EJV) diameters were significantly higher than internal JV (IJV) diameters ($p<0.001$). The IJV diameters were significantly higher in VT compared to N ($p<0.001$), and the EJV diameters were significantly higher in any methodology group (V, T, VT) compared to N (T vs. N, $p=0.0008$, TV vs. N, $p<0.0001$, V vs. N, $p=0.0045$). Operators experienced significantly fewer attempts ($p=0.0142$) and significantly higher success rates ($p=0.0167$) of introducer needle placement in VT than N. There was no significant difference in time for needle placement between VT and N. There was no significant difference in attempts between IJV and EJV introducer needle placement. Self-reported operator experience did not affect introducer needle placement outcome measures. Implementation of VT methodology for IJV and EJV venipuncture in pigs can reduce the number of attempts and increase success rates for needle introduction compared to the commonly used N methodology.

PS48 Evaluation of the Antimicrobial Effects of Blue Light and Hypochlorous Acid on the Macaque Cranial Implant Margin Microbiota

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Cranially-implanted macaques are a critical model for neuroscience research. Cranial implant complications include brain abscess, meningoencephalitis, and implant-tissue margin infections. Overuse of antimicrobials has led to increased resistance, risking macaque health and raising zoonotic risks to personnel. The goal of this project was to assess the antimicrobial effects of blue light (~415nm) and hypochlorous acid (HOCl) treatment of cranial implant margins compared to a standard HOCl treatment regimen. Blue light exerts antibacterial effects via the induction of reactive oxygen species and the inactivation of bacterial catalase. We hypothesized that exposure of cranial implant margins to a commercially available blue

light device followed by HOCI treatment would improve clinical appearance and decrease bacterial burden as assessed by aerobic/anaerobic culture and tissue margin microbiota analysis (decreased alpha- and beta-diversity and altered taxonomic composition). Eight chaired rhesus macaques with cranial implants underwent exposure to 6 minutes of blue light followed by 0.024% HOCl solution for 12 sessions (3x/week for four weeks). Swabs for microbiota analysis and bacterial cultures were collected pre- and 24 hours after the last treatment session. Control microbiota swabs were collected from a separate implant margin area only exposed to HOCl. All animals tolerated the blue light exposure but had varied improvements in margin clinical appearance. The most common bacteria identified on culture were *Staphylococcus aureus* (N=8), B-hemolytic streptococcus (N=8) and *Corynebacterium ulcerans* (N=6). Microbiota analysis of the V4 region of the 16s rRNA gene demonstrated many anaerobic operational taxonomic units (OTUs) in addition to the aerobic species cultured, highlighting the limitations of culture-based methods. All animals had unique microbiota taxonomic profiles with a mean of 84 OTUs identified and a median Shannon diversity index of 2.6. No significant differences were found between treatment groups, alpha-diversity or beta-diversity pre- and post-study. The effectiveness of blue light therapy is likely related to the power of the device and the depth of penetration into the tissue margin. While safe, future work is needed to optimize the dose and delivery methods of light-based therapies.

PS49 Identification of Multiple Plasmids in *Corynebacterium bovis*

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Corynebacterium bovis is an opportunistic pathogen of cows, rodents, and humans that can colonize the skin and mammary glands. While its route of introduction into research rodents is unknown, immunodeficient rodents are uniquely susceptible to *C. bovis* colonization. Yet, clinical signs due to infection are reported to be variable, ranging from no clinical presentation to skin inflammation, scaly skin, and weight loss. Here, we question if the genetic composition of C. bovis isolates correlates with clinical presentation or aligns with outbreak conditions. In this study, we performed long-read sequencing of twelve *C. bovis* isolates, representing eleven isolates from immunodeficient mice and one isolate from an immunodeficient rat. *C. bovis* colonies were isolated from animal swabs collected during routine bacterial culture health monitoring from academic, pharmaceutical, and industry sources. Results were correlated with host species and the presence or absence of clinical signs. Plasmid sequences were annotated and aligned to compare genomic organization. Here, we report four distinct plasmids associated with *C. bovis* isolates derived from rodents used in biomedical research. Three *C. bovis* isolates of colonized immunodeficient mice with skin hyperkeratosis contained two plasmids that were approximately 71kB and 52kB in size. A single isolate from athymic nude mice with transient signs or without disease also contained both the 71kB and 52kB plasmids. In contrast, isolates from immunodeficient models without any evidence of clinical signs (including the single rat isolate) contained two different plasmids, which were approximately 78kB and 56kB in size. A third genotype was observed, where two isolates from the same non-clinical outbreak were found with a single 56kB plasmid. This data suggests a genetic component that differentiates *C. bovis* isolates of immunodeficient rodents with clinical versus non-clinical presentations. The significance of these plasmids is not yet defined, but it reinforces previous publications recognizing host-specific genetic variation of *C. bovis* isolates and a correlation between genotype and disease. Future work, including a re-analysis of previous studies, will aim to test this hypothesis further to help define our understanding of the variables leading to clinical *C. bovis* onset.

PS50 Vaginal Impedance Measurement as an Indicator of Estrous Cycle Stage in Guinea Pigs (*Cavia porcellus*)

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Historically, preclinical studies have focused on male animals. Current National Institute of Health guidelines recommend that studies involving vertebrate animals include both male and female subjects unless scientifically justified to study only one sex. When including sex as a variable, the resulting data may be affected as a comparison of data using females could be impacted by the potential influence hormonal levels have in relation to the estrous cycle. The gold standard for determining the estrous stage in the guinea pig is vaginal cytology. However, vaginal cytology has the disadvantages of requiring technical skill to differentiate stages, as well as being a time-consuming process. Vaginal impedance measurement has been suggested in other rodents for determining estrous cycle stage, but it has yet to be validated in guinea pigs with current technology. It was hypothesized that vaginal impedance measurement would correlate with the estrous cycle in guinea pigs and, thus, present a refinement for researchers when collecting data from female animals. Vaginal impedance measurements were collected each day for three 16-day estrous cycles of six Hartley guinea pigs (Crl: HA, n = 6) for a total collection of 18 full estrous cycles. Immediately following impedance measurement, cytology samples were collected by vaginal lavage using sterile saline. The collected samples were deposited onto glass slides and stained with Diff-Quik. Cytological analysis was performed to determine the estrous stage for each guinea pig. In a linear regression model, there was a significant relationship between impedance value and estrous cycle ($P < 0.05$), with average impedance measurement at a higher value during proestrus as compared to the other estrous cycle stages. This knowledge allows researchers to use proestrus as an anchor point when collecting data in guinea pigs during the estrous cycle. Understanding the stage of estrous during data collection will help elucidate any confounding influence hormonal variations during the estrous cycle may have on studies.

PS51 Competence Assessment in a Portuguese Research Framework: Promoting a Culture of Care in Laboratory Rodents

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Compliance with Ethics, Regulatory Directives, and National Legislation is imperative when using animals for scientific purposes, ensuring the well-being of the animals involved while promoting reliability and a good research outcome. In this context, people are required to undergo a thorough process of education, training, and supervision, culminating with the assessment of proficiency in routine techniques. Thus, this process is expected to be clearly defined, reliable, and consistent across different trainers, assessors, facilities, and institutions. Based on the EU Common Education and Training Framework document, a comprehensive methodology of competence assessment was established within four Portuguese research Institutions in the Lisbon area. A dedicated Working Group defined thorough tabulated DOPS (Direct Observation of Procedural Skills) for the most prevalent laboratory techniques involving mice and rats. These tables comprise diverse items addressing learning outcomes related to skills, professional attitude, knowledge, and the application of the

3R's. All tables were validated before implementation, and clear guidelines and assessment criteria were defined, contributing to the standardization of this process across the four Institutions. The assessors in this process are members of both Rodent Facilities and Animal Welfare Bodies, with extensive experience in Laboratory Animal Science. Two years after the practical implementation of this process, it is evident that this collaborative work has enhanced the proficiency of everyone involved in the use and care of mice and rats for scientific purposes. Engagement with this new procedure has been remarkably positive, as this initiative has fostered a general culture of care, improved self-confidence, and promoted mutual help among users. Equally significant is the fact that this process facilitates the free movement of people between these four institutions and, ultimately, across other Portuguese institutions and EU countries with equivalent standards of competence. The competence assessment methodology achieved within the Working Group is reliable and consistent across different assessors and facilities, and it can serve as a valuable tool to harmonize skill validation in other Institutions. We plan to present the process of interinstitutional collaboration for the implementation of competence assessment, focusing on how the assessment tables were defined and structured, providing some examples, and the methodology used to validate these tables. Clear guidelines that contributed to ensuring a harmonized implementation of this process across the four institutions will also be discussed.

PS52 Enhancing Institutional Culture and Communication Through Implementation of a Voluntary Researcher Credentialing Program: Lessons Learned over the Past Ten Years

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As a large academic research institution consisting of six schools and nearly 500 principal investigators (PIs), with approximately 1200 active protocols involving many different species, our program continually strives to improve communication between our research community and those overseeing the animal care and use program. Specific goals are to streamline processes to reduce PI-perceived regulatory burden and to improve compliance and animal welfare simultaneously. To help achieve these goals, in 2014, our IACUC instituted a voluntary researcher credentialing program called the 'Laboratory Animal Research Coordinator (LARC) certification. This program is a nine-month curriculum offered annually to full-time members of research laboratories (with their PI's approval), such as laboratory managers or senior research technicians. The program's goals are for the LARC graduate to: 1) serve as their laboratory's "go-to" person for all information related to animal research, 2) function as a liaison between the laboratory and the campus entities responsible for the animal care and use program, 3) understand the regulations and how to be compliant in all aspects of their research, and 4) understand how to disseminate this information to other members of their lab. Over the past ten years, we have made continual adjustments to this program, including shifts toward active learning methods and a hybrid format, curriculum updates to address both known and perceived information gaps, and outreach improvements to advertise to new PIs. Over 200 LARCs have now successfully completed the program, representing 135 different labs. The program's continued popularity among the research community and resounding positive feedback from LARCs and their PIs are metrics of success. The program has been a major contributor to improved culture and collegiality between regulatory and research staff. Implementation of a similar program at other institutions, utilizing some of the lessons learned from our decade of successfully running this program at our institution, could improve researcher satisfaction and efficiency, communication, and compliance to strengthen the institution's animal care and use program.

PS53 Learning from Failure: Fostering the Culture of Care

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Working with animals in research entails a commitment to the Culture of Care (CoC). In AstraZeneca, we have pledged to support CoC, going above and beyond what is legislatively required of us in animal welfare, care of staff, transparency, and scientific quality. Focusing on errors is closely linked with staff psychosocial security, but also a natural component of securing sustained and improved animal welfare. Talking openly about what goes wrong can be a challenge for staff as it entails sharing examples and situations of "near misses" or "failures." Effectively working with learning from errors demands a pre-established culture of trust and care where staff willingly can share information on things that did not go the way it was planned without the fear of retaliation. In this talk, I will present the journey of setting up a "No-Blame, Learning from Failures" system in AstraZeneca. To do this, we adopted the "Human and Organizational Performance" (HOP) mindset from workplace safety and used it in the context of the culture of care. We developed a "leaning-log" and a method to address and take learnings from errors effectively. The log is an automated process that includes an easily accessible event reporting tool called "Learning Log" and a Root Cause Analysis practice. I will discuss what we have learned (pros, cons, requirements) and provide advice on how to address this new way of working in trustful collaboration with staff.

PS54 Take Your Kids to Work Day: Promoting Biomedical Research Awareness Through Youth Outreach

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The Central Illinois Branch of the American Association for Laboratory Animal Science partnered with the University of Illinois Animal Care Program at Urbana-Champaign for a BRAD-registered outreach event highlighting a day in the life of caring for animals who contribute to medical advancement through research. During this "Take your Kids to Work Day," employees shared their typical workday with the youth in their lives and promoted discussions surrounding the topics of biomedical research, animal welfare, and medical advancement. Stations in an animal housing facility were organized for participants to learn about different components of the program. The occupational health and safety specialist started the tour with a lesson on workplace hazards and a demonstration of personal protective equipment (PPE). Participants were shown how to properly don and doff the PPE they wore as they continued to the next stations. The husbandry staff set up a room with mock mouse cages that consisted of pipe cleaner mice and jellybeans to resemble neonatal mice. Participants were walked through a cage change and provided information regarding daily health observations of all animals. An enrichment station shared many ways to promote animal welfare with enrichment items. Participants got creative decorating boxes for rabbit hideouts and filling paper bags and cardboard rolls with hay. Additional stations focused on the veterinary care of animals and included a mock training session where general mouse information was shared, a surgical station where participants learned suturing techniques and the importance of aseptic technique, and a clinical pathology station that highlighted the use of a microscope and its importance for diagnosing conditions. Participants received gift bags containing BRAD materials and items donated by some of our vendors. The day was complete with inquisitive youth showing excitement for animal contributions to biomedical research and the professionals who make it all possible. The positive response from participants is motivation to host this event annually.

PS55 Using Visual Storytelling and Interactive Elements to Improve Engagement During Employee Training

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In this fast-paced digital age, people have become accustomed to immediate gratification and a constant stream of entertaining content. Studies have shown that over the past two decades, attention spans have been decreasing exponentially, so training must also evolve to meet the trainee's needs. People have limited patience when subject to the traditional approach of training in a lecture setting. Frequently, training is seen as a chore or, worse, punishment. Educational specialists have long known that when training is fun or interesting, it becomes "*sticky*," enabling learners to retain more information. In response to these changing demands, we have combined digital technology with established techniques used in other industries to make our educational content more engaging. Utilizing knowledge of consumer behavior from the advertising industry, we have updated signage and email blasts to increase the likelihood that staff will pause and read the content. By employing storytelling techniques from the movie industry, animated visuals have been developed that simplify the delivery of complicated training concepts in a way that is both enjoyable and easy for the learner to remember. Lastly, interactive games have been incorporated into online training material to encourage users to engage with the content actively. While animated videos and training gamification may seem expensive or complicated, new commercially available tools have allowed us to incorporate these methods into our training without a major investment. In response to these changes, we have seen an improvement in attendee knowledge and an increase in questions from the trainees who exhibit a deeper understanding of the material. This presentation will introduce these techniques and demonstrate how they can be implemented with "real-life" examples.

PS56 Organ-on-a-Chip Modeling: An Ethical and Translational Approach to CMT2S Research

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Animal model reliance for preclinical studies is a cornerstone of drug discovery and development. However, ethical concerns, translational limitations, costs, and the inability to fully simulate human physiology evidence the need for alternative approaches. For rare diseases, extensive animal testing is required to create and validate disease models. This ethical and financial burden curtails rare disease research. Microphysiological systems (MPS) are a promising solution, offering a platform that mimics human organ function *in vitro* and can be tailored to specific genetic variants. We present a neuromuscular junction (NMJ) MPS to study Charcot-Marie-Tooth Disease Type 2S (CMT2S) caused by a rare variant within immunoglobulin mu-binding protein 2. We developed a patient-specific CMT2S *in vitro* model, circumventing the need for animal model testing while providing the most representative disease model. CMT2S motor neurons (CMT2S-MNs) were differentiated from an induced pluripotent stem cell (iPSC) line generated from a CMT2S patient's fibroblasts. Patch clamp electrophysiology, phase imaging, and immunocytochemistry experiments were performed to characterize CMT2S-MNs. To determine NMJ defects, CMT2S-MNs and wild-type (WT) MNs were integrated into a dual-chamber NMJ platform with WT iPSC-derived skeletal muscle myofibers. NMJ functional defects were analyzed by number per chamber, fidelity, and fatigue index (FI). CMT2S-MNs were differentiated from CMT2S-iPSCs, confirmed by their positivity to neuronal marker MAP2 and MN marker ChAT. Imaging revealed thinner axonal processes and a higher number of varicosities in CMT2S-MNs. Electrophysiology revealed hyperexcitability and spontaneous firing of CMT2S-MNs. NMJ FI functional readout revealed a high FI in CMT2S compared to WT, without differences in NMJ number and fidelity. Tetanus characterization revealed a decay tetanus response. Using this model, we identified key aspects of the underlying neuromuscular physiological deficits of CMT2S, highlighting the translational relevance of this approach. MPS holds promise for accelerating drug discovery and personalized medicine, reducing industry reliance on animal testing.

PS57 In Line, Out of Line and Over the Line - The Many Forms of Veterinary Leadership

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Numerous laboratory veterinarians raised concerns for independent advice to the council of a European veterinary professional body (voluntary association) relating to day-to-day challenges and career pathways. The root cause of the worry was frequently cited as the institute's organizational structure or hierarchy. From the team structure to the leadership role to the current job title – many brilliant veterinarians felt they were not achieving, which, combined with their role and societal pressure on the profession as a whole, has a deeply negative impact on the Culture of Care and staff retention in the profession. Trend analysis showed a lack of awareness of other potentially more suitable roles/paths available, or commonly, a poor understanding of the reality of roles they aspired to achieve. Using the concept of 'vet-led team' and applying this to the laboratory setting helped demonstrate the various ways veterinarians could be leaders, and more often than not, were already using three broad categories: **in line** (functional/direct structures), **out of line** (matrix/influence models), and **over the line** (thought/sector leadership). 'In line' formal structures rely upon line management responsibilities with the benefits of clear accountability. 'Out of line' may include matrix or contracted roles, relying on influence rather than traditional line management-based leadership. 'Over the line' takes this further to leading topics outside of the day job. Leading is an innate skill that all working in laboratory animal care programs have. However, data showed many individuals do not take time for self-reflection, seeking feedback, or mentorship/coaching. Without this, there is little ability to articulate our value if seeking career progression or to hone skills to increase impact. Demonstrating career pathways or leadership opportunities (including less commonly considered, e.g., volunteering for professional bodies) to the organization members and others in the profession aided progression towards their personal goals whilst staying in the field of laboratory medicine or regulatory welfare compliance.

PS58 Pursuing an Open Understanding of Animal Research: Development of a Video Campaign to Highlight our Animal Care Professionals

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Employees who support animal care and husbandry efforts in research environments may find it difficult to talk about their profession to friends and family. This can negatively impact self-esteem and morale in the workplace. To better support aspects of compassion, resilience, and inclusivity, our institution created a video series that highlights colleagues involved in research animal operations. Specifically, the goal was to provide an avenue of understanding for employees navigating conversations about animal research, bolster morale and confidence, and convey the importance of animal research teams to internal research partners and to the public alike. Our department conceived, developed, and produced brief videos (n=4) that feature animal care program support positions, including perspectives from a veterinary

technician, an animal care technician, a cage wash supervisor, and a facility manager. Video footage was recorded by a professional videographer, specifically within work areas, showing activities and interviews. The footage was edited professionally, and the final clips were each ~2 min in length. These videos were shared with our entire department and have been shown at BRAD events. Going forward, video clips will be part of facility orientation modules and shared with other institutions. Internally, highlighting animal care positions has been a source of pride for our staff, some of whom expressed that they never felt their work was important enough to film. Other staff have asked if they can show the video spots to family members to help them better understand their job. This series is an innovative firsthand perspective sharing details on careers in research animal science, founded on how to convey complex narratives in a relatable manner, ideally to help others share their stories. The video series can also be used to bolster employee morale and confidence, engage stakeholders in impactful projects, and attract new talent to the field.

PS59 Bioactivity and Its Use in Captive Care and Herpetological Research

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Research into reptiles and amphibians has been more prevalent in the scientific literature than ever before, and the research is still quite limiting as even basking behaviors are not fully understood. Many reptiles and amphibians are kept in simple sterile setups, and these enclosures may generate bias in research data through the lack of environmental stimulation and the assumption that these animals are not the product of their environmental upbringing. For example, *Eublepharis macularius* are rarely given UV-B lighting in captivity due to their ability to survive on dietary D3. This prevents this species from behaving naturally, as this species is known for sticking its tail out into the sunlight while it sleeps to absorb UV-B throughout the day. The inability to do this prevents them from manipulating their environment or themselves to their environment. If you were to use this species in a study for basking behaviors, there would be issues with data as there is an inherited bias in how these animals behave due to the captive conditions. The solution to this is keeping these animals in naturalistic enclosures (known as bioactive enclosures) during non-research periods or during experimental trials if viable. These setups allow for a wide range of enrichment that caters to the needs of the animal's natural abilities. For instance, a *Testudo kleinmanni* natural environment should allow for proper grazing of dried grasses and flowers for nutrition regulatory behaviors, so mimicking natural grazing in captivity allows for natural interactions with their environment, which leads to better data. This can become quite specific, like allowing the species *Uroplatus sikorae* to camouflage against vertical pieces of wood, preferably against species of bark they would naturally exist on. A snake, or even a lizard, is unable to interact with their environment in the same way a mammal can, and the enrichment needs to be catered to the specific species in question. We need to allow these animals to express their natural behaviors to prevent bias in data and to create more confident and reliable animal models.

PS60 Best Practices for the Captive Care of Common Vampire Bats (*Desmodus rotundus*)

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The neotropical blood-feeding common vampire bat (*Desmodus rotundus)* has long been exhibited at zoos, and they are increasingly kept in captivity for research purposes because they are an emerging model species for the study of social behavior and infectious disease transmission. Behavioral ecologists study vampire bats to understand their cooperative behavior (e.g., regurgitated food donations), social foraging, and social network structure. Disease ecologists study vampire bats to understand their role in pathogen spillover events (e.g., transmission of rabies to livestock), which impact agricultural development and public health. However, much of the published literature on the captive and medical care of common vampire bats is outdated, and much of the knowledge about their care is either unpublished or siloed in either research or exhibition settings. To address this problem, we summarized information and experiences about vampire bat care from multiple colonies and caretakers across both exhibition and research institutions. We first reviewed the literature and interviewed caretakers to provide updated information on pathologies and husbandry. Next, we outline suggestions and standardized recommendations for the captive care of vampire bats, highlighting the veterinary practices and environmental, dietary, and social conditions associated with successful breeding colonies. Finally, for cases where best practices are unclear, we make suggestions based on studies of their ecology in the wild.

PS61 Challenges of Collecting, Transporting, Housing, and Managing a Variety of Non-Traditional Wild Caught Research Animal Species

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We will present a variety of shared and learned experiences managing, adapting, and adjusting conventional laboratory animal facility operations for the housing and studying of several non-traditional animal research models such as venomous snakes, tadpoles, and most recently wild, caught juvenile alligators, as well as a variety of other species from other similar research facilities. The discussion will include some of the unique challenges of developing enrichment and modifying husbandry techniques and common lab animal enrichment items repurposed or developed while housing some wild-caught species. Lessons learned along the way will be shared related to modifying traditional lab animal facility management practices, equipment, and SOPs to safely secure, accommodate, and facilitate research using wild-caught, potentially hazardous, non-traditional research animal models. In addition, we will share a variety of ideas, techniques, and considerations related to developing safe methods of capturing, transporting, housing, managing, and handling animals and developing enrichment for a variety of biological specimens that are not traditionally encountered in laboratory animal facility management.

PS62 The Nanopig Experience

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Porcine models have been at the forefront of scientific discovery for many years. Historically, farm pigs and miniature models have been the predominant choice across many different disciplines of biomedical research. Due to the relative novelty of Nanopigs, there are many unknowns when working with this model. For that reason, we aim to share what we have learned and experiences to increase the industry's understanding of their use. Developing a better understanding of this model and exchanging information among its users will benefit both the animals and the investigators we serve. Over the last year, we have explored a few critical care and use strategies. The size and temperament of the Nanopigs, subjectively enable easier sling training and fewer restraint requirements for sample collections. We assessed two blood collection sites, the auricular vein and a modified pre-caval site. We explored alternative housing strategies using two different housing environments, cages vs. run housing, with varying types of flooring, coated vs. fiberglass. Lastly, our staff compared two sedation protocols using

(Telazol/Ketamine/Xylazine and Midazolam/Butorphanol/Dexmedetomidine). During the trials of different blood collection sites, study staff found the pre-caval site more reliable than the auricular vein. While none of the trialed floorings caused significant problems, the fiberglass flooring allowed for increased animal mobility and was easiest for animal care technicians to work with. Our veterinary staff noted easier recovery in the Nanopigs when using Midazolam/Butorphanol/Dexmedetomidine due to having the reversal agent, atipamezole, that allows for a smoother recovery. While these findings indicate significant progress in understanding best practices using the Nanopigs, there is still much to learn. We plan to continue investing in Nanopigs for further method development and best practices for future use of these important models.

PS63 Is Ultra-Processed Food Appropriate for Dogs and Cats Used in Biomedical Research?

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Nutrition forms the foundation of animal health and well-being. For optimal health, it is imperative to feed each species a diet tailored to their species-appropriate needs. In the context of biomedical research, animals are typically fed a specially formulated diet to meet their nutritional requirements. However, these diets are often ultra-processed extruded diets. In all medical fields, except veterinary medicine, the emphasis is on fresh, while ultra-processed foods (UPF) are discouraged. This raises the question: Is ultra-processed food truly appropriate for dogs and cats used in biomedical research? UPFs, often used in biomedical research, are formulations made from food extracts with little or no intact food. These diets, laden with additives, preservatives, and synthetic vitamins and minerals, can lead to a range of health issues in dogs and cats, including obesity, cardiovascular diseases, inflammatory bowel disease, digestive disorders, and an increased risk of cancer. However, switching from UPFs to fresh food can be a game-changer, leading to significant health benefits in dogs and cats, including improved gastrointestinal health, better nutrient absorption, reduced risk of obesity and chronic diseases, enhanced dental health, and potential behavioral improvements. The key lies in ensuring a balanced diet with a variety of fresh food, a crucial step for maximizing these benefits. Fresh foods can be provided raw or gently cooked. Given the ongoing use of these species in biomedical research, providing them with a species-appropriate diet is crucial to avoid any potential for inconclusive results. By ensuring that animals receive diets that promote their health, we can enhance the accuracy of biomedical research and facilitate the translation of findings to human conditions. This presentation will delve into whether ultra-processed diets truly meet the nutritional needs of these animals and the potential implications for research validity.

PS64 Playing with Puppies and Kittens! Collaboration with a Veterinary School to Provide Enrichment to a Colony of Research Animals

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Canine and feline research models are highly intelligent and social creatures for which appropriate enrichment can be challenging to provide. These species particularly enjoy human interaction, which can be time-consuming and difficult when working on a limited budget. At our institution, we have the privilege of working with the College of Veterinary Medicine (CVM) to provide human interaction for our animals through their use as live teaching models, as well as a student-run socialization program. Veterinary and veterinary nursing students interact with research colony dogs and cats as part of IACUC-approved teaching protocols to learn physical examination, anatomic palpation of body systems, and appropriate restraint and handling. The students also learn about preventative health care by performing phlebotomy for heartworm tests, administering vaccines and oral parasite preventatives, providing anesthesia, and performing dental exams and prophylaxis. The CVM also maintains a student-run organization called "Walks and Wags," in which students and staff volunteer to walk the dogs and play with the cats. This is an extra level of socialization for the animals in a relaxed setting, including an outdoor enclosure for dogs. All teaching lab instructors and volunteers are required to complete IACUC and facility training before they are granted access to the facility and animals. Students are educated about the use of dogs and cats in research and the biosafety requirements related to their handling. All lab and volunteer interactions are recorded and monitored by the operations supervisor to prevent overhandling of animals. We have found that dogs and cats are easier to handle for husbandry and study use due to this extra socialization. Collaboration with the CVM has been a positive experience that provides invaluable hands-on educational opportunities for students as well as beneficial human interaction and socialization for the dogs and cats in our care.

PS65 Of Mice and Microchips: Navigating Primary Identification in Mouse Models

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Individual animal identification is a fundamental component of effective animal husbandry and robust preclinical research. When selecting an identification method for individual mice, it is essential to consider factors such as ease of application, readability, longevity, and impact on animal welfare. These considerations are particularly important for mouse strains that require specific accommodations in their management. In mouse strains that require genotyping early in life, identification prior to weaning allows for more immediate enrollment into experimental studies or breeding cohorts while mitigating the risk of post-wean misidentification. In strains that are immunocompromised, prone to barbering, or predisposed to inflammation around identification sites, the selected method should minimize the risks of complications when possible. The current gold standard is to have one primary identification modality that can be administered at any life stage and remains with the mouse for life, with minimal impact on animal health, management, or experimental outcomes. Here, we present a systematic evaluation of microchip/transponder technologies in a production setting, with a particular focus on identifying methods that may be readily applied to multiple strains, including the NSG (NOD.Cg-Prkdcscid Il2rgtm1Wjl/SzJ) and C57BL/6J, and administered as early as 10-14 days of age. Selected products were first evaluated by veterinarians and trainers and then incorporated into institute-wide training programs for technical staff. Products were applied to mice of representative strains and ages, allowing for a comprehensive assessment of retention and complication incidence. Finally, we evaluated each product for ease of animal differentiation and data collection. We found that each method is associated with advantages and disadvantages, which must be weighed against the major objectives of potential studies. Although there is no such thing as a "one-size-fits-all" mouse identification method, we show that microchip model selections can be tailored to specific ages, strains, or study designs, and we offer recommendations for evaluating available products.

PS66 Evaluation of Ketamine for the Attenuation of Pain After Stress and Full Thickness Thermal Injury in Female SD Rats

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Pain management is a significant challenge for patients with burn injuries due to the complexity and multifactorial interactions of burn

pain. Our lab has previously shown that stress exposure prior to thermal injury increases pain sensitivity post-injury. This paradigm is intended to model traumatic injury. Ketamine, an NMDA receptor antagonist, has shown potential for treating various mental health conditions, including anxiety and trauma, as well as both acute and chronic pain, when administered at subanesthetic doses. The goal of this study was to assess whether treatment with ketamine (20 or 40 mg/kg), compared to an equivalent volume of saline, would be effective for reducing pain sensitivity while maintaining measures of well-being in female SD rats using a rat model of full thickness thermal injury with prior stress exposure. Singly housed female SD rats (N=24) were exposed to forced swim stress for 20 minutes a day for three consecutive days. Following the final stress exposure, rats were anesthetized (2-4% isoflurane) and received a full-thickness thermal injury to the right hind paw. Rats were randomly assigned to receive a single intraperitoneal injection of either 20 or 40 mg/kg of ketamine or saline at the time of injury. The pain was assessed at eight time points: baseline (before stress), 24h after forced swim stress (post-stress), and then again after thermal injury (at 24h, 48h, 72h, 96h, seven days, and 14 days post-injury). Pain was assessed utilizing evoked pain measures, including mechanical allodynia and thermal hyperalgesia, and spontaneous measures of pain, including the Rat Grimace Scale, gait analysis, and static weight bearing. Wellbeing was assessed using a welfare assessment scale and body weight trends. Observers were blinded to the treatment groups. The results showed that both ketamine doses attenuated mechanical allodynia at 24 hours post-injury. Both ketamine groups had lower body weight gain at 14 days post-injury compared to the saline-treated group, but this was only significantly different at the 40 mg/kg dose. These results indicate that a single injection of subanesthetic ketamine (20-40 mg/kg) may provide adequate analgesia for acute pain 24 hours post-injury in female SD rats, with the 20 mg/kg dose having less effect on weight gain post-injury.

PS67 Pharmacokinetics and Non-Lethal Adverse Effects of Long-Acting Transdermal Buprenorphine Solution in Rats

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Rats regularly undergo surgical procedures, which may result in pain. Alleviation of unnecessary pain is an ethical and regulatory responsibility. Buprenorphine is an opioid analgesic commonly used in rats and requires dosing every 6-8 hours to be effective. Frequent administration is time-consuming and increases stress, surgical pain, and dehiscence in rats making the use of long-acting formulations an attractive alternative. A transdermal buprenorphine solution (TBS), FDA-approved for use in cats, is commercially available and effective for up to 96 hours. We hypothesize a single dose of TBS in rats will result in clinically relevant plasma buprenorphine concentrations (>1 ng/mL) for up to 96 hours. To test this, 39 rats were randomly assigned to the following treatment groups: Low dose-5 mg/kg (n=6 females, n=6 males), high dose- 10 mg/kg (n=6 females, n=6 males), vehicle control (n=7 females, n=8 males). TBS or anhydrous ethanol (vehicle control) was topically applied. Blood was collected at 4 h, 1, 2, 3, 4, and 7 days post-administration, and buprenorphine concentrations were determined via HPLC-MS. To assess adverse effects, daily fecal output, food intake, and weight gain were quantified, and observations of hematuria and skin lesions were documented. Plasma buprenorphine concentrations exceeded 1 ng/mL in all TBS rats (n=24) at 4 h, 1, 2, and 3 days. No rats experienced fatal side effects or developed gross lesions at the application site. Both male (n=6) and female (n=6) high-dose TBS groups had significant (p=0.0016, p=0.0428, independent t-test) decreases in fecal output compared to vehicle control groups. All TBS groups had reduced weight gain compared to control groups with p values <0.001 (independent t-test). These results suggest that TBS dosed at 5-10 mg/kg could provide analgesia for up to 3 days in rats, and administering a lower dose mitigates some adverse effects.

PS68 Meloxicam as an Immersion Analgesic for Post-Operative Zebrafish (*Danio rerio*)

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Zebrafish are becoming a popular surgical model in biomedical research, and literature shows they perceive noxious stimuli; therefore, they likely experience pain post-operatively. NSAIDs, commonly used for analgesia in other species, are underutilized in zebrafish. Small body size makes administering injectable and oral medications challenging. Providing analgesia as an immersion is a feasible option for zebrafish, minimizing handling and its associated stress for the fish. The objective of this study was to assess whether administering injectable meloxicam via an immersion at doses of 1.0mg/L or 10.0mg/L to post-operative zebrafish decreases stress and provides a positive analgesic effect with minimal adverse effects. In part one of this study, water samples were collected from static tanks containing singularly housed zebrafish (*n=150*) to measure water cortisol concentrations 1h pre-operatively, 1h, 12h, and 24h post-coelomic incision surgery. Part two of the study used commercially available behavioral analysis software to quantify the average total distance swam and position in the water column at 5m, 1h, 12h, and 24h post-operatively. Part three of this study consisted of histopathology of 5-10 randomly selected fish (*n=75*) from each study group. Water cortisol results show cortisol concentrations >1,000pg/mL at the 24h timepoint in the group that received no analgesia post-operatively; fish that received meloxicam from both dosage groups had cortisol concentrations ranging from 700-800pg/mL before to the 24h timepoint. Behavior analyses showed an increase in average total distance swam and greater variation in water column position in post-operative fish that received meloxicam at both doses versus no analgesia at the 5m, 1h, and 12h time points. Histopathological examination showed that 31/75 fish had no pathological findings; pathologies observed in the remaining fish were deemed incidental, unrelated to meloxicam administration. These results support the use of meloxicam as an analgesic for post-operative zebrafish and a potential refinement in the administration technique of meloxicam for zebrafish.

PS69 Aquatic Advancements: Validation of a Novel Zebrafish Survival Blood Collection Technique

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The zebrafish has emerged as an important model organism to study vertebrate development and disease. Due to high genetic overlap with humans, we are presented with an array of opportunities to examine organ development, drug discovery, toxicology, cancer, and various metabolic disorders. A critical part of this research is the need for repeated collection of blood samples for analyses. Due to the small size, aquatic environment, lack of easily accessible vasculature, and blood volume of this species, zebrafish blood sampling presents a unique set of challenges. While many methods exist for terminal bleeding, a standardized successful protocol for repeated survival blood collection has yet to be elucidated in this species. To determine a standardized methodology, we evaluated the dorsal aorta in the caudal half of the body as a site for serial non-lethal blood collection. Zebrafish were bled once a week for up to four weeks at three differing volumes calculated as a percentage of body weight (0.5%, 1%, 1.25%). Hemoglobin was measured as an indicator of anemia, while clinical observational assessments, including behavioral analyses, were performed throughout the study. Histopathology was included for assessment of potential local tissue damage as well as systemic organ changes caused by this procedure. Quantitative results showed decreasing hemoglobin values with an increased number of blood draws, as expected, while clinical assessment and behavioral analyses indicated that zebrafish may tolerate significant

anemia with no clinically detrimental effects. Histopathological analyses showed no significant difference between one or up to four sampling events at a local or systemic level. In summary, blood collection of up to 1.25% at the site of the dorsal aorta once a week may provide a feasible methodology for repeated survival blood collection in the zebrafish model.

PS70 Effects of Short-Term Social Pairing on Measurable Disease Outcomes in Hospitalized Group-Housed Rhesus Macaques (*Macaca mulatta*)

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At our research institution, group-housed rhesus macaques (*Macaca mulatta*) in the outdoor breeding colony are observed daily to identify clinical and behavioral concerns. Animals with identified concerns are transported to an indoor hospital setting to receive appropriate examination, diagnostics, and treatment. Previous studies have documented the benefits of socially housing macaques in indoor caging to promote psychosocial well-being. The aim of the present study was to determine whether short-term pairing during hospitalization affects measures of disease progression. A prospective study was designed to compare the prevalences of enteric pathogens in paired and unpaired animals. We hypothesized that there would be no difference in the change in the number of pathogens as measured by multiplex polymerase chain reaction (PCR) gastrointestinal panel testing. The study population consisted of 40 rhesus macaques presenting to the colony hospital with either diarrhea (n=20) or wound (n=20) as their presenting complaint. Ten animals of each presentation type were paired with a conspecific, while ten remained unpaired. Fecal samples were collected on day zero (the day of pairing for the paired group) and day seven, and PCR testing was performed. The change in the number of pathogens between days zero and seven was determined, and statistical analysis showed no significant difference between the changes for the paired and unpaired groups. Relative risks and odds ratios calculated for each pathogen indicated no significant differences in the likelihood or odds of gaining or losing the pathogen between the paired and unpaired groups. These findings support our hypothesis and suggest that enteric pathogen transmission between conspecifics is not a significant reason to limit social housing in the hospital setting. Further analysis of clinical outcomes, including incidence of hospital-acquired diarrhea and length of treatment time with respect to pairing status, is currently ongoing using retrospective data sets encompassing hospital cases presenting between 2018 and 2024. Preliminary data analysis suggests differences in the incidence of diarrhea and length of treatment time are not significant between paired and unpaired groups. Results are expected to be presented alongside the above findings.

PS71 Long-Term Impact of Early Anesthesia Exposure in a Rhesus Macaque Model

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Every year, approximately six million children in the United States undergo procedures requiring general anesthesia. Despite its widespread use, there are concerns about anesthesia-related neurotoxicity in pediatric patients, and its long-term effects on the developing brain are still in question. Investigations in animal models have shown the presence of apoptosis and increased inflammatory markers in the brain after prolonged anesthetic exposure. Dexpramipexole (RPPX), a synthetic aminobenzothiazole, has been shown to be neuroprotective and prevent anesthesia-induced cognitive impairments in rats. We hypothesized that infant rhesus macaques with multiple prolonged exposures to sevoflurane would show chronic pro-inflammatory effects at one year of age, which would be mitigated by co-administration of RPPX. Infant rhesus macaques were divided into three groups: the Control group with no anesthetic exposure, the Sevo group with three episodes of sevoflurane exposure on post-natal days 7, 21, and 35 for four hours each along with saline administration, and the Sevo+RPPX group with three episodes of sevoflurane exposure on post-natal days 7, 21, and 35 for four hours each with RPPX administration. Animals undergoing anesthetic exposure were induced with sevoflurane to effect and maintained on 2.5% sevoflurane. To profile chronic changes in the innate immunity of the animals, a whole blood LPS-stimulation test was performed when subjects reached approximately one year of age. Various inflammatory plasma cytokines, including IFN- γ, IL-1β, IL-6, IL-2, and IL-10, were measured using the Meso Scale Discovery V-plex Proinflammatory NHP kit. Plasma IL-6, IL-1β, and IFN- γ levels did not show a statistically significant difference between the three study groups at one year of age. However, IL-2 and IL-10 levels were significantly higher in the Sevo+RPPX group compared to the Control and Sevo group. These results suggest that RPPX may be protective against chronic anesthesia-associated neurotoxicity by modulating inflammation (IL-2) and promoting anti-inflammatory cytokines (IL-10).

PS72 Injectable Anesthesia in Microbats

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Anesthesia in microbats is desirable to conduct minor procedures in the field and in the laboratory. The goal of this study was to determine drug protocols that resulted in a rapid induction, maintain anesthesia for ten minutes, and enable return to flight (RTF) within 1 hour. RTF ranges only include bats that were anesthetized. If RTF was not established after 60 minutes, RTF was not determined. RTF was < 10 minutes in bats that were not anesthetized and were not included in RTF ranges. Bats were given ketamine (15 mg/kg) and dexmedetomidine (0.05 mg/kg) i.p., (KDX1, n=6); ketamine (13.5 mg/kg) and dexmedetomidine (0.075 mg/kg) i.p., (KDX2, n=6); alfaxalone (25mg/kg) and dexmedetomidine (0.05mg/kg) i.p., (ADX, n=4); or 3-5% inhaled isoflurane via mask and maintained for ten minutes (ISO, n=5). Anesthesia was defined as a loss of withdrawal reflex to digit pressure and nonresponsive to dermal or intramuscular stimulation. Bats that were anesthetized for 10 minutes using dexmedetomidine were reversed (atipamezole 0.8 mg/kg i.m.). All six bats dosed with KDX1 were anesthetized within 2-6.3 min, and all maintained anesthesia for 10 min. None could fly after 60 minutes. Four bats dosed with KDX2 were anesthetized within 2.3-8.6 min and maintained anesthesia for 10 min, while two did not achieve anesthesia. RTF ranged from 42-48.6 min, with two bats RTF in <10 min, and 2 with RTF > 60 min. The 4 ADX bats were anesthetized within 2.3-9.3 min and maintained anesthesia for 5-10 min. RTF ranged from 39.4-46.1 min with one bat RTF in < 10 min and one with RTF > 60 min. Time to anesthesia with ISO ranged from 1-6.7 min, and anesthesia was maintained between 2.1-10 min. RTF ranged from 18.5-35.4 min. In conclusion, ISO provided the most rapid induction and RTF time, KDX1 provided a consistent duration of anesthesia, but RTF was prolonged, and KDX2 and ADX were not appropriate for anesthesia due to inconsistent durations of anesthesia and recovery.

PS73 Evaluation of Commercially Available Class A Water-Based Foam Concentrates for Swine Depopulation

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Currently, we lack an effective method for efficient, large-scale emergency depopulation of production and laboratory swine.

Depopulation using Class A water-based foam has previously shown efficacy for swine in all stages of development. However, previous studies used foam products available in the National Stockpile due to their availability and demonstrated efficacy in floor-raised poultry. Here, we investigated additional options for depopulation suitability in swine. Fifteen commercially available Class A foams were evaluated for performance (fill success, fill time, and decay rate) at 0.5, 1, and 3% concentrations. The Stockpile product and select foams, based on fill success, time to fill an 11.5 m^3 container, and minimal decay rate, were further evaluated in an exposure test in nursery-stage pigs (n=15/foam). For each product, the animals waded in shoulder-deep foam for 15 minutes and were observed for signs of aversion to the foam (vocalization, escape attempts) before depopulation with the application of additional foam. Physiological effects were assessed via gross and histopathology. The foams were also tested for efficacy in market-ready hogs (n=50/foam) in a large-scale trial. Five animals per replicate had subcutaneous dataloggers (n=15/foam) used for calculating time to cessation of movement, an approximate analog for loss of consciousness. We selected four of the 15 foam products that, at a 1% concentration, had a mean (±SE) fill time of 62.4s (±14.9) and decay rate of 0.5 (± 0.66) cm/min compared with the Stockpile product at 50.0s (±3.5) and 0.2 (±0.1) cm/min, respectively. Exposure testing elicited no vocalizations or escape attempts, and depopulation yielded a 100% death rate. Gross necropsy revealed no foam-associated lesions, and no histologic differences were observed. In the large-scale trials, the mean (± SE) time to cessation of movement was 145.6s (±10.5) across all foams with a 100% mortality rate. Our results demonstrate that at least four Class A water-based foams comparable to the product available in the National Stockpile are currently on the U.S market, which may facilitate swine depopulation in regions with limited access to the Stockpile or mitigate supply bottlenecks during emergencies, including infectious disease outbreaks.

PS74 Pilot Study of Effects of PTSD on Maternal Behavior and on Anxiety-Like Behavior and Neuroendocrine Changes in Offspring

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Maternal care plays an enormous role in offspring's emotional development in both humans and animals. Post-traumatic stress disorder (PTSD) is a frequent issue related to women facing domestic violence and could affect the level of anxiety in their children. Prior studies suggest there is an increased incidence of PTSD and anxiety-like behaviors in offspring raised by PTSD mothers, likely due to stress/trauma experienced during gestation. There is currently little to no research in animals assessing if trauma to the mother prior to conception is associated with a heightened incidence of anxiety in the offspring, and we hypothesize that this is the case. To determine the effect of PTSD on the offspring of mothers who faced a traumatic event prior to conception, female Sprague Dawley rats were exposed to a single prolonged stress paradigm (SPS), a model for eliciting PTSD-like symptoms in rats, or maintained in their home cage as an unexposed control. The unexposed and exposed females were bred to control males 14 days post-SPS and were allowed to raise their pups undisturbed. To compare anxiety-like behaviors of the offspring of PTSD and control mothers, pups (n=10-11 per group) were assessed using Elevated Plus Maze (EPM) and Open Field (OF) tests at 28-37 days of age. While EPM results did not differ between groups, distance traveled in the center of the OF, and distance traveled in the center as % of total distance traveled were significantly lower in pups of the PTSD mother compared to the pups of the control mother ($p = 0.0003$ and $p = 0.0005$, respectively). Freezing time was significantly higher in the pups with PTSD than in the control ($P = 0.0379$). The OF test results suggest that the offspring of PTSD-exposed mothers experience higher levels of anxiety than those of unstressed mothers. This is similar to results seen in previous studies of offspring from mothers who experienced trauma during pregnancy. These results, though preliminary, support the conclusion that trauma prior to motherhood increases the likelihood of anxiety in offspring conceived after trauma.

PS75 The Vagus Nerve and its Role in Stress Sensitivity

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The vagus nerve, a key component of the parasympathetic nervous system, and the hypothalamic-pituitary-adrenal (HPA) axis are crucial in regulating the body's stress response. The interaction between these systems modulates acute stress responses, influencing both physiological and behavioral reactions. Understanding the interplay between the vagus nerve and the HPA axis provides insights into stress sensitivity and resilience. To investigate the vagus nerve's role in stress sensitivity, male Sprague Dawley rats (250-275g) underwent either selective gastric branch vagotomy (gVX, n=7) or sham surgery (n=7). Body weight was evaluated on postoperative days two, four, and seven. After seven days of recovery, both groups underwent a two-hour restraint test where the amount of distress ultrasonic vocalizations was evaluated. In a subset of rats, plasma corticosterone levels (sampled before and during stress, n=3/group) and fecal corticosterone levels (sampled before and 24 hours after stress, n=3/group) were evaluated. Results showed comparable body weight changes post-surgery in both groups ($p=0.61$), indicating that gVX does not significantly alter food intake or weight gain in the basal state. During the restraint, the gVX group exhibited a significantly higher plasma corticosterone response ($p=0.028$) compared to sham surgery rats. Additionally, the gVX group displayed significantly increased distress vocalizations compared to the sham group when re-exposed to restraint stress the following day ($p=0.0033$). Corroborating these findings, the gVX rats trended towards higher stress-induced increases in fecal corticosterone levels 24 hours after the restraint stress ($p=0.07$). These findings suggest that the vagus nerve, specifically the gastric branch, plays a pivotal role in stress sensitivity. The heightened acute stress response in gVX rats highlights the connection between the gastrointestinal system and stress pathways mediated by the vagus nerve. Replicating this study with a larger sample size and delving deeper into the underlying mechanisms of stress modulation could facilitate the development of more effective therapeutic strategies for addressing stress-related disorders.

PS76 Acupuncture Treatment of Forelimb Paresis in a Sheep

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A 1-year-old, 97kg female Polypay sheep (*Ovis aries*) presented with lameness of the right forelimb following an MRI scan. A physical exam revealed no swelling, heat, or sensitivity along the right forelimb, but she was unable to ambulate normally or extend the fetlock or pastern. She had normal vital signs and mentation. CBC and blood chemistry were within normal limits. Radiographs of the affected limb revealed no bony or soft tissue abnormalities. A presumptive diagnosis of partial paresis was made, with the most probable cause being right radial nerve impingement due to positioning during the MRI scan. Banamine (1mg/kg IM) and buprenorphine (0.1mg/kg IM) were administered, and the animal was put on stall rest overnight. The following morning, the paresis of the forelimb was somewhat improved, with the animal occasionally correcting fetlock and pastern placement. Traditional Chinese Veterinary Medicine (TCVM) diagnosis was Bi Syndrome from Qi stagnation at the pastern joint. Acupuncture treatments were

administered to promote Qi flow once per day for the following three days at Liu Feng, TH-4, PC-8, TH-14, LI-15 on the right forelimb, and ST-36 on the left hindlimb. Banamine was also continued daily for these three days. Immediately after acupuncture sessions, the animal showed marked improvement by placing the fetlock and pastern correctly and consistently. Over the subsequent days, the paresis steadily improved until she was able to utilize the forelimb normally, and treatment was discontinued. The sheep was euthanized at the end of its study, and the forelimb was dissected. Using needles and dye, the placement of the acupuncture needles was illustrated. Acupuncture was effectively used in conjunction with Western medicine to resolve lameness when Western medicine alone was not effective. Based on this and similar case examples, we support the use of acupuncture as part of a multimodal approach to treating paresis in lab animal medicine.

PS77 Deer Oh Deer, What Happened to Your Leg!

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Abstract: An approximately 4.5-month-old female white-tailed deer (WTD) (*Odocoileus virginianus*) arrived at the facility in a group of 12 mixed-sex deer and was housed in an indoor deer facility with one other female deer. Fourteen months after receipt, during routine room cleaning, the deer obtained a traumatic "L" shaped laceration on her left front lateral pastern just above the lateral claw and extended into the hoof wall. The deer was sedated with 0.01-0.2mg/kg medetomidine and 2-8mg/kg ketamine IM for wound examination and cleaning. On initial exam, during flexion on the proximal interphalangeal joint, clear liquid was observed seeping out of the suspected joint space. Initial treatment consisted of intravenous flunixin meglumine (1.1mg/kg) and two doses of intramuscular ceftiofur (6.6mg/kg) 6 days apart. Fourteen days after the initial injury, there was significant swelling of the distal left front limb and a drainage tract proximal to the injury from which purulent discharge was expressed. Differentials included cellulitis, pathologic fracture, septic synovial structures, and osteomyelitis. Additional flunixin meglumine (1.1mg/kg) was administered, antibiotics were restarted, and subcutaneous florfenicol (40mg/kg) was administered every four days. Twenty-one days after the initial injury, an ultrasound of the left front limb showed distension of the tendon sheath, which, when aspirated, was confirmed to be purulent material, and radiographs showed evidence of osteomyelitis. The left front tendon sheath and lateral pastern were confirmed to be septic. Florfenicol was discontinued, and a new treatment plan was implemented, consisting of lavages of the tendon sheath and lateral pastern joint and administration of amikacin (500mg) into those structures every other day for one week and subcutaneous tulathromycin (0.2mg/kg) in addition to analgesics and NSAIDs weekly for one month. Radiographs were repeated ~1 month after the tulathromycin treatment ended and showed osteoarthritis of the left lateral pastern as well as evidence of joint fusion. Approximately four months after the initial injury, the deer has no significant lameness and has recovered from her septic tendon sheath and pastern joint.

PS78 Malignant Iridophoroma in Super Lemon Frost Geckos (*Eublepharis macularius)* Demonstrating Pre-Ovarian Follicular Stasis

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Upon intake, two four-month-old female super lemon frost leopard geckos (*Eublepharis macularius*) presented for hyporexia and lethargy. This persisted despite husbandry alterations to diet, housing, enrichment, and humidity. Both animals then began to develop weight loss with asymmetric coelomic distension regardless of supportive measures. At this time, differential diagnoses for distension included gastrointestinal impaction, reproductive stasis, neoplasia, and infective etiologies affecting the digestive tract, such as cryptosporidiosis. Within a month, one gecko developed a focal dermal irregularity of the flank, whereas the other developed a soft, freely movable submandibular skin mass. Fine needle aspiration and cytology of this mass revealed multiple clusters of fusiform cells characterized by oval nuclei, abundant cytoplasm, and numerous intra- and extracellular, blue-black pigmented, birefringent granules. Clinical ultrasonography demonstrated post-follicular egg development and heterogenous hepatic echogenicity in one gecko. Follow-up ultrasonography suggested delayed secondary clutch development and hepatic displacement associated with a cystic heterogenous mass appearing to originate from the caudal coelom. The other gecko demonstrated subtle but similar hepatic changes. Four months after the initial presentation, euthanasia of both animals was elected due to continued decline in clinical condition and poor prognosis. A diagnostic necropsy was performed and confirmed the final diagnosis of malignant cutaneous iridophoroma with metastasis to the liver, alongside preovulatory follicular stasis in both animals; neoplastic metastasis was also found in ovary, lung, and intestine in one animal, whereas egg yolk-associated hepatitis and coelomitis was found in the other animal. Iridophoroma, derived from pigmented skin stem cells, has been found with high frequency in super lemon frost morphologies due to heritable SPINT1 mutation. While abundant iridophores give the morph its characteristic coloration, SPINT1 is a tumor suppressor gene, and mutation is associated with melanoma in people. This report describes the clinical progression of iridophoroma in two leopard geckos, with POFS as a possible clinical complication of that malignancy.

PS79 PUFA Administration in Treatment of Alopecia in Cynomolgus Macaques

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Alopecia is a common problem among captive nonhuman primates. Polyunsaturated fatty acids (PUFA) administration has been used to treat alopecia in several species. In this study, we evaluated the effectiveness of a monthly application of a topical PUFA (Dermoscent 6, $n = 100$) compared to a previous study of a daily oral form of PUFA (Lil Critters Omega 3 gummies, $n = 97$) in treating moderate to severe alopecia in cynomolgus macaques. All cases had been reviewed by a veterinarian and were assumed to be idiopathic and/or behavioral in origin. Alopecia was scored monthly, and the score was used to determine severity. Treatments continued until the animal decreased to mild alopecia severity or resolved. The treatment duration of the PUFA dose varied from 1 to 22 months (mean four months for topical and three months for oral). For the study with topical application, 46% of the animals improved in alopecia severity, 50% had no change, and 4% worsened. For the study with oral dosage, 34% improved, 58% had no change, and 6% worsened in severity level. Results were compared via Chi-Square, and outcomes did not differ between the two formulations of PUFA administration. Outcomes of both treatment modalities were then compared to a third trial of animals not treated with PUFA ($n = 87$) in which 38% had improved, 9% had no change, and 52% worsened. The results were significant; χ^2 (1, $N = 284$) = 99.919, $p < .001$. These results support the use of PUFA as a favorable modality for treating moderate to severe alopecia in NHPs compared to no treatment. The benefits of a topical administration compared to oral treatment are increased treatment compliance and less frequency of administration.

PS80 Tumor Lysis-Like Syndrome in a Cynomolgus Macaque in a Transplant Model

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A 7-year-old, 7.0 kg intact male cynomolgus macaque *(Macaca fascicularis)* presented with prolonged recovery following routine sedation. The primate had undergone a heterotopic allograft heart transplant and bone marrow transplant approximately one month prior, a CAR Treg infusion approximately seven days prior, and a blood transfusion approximately four days prior to presentation. He was currently being treated with an immunosuppressive regimen consisting of aCD154 and rapamycin. He was also receiving erythropoietin for mild anemia. On exam, the primate was extremely quiet, and vitals were within normal limits. A CBC and iSTAT showed leukopenia and thrombocytopenia, mild azotemia, hypoglycemia, and an unreadable hyperkalemia. Saline and 50% dextrose were administered intravenously. Differential diagnoses at this time included transplant rejection, acute kidney injury, urethral obstruction, thrombosis, and neoplasia. The primate failed to recover, experienced a bout of hematuria, and was euthanized. Diagnostic testing at the time of euthanasia showed continued leukopenia and thrombocytopenia, worsening azotemia, severe hyperkalemia, hyperphosphatemia, hypocalcemia, and elevated LDH. Post-mortem, donor-specific antigen tests revealed the blood donor's reactivity to the CAR Tregs. It is, therefore, probable that antibodies from the blood donor led to lysis of the CAR Tregs. Massive cell death resulted in the release of cellular contents into the bloodstream with characteristic electrolyte abnormalities and clinical manifestations, as observed in this case. This etiology is similar to tumor lysis syndrome, a syndrome that is a rare occurrence in human patients receiving CAR T cell therapy for hemolytic malignancy.

PS81 Dissecting the Roles of *Corynebacterium bovis* and *Demodex cricetuli* in Scaly Skin Disease and Mortality in Neonatal Armenian Hamsters (*Nothocricetulus migratorius*)

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Corynebacterium bovis (Cb) causes skin disease in immunocompromised mice and possibly rats. Cb has not been reported to affect other rodents. In 2022, scaly skin and mortality were observed in 7 to 11-day-old neonates (n=8) from a primiparous Armenian (*Nothocricetulus migratorius*) hamster breeding pair in a newly established breeding colony. Differential diagnoses included bacterial or fungal skin infection, ectoparasite infestation, autoimmune disease, and contact dermatitis. Cb was detected by culture and PCR and affected animals had moderate to severe acanthotic and hyperkeratotic lesions, with intralesional Cb confirmed by in situ hybridization. Additionally, a few intrafollicular *Demodex cricetuli* (Dc) mites, an ectoparasite found in all laboratory-maintained Armenian hamsters, were also identified. To elucidate the role of Dc on Cb-associated disease and maintain adult hamsters without the need for sustained mite treatment, a Dc-free colony was generated by treating breeding pairs and their 1 to 3-day-old neonates with topical fluralaner (35 mg/kg). Subsequently, a Dc-free, 12 to 14-day-old litter (n=5) from a primiparous pair developed severe skin disease and mortality as was observed in the Dc-infested hamsters. To better understand the relationship of Cb and Dc, two cohorts (Dc-infested and Dc-free) of 1-2, 3-5, 7-9, 10-12, 14-16, 18-20, 21-22, 60-90, 120-150 and 210-240-day-old hamsters (n = 3/age group; no scaly skin or mortality developed) were evaluated by culture, PCR, deep scrape, fur pluck, and histology of the skin. Cb and Dc were first detected in the Dc-infested cohort in 7 to 9-day-old pups in association with mild acanthotic hyperkeratosis, which progressed to peak at moderate severity in 15 to 22-day-old pups. A low bacterial burden remained in most animals with age, but the acanthotic hyperkeratosis diminished; however, the mite burden increased, requiring treatment. In comparison, the Dc-free cohort became culture positive and developed similar histologic lesions earlier in 3 to 5-day-old pups with similar progression; low bacterial burden and mild lesions persisting into adulthood. Given these findings, Dc does not appear to contribute to Cb colonization and disease severity. However, it remains unclear as to why only a few primiparous litters (2/70 litters) develop severe disease and mortality.

PS82 Full Disclosure to Promote Animal Welfare – Fenbendazole Toxicity in a Transgenic Mouse

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An international shipment of transgenic mice (*n*=18) was received at our quarantine facility, arriving in good condition. Mice were housed in individually ventilated, sterile cages on corncob bedding and provided with a medicated fenbendazole diet and acidified water as standard. Two weeks into the quarantine period, mice were paired and allowed to breed (3 pairs/line). Testing results unrelated to clinical issues required us to extend the quarantine period. Unexpectedly, failure to thrive and mortality in the weanlings was observed at 8-10 weeks of age, followed by the death of 3 adult breeders. Morbidity/mortality was initially attributed to difficulty with the water source/hydropac, and supportive care was initiated. At this time, cages were also transitioned from a fenbendazole diet to a standard rodent diet. Gross necropsy and histopathology, performed on an euthanized weanling, demonstrated severe diffuse hepatopathy characterized by hepatocyte enlargement and karyomegaly. Overall, mortality occurred in 57% of the Bcrp1 (-/-) weanlings; 67% of the Bcrp1(-/-);Mdr1a/b(-/-) weanlings and 50% of the Bcrp1(-/-);Mdr1a/b(-/-) breeders. No mortality was observed in the Mdr1a/b(-/-) line. Notably, the three separate lines were deficient for genes involved in the ATP-binding cassette (ABC) efflux transporter. A follow-up discussion with the originating institution noted that the transgenic lines were known to be particularly sensitive to fenbendazole and similar benzimidazole drugs, which was consistent with pathology findings. No further morbidity or mortality was observed following the transition to a standard diet. Although the lengthened quarantine period contributed to the clinical issues, this case highlights the importance of clear communication for rodent imports and full disclosure about any potential impacts of genetic manipulation. The identification of special phenotypes and/or other health concerns in advance of shipment directly benefits animal welfare. Subsequently, our communication has been modified to make clear that mice are provided a fenbendazole diet when requesting information on expected phenotypes and/or requiring special food/water.

PS83 Geriatric Pathology of Grasshopper Mice

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The southern grasshopper mouse, *Onychomys torridus,* is used in neurophysiology studies due to its adaptations to chemically defended prey. The grasshopper mouse is a carnivorous rodent that has a geographical range from the western United States to northern Mexico. In these prairies and brushlands, the mouse feeds primarily on arthropods, including the lethal Arizona bark scorpion.

Few opportunities arise to understand age-related changes in wild species. Often, these species do not survive long enough in the wild to develop lesions or diseases related to the aging process. A population of wild-caught grasshopper mice was successfully bred in the laboratory animal facility. The captive-reared descendants were maintained long enough to become geriatric. This geriatric population of captive-reared grasshopper mice was evaluated for the most common postmortem findings and histopathological diagnoses. Fourteen clinical cases were submitted for histological evaluation. All animals were approximately 2-3 years old. The most common necropsy findings included polycystic ovaries, uterine enlargement, liver nodules, and gallbladder abnormalities. The histopathological diagnoses include polycystic ovaries, hydrometra, hepatocellular carcinoma, and choleliths. There is potential that geriatric grasshopper mice could be potential models for these diseases. The results allow us to better understand the age-related changes and diseases of grasshopper mice and possibly other species of wild mice.

PS84 Otitis as a Sequela of Salivary Gland Irradiation in a Cohort of Sprague Dawley Rats

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Nine 7- to 8-month-old Sprague Dawley rats (*Rattus norvegicus domestica*) presented with neurological symptoms of varying severity, including head tilt, circling, head bobbing, and ataxia, over a 2-month period. These rats came from a cohort of 39 rats who underwent a single session of parotid salivary gland irradiation (22 Gy) at approximately two months of age. Some had also undergone subsequent salivary gland cannulation procedures. The affected rats were euthanized following symptom onset. Differential diagnoses included otitis media/interna, central or peripheral nervous system disease, and neoplasia. Three rats were necropsied to investigate the underlying cause of their neurological signs. The salivary glands were retained by the lab and were not available for examination. All three rats had thick, white-to-tan, caseous-to-flakey material in one or both tympanic bullae. Culture and polymerase chain reaction testing identified mixed bacteria, most significantly group B *Streptococcus* and *Staphylococcus aureus*. Histopathology confirmed otitis media in all three rats, with two cases also involving the external and/or internal ear canals. Additionally, marked hyperkeratosis distended the auditory canal in all three rats, resembling the histologic features of cholesteatoma. While otitis is common in rats—particularly that caused by *Mycoplasma pulmonis* in pet rats—*Mycoplasma* was not detected in any of the submitted samples. Instead, infections were dominated by opportunistic commensal bacteria along with the development of hyperkeratosis/cholesteatoma. Both bacterial otitis and external auditory canal cholesteatoma have been reported in human patients with head and neck cancer following radiation therapy. Laboratories performing radiation in this area should be aware of the potential for delayed-onset otitis, which may impact their experimental endpoints and research outcomes. This case highlights the unique presentations and challenges that laboratory animal veterinarians frequently encounter, where the expectations of classic disease presentations can be subverted by experimental settings.

PS85 Analgesic Efficacy of a Long-Acting Transdermal Buprenorphine in Rats

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Zorbium ® is the first long-acting transdermal buprenorphine (LAT-bup) approved by the FDA for relieving postoperative pain in cats for four days. This study aimed to investigate LAT-bup's efficacy for attenuating postoperative mechanical and thermal hypersensitivity in a rat incisional pain model. We hypothesized a low and high dose of LAT-bup would attenuate mechanical hypersensitivity in rats for four days postoperatively. A total of 34 Sprague-Dawley rats, 16 females and 18 males, were randomly assigned into one of four treatment groups: 1) Saline (Saline, 0.9% NaCl, 5 mL/kg, transdermal); 2) buprenorphine extended-release (Bup-ER; 1.2 mg/kg, SC); 3) low dose long-acting transdermal buprenorphine (LAT-bup low; 5 mg/kg, transdermal); or 4) high dose long-acting transdermal buprenorphine (LAT-bup high; 10 mg/kg, transdermal). One hour after drug application, under isoflurane anesthesia, a 1-cm longitudinal skin incision was made on the left plantar paw. Mechanical and thermal hypersensitivity assessments were performed on D-1 (24 hours prior to surgery) and at D0 (4 hours after surgery), D1, D2, D3, and D4 afterward. Clinical observations were recorded daily, and a gross necropsy was performed following the final testing hypersensitivity testing time point. The saline group exhibited mechanical hypersensitivity from D0-D2 and thermal hypersensitivity from D0-D4. Results indicated Bup-ER and LAT-bup high effectively attenuated mechanical hypersensitivity from D0-D2. Bup-ER attenuated thermal hypersensitivity only on D4. LAT-bup low and LAT-bup high did not provide attenuation of thermal hypersensitivity. There were no abnormal clinical signs, but injection site reactions were noted in the Bup-ER group. This study indicated that LAT-bup had high attenuated postoperative mechanical hypersensitivity, as effectively as Bup-ER for 48 h in an incisional pain model in rats. We recommend LAT-bup at 10 mg/kg for minor incisional pain procedures in rats.

PS86 Pharmacokinetic Evaluation of a Topical Extended-Release Analgesic in Mice

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Mice often undergo painful procedures and surgeries as part of biomedical research protocols. Buprenorphine, a partial μ-opioid receptor agonist and kappa receptor antagonist, is commonly used to alleviate the pain associated with such procedures. Due to its pharmacokinetic profile, buprenorphine requires frequent dosing, resulting in handling stress that can impact animal welfare and study data. A long-acting transdermal buprenorphine formulation (LA-bup) was recently approved for use in cats to provide up to 4 days of postoperative analgesia. In this study, we characterized the pharmacokinetics of a single topical dosing of LA-bup in male and female CD-1 mice administered a 0.36mg or 18 μl topical dose at select time points. Plasma buprenorphine concentrations were evaluated at 0.25, 0.5, 1, 1.5, 2, 4, 8, 24, 48, and 72 hours (n=3 mice/time point) and remained above the purported therapeutic threshold (1 ng/ml) from 1 to 24 hours post-administration. Repeated daily dosing at 24 and 48 hours demonstrated plasma levels above one ng/ml for up to 72 hours with minimal accumulation or changes in maximal concentrations over time. Inadvertent transfer of the topical drug to non-dosed mice in the same cage was evaluated by measuring plasma buprenorphine concentrations in non-dosed mice co-housed with a single-dose mouse. Male mice did not demonstrate the transfer of drugs via grooming or interactions. However, 2 out of 26 non-dosed female mice had detectable buprenorphine plasma levels, indicating a relatively low incidence of cross-ingestion in co-housed female mice. This study demonstrates that LA-bup is a promising analgesic in mice that could be utilized for tailored analgesia strategies, depending on the surgical model or duration of analgesic therapy.

PS87 Daytime Exposure to LED Light Reduces the Risk of Obesity in Mice

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Approximately 40% of the US population is obese due in part to elevated daily intake of dietary fats, leading to an increased incidence of diabetes, heart disease, and stroke. Our previous studies demonstrated in research rats that exposure to blue-enriched (465-485 nm) light-emitting diode (LED) light at daytime (bLAD) elevated plasma levels of the pineal circadian nighttime neurohormone melatonin that suppressed dietary total fatty acid (TFA) uptake by inguinal fat stores (IFS). Here, in our *GLAS*-supported investigation, we examined the hypothesis that mouse exposure to bLAD, compared with broad-spectrum (400 – 740 nm) white fluorescent (WF) vivarium light, elevates nighttime plasma melatonin levels and attenuates TFA uptake by IFS and prevents obesity. Male and female SPF C3H inbred, pigmented mice (n=60/gender) were maintained in an AAALAC-accredited facility under an IACUC-approved protocol for 12 weeks on a common lighting regimen of 12L (72.5 ± 3.5 lux; 29.6 ± 2.7 µW/cm^2, within cage); lights on 0600 h):12D (0 lux) on either WF or bLAD lighting, and were provided standard lab chow (LabDiet 5053) and acidified water ad libitum. At week 12, mice were anesthetized with ketamine (80 mg/Kg)/xylazine (9.80 mg/kg) using a 1-mL, 25g tuberculin syringe at six circadian time points (1200, 1600, 2000, 2400, 0400, and 0800). The right carotid artery was ligated, and 1 mL of whole blood was collected in a heparinized vial during exsanguination. Results revealed that nighttime plasma melatonin levels were significantly elevated ($P < 0.001$) by over 6-fold in the bLAD versus WF light group, while daily dietary and water intake (per 100 g body weight), body growth rates, final body weights, IFS weights and plasma TFA, glucose, insulin levels were significantly lower by 8%, 7%, 8%, 13%, 115%, 40%, 13%, 41%, 50%, respectively, in C3H mice (male > female) in the bLAD group compared with the WF group. The data show that exposure of C3H mice to vivarium bLAD compared with WF light amplifies the nighttime circadian melatonin signal and markedly abrogates IFS TFA uptake and metabolic factors associated with obesity and poor health outcomes.

PS88 The Effects of Vivarium LED Lighting on the Retinal Health of Albino Rats

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Numerous studies have demonstrated the harmful effects of high-energy blue light wavelength (approximately 400-480nm) on retinal health. Commercially available Light Emitting Diode (LED) lighting systems emit a peak emission in this blue light spectrum. Albino rodents, known for their heightened light sensitivity, are particularly susceptible to light-induced retinal damage, including a reduction in photoreceptor numbers, thinner nuclear layers, and decreased cell density in the retinal ganglion cell layer. While many studies have examined acute exposures to extremely high luminance lighting systems, there is a lack of research on the chronic effects of LED light in a vivarium setting. To address this research gap, we conducted a study using 120 Wistar Han rats (60 male and 60 female, eight weeks old, Crl: WI(Han), Charles River Laboratories) housed in different rooms with high and low lighting intensities, including traditional fluorescents and LED lights with or without a blue spectrum filter. Rats (6 groups, 20 (10 male and ten female) per group) were longitudinally housed at two different heights on the rack per room to represent two different intensities of lighting exposure for three months. Optical coherence tomography (OCT) imaging was conducted monthly, and functional retinal assessment with electroretinography (ERG) and histological evaluations were conducted at three months to determine functional and morphological effects of the lighting environment. The findings of this three-month study highlighted the detrimental effects of high-energy blue light on retinal health. Rats exposed to maximum unfiltered LED lighting (blue-rich LED light, Group 1) exhibited significant thinning in total retinal thickness (TRT) and outer nuclear layer (ONL) thickness compared to other lighting conditions. No significant differences in retinal degeneration were observed between rats exposed to medium lux (lumen/m^2) unfiltered LED lighting (Group 2), LED blue spectrum filtered lighting group (maximum lux in Group 3, medium lux in Group 4), and fluorescent lighting group (maximum lux in Group 5, medium lux in Group 6). Furthermore, the weakening of both A and B wave responses in the ERG tests was also observed in Group 1. Histopathological evaluation confirmed a significantly higher incidence and severity of retinal degeneration in rats exposed to maximum lux unfiltered LED lighting (Group 1) compared to the other groups.

PS89 LED Light: An Extrinsic Factor that Positively Enhances Research Rat Health and Wellbeing

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Light is an extrinsic factor that profoundly influences circadian, neuroendocrine, and neurobehavioral regulation in research animals. Our previous studies demonstrated that exposure of mice to blue-enriched (465-485 nm) LED light at daytime (bLAD), compared to broad-spectrum (300-700 nm) white fluorescent (WF) light, amplified nighttime circadian pineal melatonin production and positively influenced metabolism and physiology. This response to light was mediated via the retinal photoreceptors, including the classical rods and cones involved in vision and the recently discovered melanopsin-expressing intrinsically photosensitive retinal ganglion cells (ipRGCs) of the non-visual system. Here, in our *GLAS*-supported study, we tested the hypothesis in rats that bLAD, compared to WF light exposure, positively enhances integrated visual and non-visual system photic responses, shown to promote the circadian regulation of neuroendocrine and neurobehavioral parameters associated with optimizing animal health and wellbeing. Non-pigmented Buffalo (BUF/CrCrl) and pigmented nude (Crl: NIH-*Foxn1^{rnu}*) rats (n = 60 males/60 females per strain), maintained under an IACUC-approved protocol for eight weeks on a common lighting regimen of 12L (72.5 ± 3.5 lux; 29.6 µW/cm^2, within cage); lights on 0600 h):12D (0 lux) on either WF or bLAD lighting, were assessed for retinal photon flux (cm^2/s), radiometric (µW/cm^2), photometric (lux), and photopigment illuminances (α-opic lux). Results (mean ± 1 SD) revealed no gender or strain photon flux (photons/cm^2/s) differences between bLAD (2.33 x 10^3) and WF (2.39 x 10^3) light. However, α-opic lux stimulation of the non-visual ipRGCs and the visual S cones, rods, and M cones was 30 ± 0.5%, 800 ± 1%, 17.1 ± 0.2%, and 10.4 ± 0.1% higher ($P < 0.001$), and nighttime melatonin levels were over 7-fold higher in both strains maintained under bLAD compared to WF, respectively. The data show that rat exposure to bLAD compared to WF light positively enhances retinal photic responses regulating the circadian, neuroendocrine, and neurobehavioral parameters associated with the promotion of health and wellbeing.

PS90 Impact of Forage Enrichment on Standard Toxicology Endpoints in Sprague-Dawley Rats

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While a large body of research describes the impact of macronutrient changes on morphology and physiology in the rat, there is little information on the effect of small dietary changes, such as those created by providing edible enrichment. Our institution wanted to determine whether providing edible enrichment to rats on toxicology studies would alter standard toxicology endpoints. To that end, we divided 16 male and 16 female Sprague-Dawley rats into two groups, maintaining one under standard conditions (Teklad Global Certified 2014C diet *ad libitum*) and providing the other with additional food enrichment (Bio-Serv certified dried fruit and vegetable mixture, targeted to make up no more than 5% of the animal's weekly caloric intake) three times/ week for 28 days. Food consumption, enrichment consumption, behavior, body weight, clinical pathology, histology, and organ weights were evaluated. 100% of the enrichment was consumed after the first day it was offered. The enriched group had a non-statistically significant increase in total calories consumed over the course of the study (1445 ± 300 kCal/cage vs. 1271 ± 283 kCal/cage) and a higher proportion of calories from fat, and lower proportion of calories from protein and carbohydrate when compared to the group that did not receive food enrichment. However, no statistically significant changes in body weight were present at the conclusion of the study, with the enriched group slightly lighter (357.9 ± 114.9 g vs. 379.6 ± 113.2 g). No biologically relevant changes in clinical pathology, histology, or organ weight were observed. Statistically significant differences in behavior were not observed. However, there was a trend towards more active behaviors in the food-enriched animals. The results of this study suggest that food enrichment can be provided to rats in toxicology studies without altering standard endpoints.

PS91 Lower Relative Humidity in Breeding Cages of C57B6/J Mice is Associated with Higher Pup Mortality

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Relative humidity (RH) is commonly measured in mouse housing rooms, with a broad range of acceptable levels. This wide range accommodates seasonal variation in RH, which is typical in animal facilities located in northern regions of the United States. However, there is limited data exploring how RH variation, particularly that within the home cage, impacts the mice. Notably, it is common for laboratory mice to breed poorly during colder times of the year, which may be explained by an uncontrolled decline in vivarium RH. The purpose of this study was to examine how RH in the home cage of breeding trios fluctuates across seasons and how that impacts breeding success. RH was measured using a temperature/ humidity sensor attached to a solid top caging lid. The lid was rotated across N=48 C57BL/6J breeding trio cages, equally divided across litter ages (no litter, PD1, PD10, PD18; n=12) and seasons (summer, winter; n=24). An additional group of cages was used to observe weekly changes in humidity across transitional weather. Once a week, a single breeding trio was selected for observation if it contained a single litter of 4 to 8 pinkies (N=23). This humidity data was compared to weekly rates of pup loss in our breeding colony. All measurements lasted for five minutes, following a two-minute acclimation period in each location. Data were analyzed with general linear models and post hoc contrasts. In breeding trios, RH was impacted by season ($F_{1,12.10} = 225.83$, $P<0.001$) and litter age ($F_{3,38.82} = 10.18$, $P<0.001$). Humidity was higher in the summer and in cages that had any litter present compared to cages without litter. Weekly home cage RH ranged from 31.37 to 68.61%. This variation impacted pup loss ($F_{1,20} = 11.65$, $P=0.003$). As cage level humidity increased, the proportion of pups lost each week decreased in a linear relationship. No threshold for decreased mortality could be identified. These data highlight RH as a potential extrinsic factor in the vivarium. While these patterns are correlational, they warrant further research focused on the causative role of RH on mouse welfare.

PS92 The Effect of Age on Habituation to Human Handling in CD-1 Mice

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Mouse socialization towards humans is poorly understood. Efforts to understand how habituation to human handling affects anxiety in mice is currently a topic of great interest, yet no known research has examined whether the age when handling begins has an impact on the habituation process. This study sought to determine how mice of different ages and sex responded to nine consecutive days of handling. A total of 36 mice (18 males, 18 females) were assigned to either the treatment (frequent handling) or control (minimal handling) group and one of three life stage groups: 1) post-wean (22-28 days); 2) pre-pubescent (29-35 days); or 3) pubescent (36-46 days). The treatment mice were individually handled by being guided into their home cage tunnel, gently slid out backward into a loosely cupped hand, held for 60 seconds (allowed to explore the researcher's hand freely and were gently stroked on the flanks, head, and tail), then returned to their home cage. Measures of voluntary approach and interaction behaviors with the researcher were made both before and after handling sessions at the beginning, middle, and end of the handling period. This was followed by a four-week period with no handling other than for cage change using a tunnel for the cage transfer; then, one final handling and test session was conducted to assess the long-term impact of the previous handling. Stress indicators (urination, defecation, escape attempts) were documented during handling sessions to help determine positive or negative emotionality. The study indicated that a voluntary approach into the front half of the cage (nearer to the researcher) was not impacted by handling treatment, animal life stage, or sex. A complex interaction between the life stage and the number of handling sessions was found in the amount of voluntary interaction with the researcher's hand. Mice first handled between 22 and 28 days of age were more likely to interact, required less time to habituate, and retained the positive effects of earlier handling compared to mice first handled when older. This information is valuable for designing mouse handling protocols, which could be employed to improve the welfare of mice in the laboratory or as companion pets and may be used to bolster the mouse-human relationship.

PS93 Investigations of EV-D68 Infected C57BL/6J Mice Deficient in α/β/γ IFN Receptors as Mouse Models for Acute Flaccid Myelitis

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Since August 2014, there have been over 729 cases of acute flaccid myelitis (AFM), a rare but serious polio-like neurological condition affecting adolescents. Enterovirus D68 (EV-D68) is the primary candidate believed to cause AFM. To determine suitable models for understanding AFM and testing therapeutics, we infected mice with knockout mutations in α/β and/or γ-interferon (IFN) receptors, crucial to viral pathogenesis. We assessed how mice's genetic background affects viral responses by infecting various strains at postnatal day 10 with EV-D68 doses ranging from 10^6 to 10^3 pfu. The strains were AGB6 and AG129 (knockouts of the IFN- α/β and –γ receptors on the C57BL/6 (B6) and 129 genetic backgrounds, respectively), AB6 and GB6 (single knockouts in IFN-α/β or –γ receptors respectively), and B6 parental strain. Kaplan survival curves at 10^5 pfu demonstrate the median survival was four dpi for AGB6 and six dpi for AG129. At 10^4 pfu, some AG129 mice survived to 14 dpi, but all AGB6 mice succumbed by six dpi. Compared to AB6 mice, AGB6 showed significantly lower survival rates at 10^3 pfu. Among single knockout strains, AB6 mice were significantly more susceptible than GB6 at 10^6, 10^5, or 10^4 pfu. But there were no significant differences between GB6 and B6. Recent investigations implicate the actin histidine methyltransferase, SETD3, in *in vivo* replication and pathogenesis of EV-D68. To explore this, we compared AGB6 mice with a *Setd3* knockout (AGB6-*Setd3*$^{-/-}$) to heterozygote (AGB6-*Setd3*$^{+/-}$) and wildtype (AGB6-*Setd3*$^{+/+}$) littermates. At the lethal dose of 10^6 pfu, AGB6-*Setd3*$^{+/-}$ and AGB6-*Setd3*$^{+/+}$ had a mean survival of 4 dpi, and all succumbed by six dpi, whereas all AGB6-*Setd3*$^{-/-}$ mice survived to 14 dpi. In summary, AGB6 is the most susceptible to EV-D68 of the tested strains, and loss of SETD3 confers protection. AGB6 mice are a fitting model for further EV-D68 and SETD3 studies.

PS94 Refinement of Chimerism Assessment of Humanized Immune System Mice Utilizing Digital PCR

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Humanized immune system (HIS) mice contain human and murine immune cells. Analysis of chimerism in HIS mice is typically accomplished via flow cytometry of anticoagulated peripheral blood, but this requires collecting a 75 µL blood sample, access to a flow cytometer, and immediate analysis. Repeated bleeding can negatively affect the health of HIS mice, limiting serial analyses. Refinement of this technique was proposed by greatly reducing the blood volume required for analysis, allowing more frequent monitoring and resulting in less overall impact on the health of the mice. In this study, we hypothesized that humanization detection could be performed accurately via a small blood volume sample analyzed via digital PCR. Juvenile NOG-EXL female mice (n=24) were myeloablated and engrafted with cord blood-derived human hematopoietic stem cells. At ten weeks post-engraftment, peripheral blood was analyzed via flow cytometry after being stained with anti-murine CD45 and anti-human CD45 antibodies, and results compared to digital PCR analysis of human genes SRSF4, SF3A1, IPO8, and mouse beta 2 microglobulin targets on extracted total nucleic acids using 10µL of the same sample. Linear regression and difference of means were calculated with GraphPad Prism. A tight correlation was found when linear regression was analyzed, with y=1.004x+2.470 and R^2=0.9529. Bland Altman plot shows a slight negative bias of ddPCR relative to the flow. The difference of means was 2.65%, with flow cytometry reporting a higher percentage of human components. Despite this, the correlation coefficient (r) was 0.9762 (two-tailed p-value <0.0001), indicating very strong and significant agreement between the two methods. Digital PCR offers a novel method for chimerism analysis, requiring much smaller blood volumes (as little as 10 µL) that allow for serial analyses and can be performed on sample types unsuitable for flow cytometry. This allows evaluation of humanization at nearly any time point, facilitating proper mouse selection for study design. This method may serve as a refinement to avoid the health impacts of larger volume blood draws.

PS95 Analysis of IVC Microenvironments During 21 Day Cage Change Frequency Using Two Different Rodent Bedding Types

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The *Guide for the Care and Use of Animals* provides recommendations on sanitation frequencies for rodent caging equipment; however, it allows for performance standards to be utilized when extending this frequency for IVC caging. Our institution wanted to reexamine our current standards of care for mouse IVC caging, which includes a 14-day cage bottom and bedding change as well as the use of corn cob bedding. This is due to the fact that cage change is a stressful procedure for mice, and recent literature has described a potentially improved absorbency and multiple health and welfare benefits of paper pulp cellulose bedding products. Therefore, this study sought to compare the impact of different rodent bedding types (paper pulp cellulose and corncob) on the mouse IVC microenvironmental parameters over a 14-day versus a 21-day cage change frequency. This study was performed utilizing cages that contained 4-5 mice housed by sex, and no breeding cages were utilized. Ammonia levels, temperature, humidity, soiling/wetness of the bedding, and the animals' overall condition were assessed throughout the 21-day period. Ammonia, specifically, was measured in each cage using an ammonia gas detection tube attached to a sampling pump while the cage remained on the ventilated rack by the use of a pre-drilled hole in the front of the cage. Our data indicates that IVC cage bottom and bedding change can be extended to 21 days for either paper pulp cellulose or corncob bedding based on ammonia levels, temperature, humidity, and the animal's overall condition. However, based on the bedding soiling/wetness criteria, an early cage change may be warranted before 21 days in cages with corncob as there was a significantly increased urine latrine size in cages with corncob bedding compared to paper pulp cellulose bedding.

PS96 Reducing our Reliance on Animals through Advanced Technologies: Today and Tomorrow

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The success of preclinical pipelines relies on the ability of the models used to predict the safety and efficacy of drug candidates in patients. Modern drug development relies on advanced technologies such as complex imaging and New Approach Methodologies (NAMs) to complement traditional in vivo and in vitro models. This presentation will outline examples of such complementarity where advanced technology helps improve the predictivity and translatability of preclinical work. Imaging technologies have become a key aspect of the pharmaceutical research & development process to understand the complexity of biological events happening within tissue. The growth of spatial-omics gives access to transcriptomic, proteomic, and metabolomic data in a single tissue slice. At AstraZeneca, we're integrating those data with gold-standard histological methods to generate a holistic understanding of drug action using Mass Spectrometry Imaging (MSI) and Imaging Mass Cytometry (IMC). The integration of both technologies plays a pivotal role in modern drug development by providing pharmacokinetics, pharmacodynamics, safety, and target engagement (TE) information that is crucial for decision-making. Through their use, we maximize data and insight from every study, refine models better, and accelerate decision-making. NAMs are human-derived in vitro or in silico models developed to act as primary proxies for human biology. Examples of complex, self-organizing cellular

models such as organoids or microphysiological systems (MPS) will be outlined to illustrate ways by which improved predictivity helps implement the 3Rs and reduce our reliance on animal use.

PS97 Refining Survival Blood Collection in Zebrafish

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A critical component of research involving laboratory animals is the need for repeated collection of blood samples for analyses. Zebrafish blood sampling presents a unique set of challenges due to the small size, aquatic environment, lack of easily accessible vasculature, and blood volume of this species. A previous publication examined a survival blood collection technique in zebrafish utilizing the dorsal aorta for vascular access. Due to several factors affecting the practical applicability of this procedure, a refined and standardized protocol for survival blood collection is much needed and has yet to be elucidated in this species. Therefore, we attempted to refine aspects of the methodology performed in the publication. FDA-approved tricaine methanesulfonate was utilized for anesthesia instead of 2-phenoxyethanol. Parameters for creating appropriate glass pipette needles were established due to the small size of the needle gauge required. Capillary effect combined with bulb dispensers were used to avoid mouth-pipetting due to widely accepted health and safety guidelines. Male and female specimens were utilized to account for sexual dimorphisms and elucidate necessary technique adjustments. Finally, no study has performed repeat survival blood collections beyond one week in duration. Given the longitudinal aspect of research that may benefit from repeated survival blood collection, we attempted to repeat collections on a weekly basis for a total of four weeks to examine longer-term viability. Our results indicate that this refined and novel methodology may provide a viable technique for repeated survival blood collection in zebrafish.

PS98 Progressive Weight Loss and Intermittent Regurgitation in a Laboratory Beagle

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Peanut, an intact 1-year-old male laboratory beagle, was examined for regurgitation. He weighed 7.4 kg with a BCS of 4/9, and the physical examination was normal. He was monitored closely for one week following the event with no further regurgitation. Four weeks following, a marked weight change and further episodes of regurgitation were reported to the veterinary staff. On examination, he weighed 6.5 kg with a BCS of 2/9. He had a rectal temperature of 102.0 F, HR of 88 BPM, and RR of 32 BRPM. Cardiothoracic auscultation was normal, with no murmurs, arrhythmias, crackles, or wheezes. Abdominal palpation was unremarkable, but hypersalivation was noted. Thoracic radiographs revealed a marked, food-filled distention of the esophagus cranial to the cardiac base. Fasted radiographs the following morning revealed the feed material was no longer present in the esophagus. Differential diagnoses included congenital megaesophagus and megaesophagus secondary to a vascular ring anomaly or stricture. A videofluoroscopic swallow study was performed at the University of Missouri Veterinary Health Center. The upper esophageal sphincter was noted to open appropriately, but ingesta filled an esophageal diverticulum. The esophagus narrowed at the cardiac base, and the lower esophageal sphincter intermittently opened to 50% of the normal diameter, allowing passage of some ingesta. The study was repeated with the patient being held upright, with similar results observed. Differential diagnoses included a vascular ring anomaly and mild gastroesophageal reflux. Due to the intensive therapy required to treat a vascular ring anomaly and the possibility of persistent megaesophagus, Peanut was humanely euthanized. Gross necropsy results confirmed a persistent right aortic arch (PRAA) and megaesophagus. PRAA is a congenital anomaly that results from a developmental abnormality in which the right aortic arch does not regress after birth, encircling the esophagus and leading to esophageal dilation. Although certain breeds are predisposed to this condition, it is uncommonly reported in laboratory beagles. Without treatment, PRAA can lead to malnutrition and secondary aspiration pneumonia. Gold standard treatment for PRAA includes surgical vessel ligation, but megaesophagus may persist after treatment.

PS99 Chronic Vomiting and Weight Loss in a Common Marmoset

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A 5-year-old intact male common marmoset (*Callithrix jacchus*) was examined for vomiting and weight loss. This animal had a previous episode of intermittent diarrhea and vomiting approximately a year earlier that resolved with symptomatic care. On initial examination, the patient was underweight with a low body condition score. A marked hypoalbuminemia was detected, and subsequent examination revealed mild to moderate abdominal distention. Differentials included chronic lymphoplasmacytic enteritis, Clostridial-induced protein-losing enteropathy, gastrointestinal ulceration, and neoplasia. The anaerobic rectal culture was positive for *Clostridium perfringens* and *Clostridium difficile,* and the fecal occult blood test was negative. The treatment plan included metronidazole and tylosin for the clostridial infection; budesonide and sulfasalazine for suspect Marmoset Wasting Syndrome; and bismuth subsalicylate, famotidine, ondansetron, subcutaneous fluids, and simethicone as needed with a bland diet for symptomatic management of nausea, vomiting, and abdominal gas distension. Despite treatment, the patient continued to vomit intermittently with variable abdominal distension and hypoalbuminemia and developed a nonregenerative anemia. An abdominal ultrasound was performed, and no significant intestinal dilation or obstruction, peritoneal fluid, organomegaly, or masses were identified. Over six weeks, the vomiting and abdominal distension slowly decreased, albumin levels increased, erythrocyte regeneration developed, and the patient's weight and body condition score improved. Despite long-term supportive care and treatments, however, clinical signs never fully resolved, and the patient slowly declined. Due to the poor prognosis, the patient was euthanized and necropsied. Histopathology revealed a duodenal mucinous adenocarcinoma with regional lymph node metastasis, as well as mild chronic lymphoplasmacytic enteritis. Pathology findings concluded that intestinal neoplasia was the main cause of weight loss and other associated clinical signs.

PS100 Dermal Lesions in Two Olive Baboons

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Two pair-housed 10-month-old, male, experimentally naïve olive baboons intended for a xenograft study presented for developing acute skin lesions affecting the palmar and plantar aspects of the hands and feet with involvement of their ischial callosities. During

routine pre-study sedation, both animals were noted to have multifocal to coalescing areas of whitened skin with numerous cavitations and mild erythema on the palmar and plantar aspects of their hands and feet. In both animals, the feet had more severe lesions than the hands, with one baboon being more affected than the other. A review of their history showed that no new cleaning agents were used to sanitize their enclosure, and only two of these two were affected in a room housing other baboons. Husbandry was provided by veteran care staff experienced with proper cage cleaning procedures, but as an additional precaution, the cage was replaced with a new, clean cage. Differentials included contact, fungal, or bacterial dermatitis. Animals were sedated, and an 8 mm punch biopsy was used to sample from the left foot and the left aspect of the ischial callosity in the more severely affected baboon. Animals were then treated three times a week with chlorhexidine scrub and topical triple antibiotic ointment for two weeks. Five weeks after the presentation, the condition had fully resolved in both baboons. The biopsies were stained with H&E, revealing areas of multifocal stratum corneum erosions of moderate severity, with intralesional bacterial coccobacilli and filaments and scattered hemorrhage. These lesions were consistent with pitted keratolysis, a condition known to occur in humans but never reported in non-human primates. This condition should be considered a differential in any nonhuman primate that presents with similar lesions.

PS101 Lymphadenomegaly and Leukocytosis in an Olive Baboon (*Papio anubis*)

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An 8-year-old female olive baboon (*Papio anubis*) presented with mild facial pallor, mild lethargy, and heavy menstruation. Over the preceding three months, the animal had three reported episodes of loose stool. Tuberculosis skin tests and complete blood counts conducted bi-annually throughout the life of the animal were within normal limits. On sedated physical exam, there was diffuse gingival bleeding, pale mucous membranes, moderate vaginal bleeding, and bilateral inguinal and axillary lymphadenomegaly. The animal had lost 4.6 kg over approximately four months. Serum chemistry was within normal limits. A complete blood count revealed severe leukocytosis (129k), severe nonregenerative anemia (hematocrit 10.4%), and severe thrombocytopenia (6k). Blood smear revealed marked leukocytosis characterized by lymphocytosis with 96% small lymphocytes, 1% neutrophils, 2% monocytes, and 1% intermediate round cells. The primary differential diagnosis was neoplasia, with infectious, inflammatory, and parasitic etiologies considered less likely. The animal was humanely euthanized due to poor prognosis. A gross necropsy revealed multifocal mesenteric lymphadenomegaly, multifocal petechiation of the serosal surfaces of the uterus and kidneys, a diffusely pale, yellow liver, an enlarged thymus with multifocal petechiation, and multifocal meningeal petechiation. Histopathology of the lymph nodes revealed diffuse infiltration of medullary sinusoids with a monomorphic population of lymphoid cells with prominent mitotic figures and apoptotic bodies. The lungs and interstitial and sinusoidal areas of the liver had a marked to massive influx of neoplastic lymphoid cells, often with an angiocentric distribution. Various other tissues were infiltrated with neoplastic lymphoid cells. Western blot on serum was positive for simian T-lymphotropic virus (STLV). These findings were consistent with lymphoblastic lymphoma/leukemia secondary to STLV infection. Inverse polymerase chain reaction could be utilized to detect integrated STLV provirus and further confirm the diagnosis. STLV seropositivity is common in conventional baboons, and 1-2% of affected animals develop lymphoma leukemia. To avoid confounding research variables and to prevent the development of clinical disease, baboons should be sourced from colonies that exclude STLV.

PS102 Effectiveness of Combined Pioglitazone and Insulin Therapy in Managing Diabetes in Geriatric Macaques

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Diabetic management in geriatric non-human primates (NHPs) requires a multifaceted approach depending on the stage of clinical disease. Diabetes in NHPs is categorized into sequential phases in which individuals first exhibit a period of insulin resistance despite normal glucose tolerance, followed by a progressive decline in beta-cell function and insulin secretion, resulting in compromised glucose tolerance. Insulin resistance is the therapeutic target of early-stage diabetes, whereas insulin supplementation is the cornerstone of therapy for late-stage diabetes. However, insulin therapy alone ultimately becomes insufficient in the face of continued insulin resistance. Pioglitazone has been shown to improve insulin resistance and has been employed as a therapeutic for early-stage diabetics. Here, we evaluated the efficacy of diabetic management in geriatric Rhesus macaques (22-28 years; 2 males & 6 females) treated with 3-5mg/kg pioglitazone (n=6), 7-18 units of insulin therapy (n=4), or a combination of both (n=4). Fasting glucose, HbA1c, lipid profiles, and liver enzymes were assessed across treatment groups using hypothesis tests for percentage changes relative to baseline readings. Pioglitazone, as a sole therapeutic, significantly reduced fasting glucose levels (193.0±54.5 mg/dL to 126.3±35.3 mg/dL; P=0.003) and resulted in a trend for reduced HbA1c (0.3% mean reduction). Insulin therapy alone did not significantly reduce fasting glucose levels (P=0.298) and led to an overall 0.9% mean increase in HbA1c from baseline. Individuals treated with combination therapy displayed trends for reduced fasting glucose levels and HbA1c (0.4% mean reduction). Pioglitazone reduced mean cholesterol from 244.7±145.6 mg/dL to 162.6±17.9 mg/dL and triglycerides from 858.6±525.4 mg/dL to 438.6±54.0 mg/dL, with further reductions noted with combination therapy. Elevated ALT and AST levels observed at the start of insulin therapy were reduced by 32% (P=0.093) and 29% (P=0.027), respectively. These results support that pioglitazone is an effective therapeutic for reducing fasting glucose and improving lipid profiles in diabetic geriatric macaques. Further, the combination of pioglitazone and long-acting insulin supplementation may be superior to insulin therapy alone in the management of late-stage diabetes in geriatric macaques.

PS103 Neuroendocrine Carcinoma in a Clownfish

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A 6-year-old clownfish presented with unilateral exophthalmos of the left eye. Following unsuccessful medical management for treatment of potential bacterial or fungal causes by research staff, the decision was made to perform an enucleation of the left eye. Potential differentials included inflammatory/infectious, neoplasia, or trauma. The patient was anesthetized using tricaine methanesulfonate (200 mg/L immersion). Once a surgical plane of anesthesia was reached, the clownfish was moved to a shallow surgical "pool" where the anesthetic solution was present for partial immersion during the procedure. Fresh system water and anesthetic immersion solution were "flushed" over the gills to maintain proper anesthetic depth. A local block with bupivacaine (2 mg/kg) was given in the retrobulbar region. Forceps were utilized to gently grasp the globe and provide access to the conjunctival tissue located caudoventral to the left eye. Iris scissors were utilized to incise this tissue and then bluntly dissect the globe from the orbital socket. The globe and attached musculature were incised to free all attachments other

than the optic nerve and associated retinal vasculature. Micro-hemostats were placed across the retinal vessels and optic nerve prior to transection distally. The hemostats remained in place for ~30 seconds to ensure hemostasis. The nerves and vessels were released and checked for bleeding. The fish recovered in fresh system water until they swam normally and then were returned to the home tank. Meloxicam (5mg/kg IM) was administered 24 hr and 60 hr postoperatively. The eye was submitted for histopathology. Due to poor recovery (lethargy, inappetence, trouble righting) and prognosis, the decision was made to euthanize 96hr after surgery. Histopathology of the eye diagnosed a neuroendocrine carcinoma with invasion into the brain, pituitary, and right eye, and the primary differential being a retinoblastoma. Current literature only identifies this in Pajama cardinalfish, Porkfish, and Brown Bullhead.

PS104 Comparison of Premedication Strategies in Turkeys (*Meleagris gallopavo*) Utilizing Midazolam, Midazolam/ Butorphanol, or Saline Control

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Avian anesthesia for translational medicine requires considering a diverse range of species, with limited published research addressing poultry, specifically turkeys, used in scientific investigations. Premedication is commonly used in veterinary medicine to produce smoother anesthetic events for patients and staff. This study assessed intramuscular premedication of turkeys with either Midazolam or Midazolam/Butorphanol compared to saline control to improve handling/patient behaviors at time points, including post-premedication, induction /intubation, and recovery. Thirty-five female turkeys undergoing a surgical procedure for an orthopedic study were randomly divided into groups (Midazolam 2 mg/kg IM, Midazolam 2mg/kg IM, and Butorphanol 1 mg/ kg IM, or saline control 0.45 ml/kg). Blinded observers scored the birds for criteria at 10 minutes post premedication (body position, restraint required, and muscle relaxation), at induction (intubation attempts/ease, and apnea), and at recovery (restraint required, tremors, wing flapping). Relevant time points tracked include time of premedication, induction, intubation, inhalant end time, and recovery. Premedication with either Midazolam or Midazolam/ Butorphanol scored significantly higher for premedication-induced body position, restraint required, and muscle relaxation when compared to saline (control). Premedication with either Midazolam or Midazolam/Butorphanol significantly decreased the time from premedication to intubation when compared to control. Saline produced significantly faster recoveries from the end of isoflurane administration to extubation and a significantly shorter time to recovery compared to the premedication groups. When considering anesthetic premedication protocols, many factors come into play in determining the best choice. Turkeys premedicated with either Midazolam or Midazolam/Butorphanol produced significantly better scores at induction, leading to easier handling and restraint for staff. Due to significantly longer recovery times, Midazolam or Midazolam/Butorphanol combinations could increase turnover between patients and staffing needs but may provide a smoother and less traumatic recovery. Both considerations are important in lab animal medicine to uphold the 3Rs and provide high-quality animal welfare.