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Abstracts of Scientific Presentations

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PS1 Risky Business: A 'Grassroots' Approach to Animal Personnel Safety

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Working with research animals can be both rewarding and dangerous at the same time. Bites, scratches, kicks, and allergy development are among the many injuries that can occur while handling biomedical animals. Many institutions rely on their Environmental Health and Safety (EH&S) department to provide feedback and guidance on how to provide husbandry and data collection while maintaining a safe environment. The problem with this scenario is most EH&S professionals have expertise in chemical safety and industrial hygiene, but very little experience with the inherent dangers of handling animals, both large and small. This very issue developed at our university. We are unique in that our institution has vast research programs focusing on medical, agricultural, and veterinary health issues. There is a broad array of individuals from full-time animal care technicians, to veterinary students, to undergraduate and graduate lab staff with differing skill levels and backgrounds that work with a very diverse range of species. We have traditional housing situations (vivariums), but also several farms and wildlife stations where research is conducted. This diversity demanded a structured program where people could seek out guidance from experienced individuals who could make recommendations to improve their safety while working with animals. To meet this need, we created a very 'grassroots' group of animal technicians, supervisors, professors, lab technicians, and OHSP professionals, each representing different areas of campus and farms, to collaborate with EH&S to form the Committee for Animal Personnel Safety (CAPS). We use work injury reports, surveys, newsletters, email correspondence, and personal interaction to identify and help resolve safety issues. We have found that staff is more likely to approach these representatives because they work side-by-side with them and see them every day in the animal facilities and their experience lends them credibility when dealing with safety issues. This committee has helped resolve many issues in its first year of creation and plans to expand as safety issue, trends, and topics are brought to its attention.

PS2 Proof of Identity and Paternity of Nonhuman Primates by Noninvasive Sampling Methods and DNA Profiling Using Microsatellites

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There is a need for DNA-based tests for proof of identity and paternity of nonhuman primates. In scientific studies, we must assure that laboratory animals are breeds from distinct subpopulations or breeding lines, and they are not captured from wild populations. Breeders need a method to demonstrate how closely related breeding lines are, and it is necessary to distinguish between breeding animals and captured animals. DNA profiling by microsatellite analyses is routinely used in human paternity testing for the generation of reference databases and forensic casework DNA analysis. Several of the human markers which are included in international standard marker sets could also be used for DNA profiling of higher primates like orangutan or chimpanzee. For other nonhuman primates like *Macaca* or *Callithrix* species separate informative DNA markers are already described. Normally, genetic information is identical in all somatic cells of an individual. Therefore, DNA profiling could be performed from different sample material, including blood samples and buccal swabs. However, for some species, a chimeric blood status is described. From analyses of human stem cell recipients, it is known that this chimeric blood status also affects the results of buccal swab samples. Therefore, we recently evaluated DNA profiling based on other sample material like hairs or stool samples to circumvent DNA profiling biases based on this special blood status. Hence, DNA profiling can now also be used for parentage verification, individual identification, and population or subspecies determination of chimeric nonhuman primates.

PS3 The Price is Right: Per Diem Rate Increases

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Directors and Managers of animal facility operations may find determining per diem rate increases a complex, daunting, and arduous task. Moreover, decision makers are often burdened with justifying and defending rate increases to researchers and institutional administrators. Without quantitative analysis or concrete figures this exercise may be futile and even contentious. A year-over-year trend analysis providing a clear understanding of animal care expenses relative to total operating expenses can provide a framework for rate increases. To facilitate this, the use of common-size ratio analysis can provide a standardized and objective measure to develop a simple formula to base such rate increase decisions.

PS4 PET/CT and MRI in Animal Biosafety Levels 3 and 4: Challenging Primate Models

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Central to the core mission of the National Institute of Allergy and Infectious Diseases' biocontainment facilities is the use of hospital tools, including computed tomography (CT), magnetic resonance imaging (MRI), and positron emission tomography (PET) to systematically evaluate the pathogenic processes and clinical course of disease in animal models exposed to emerging infectious pathogens. A key goal of our work is to correlate the transmission, virulence, and invasiveness of high consequence microbial agents with clinical tests and diagnostic imaging. Nonhuman primates in particular present unique challenges in ABSL3 and 4 environments. Highly compromised animals frequently undergo multiple imaging procedures using radioactive materials for time periods of up to 7 h, presenting radiation safety, anesthesia, and patient support issues. Prevention of cross-contamination between animals on different studies using the same space is a constant battle when working with dangerous, exotic microbial agents. Personnel safety and accident prevention must also be considered in the high containment setting. In order to address the

complexity of these challenges, we incorporated engineering design solutions into the construction of the imaging facilities and developed innovative materials for primary containment devices for use in shared spaces. The techniques, equipment, and procedures presented describe novel methods for addressing the challenges of imaging in biocontainment laboratories.

PS5 Virtual Tour of a Laboratory Animal Resources Department

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Biosecurity is an ever increasing concern in animal research. Persons not directly involved with animals or research do not often get to see the workings of an animal facility. Our institution offers courses that teach undergraduate, graduate, and veterinary students about laboratory animal medicine; these students will not have the opportunity to visit the animal facilities as granting access to hundreds of people is not only logistically limiting, but compromises the animals as well as the research. It was proposed that a virtual tour of the animal facilities be created to show students, as well as employees and visitors, how animal facilities are managed at ISU. The Laboratory Animal Resources' Virtual Tour (Tour) was designed to meet 2 broad criteria. First, it needed to be created using basic software that would make it attainable for other institutions. Second, it needed to protect the welfare of the animals and integrity of the research while remaining an efficacious teaching tool. Every animal facility at ISU was photographed, edited, narrated, and made into a movie using a slide presentation software and a movie-making software. The Tour was shown to people both familiar and unfamiliar with the animal facilities at ISU, and within the ISU Laboratory Animal Resources department. All who viewed the Tour were given an identical questionnaire regarding the movie. Comments and concerns were considered and changes were made accordingly. The Tour has been received positively and has been shown to employees, administrative staff, as well as various veterinary student organizations. In general, people find it insightful, educational, and an accurate depiction of the facilities at ISU. The Tour has proven to be a great tool illustrating the fine details of an animal facility while maintaining biosecurity measures and can be easily adapted at other institutions.

PS6 Maintenance Mode: A Novel Approach to Establishing and Maintaining Health Standards in a Laboratory Animal Facility

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Establishing health standards in a laboratory animal facility is a challenge to determine which agents should be excluded. Maintaining these standards is complicated by trading strains between institutions. Because disease outbreaks cost institutions unnecessary financial resources, animal loss, and research delays, to ensure the highest animal health status possible, this lab animal facility took a novel approach to establish a "No Tolerance" health standard. The exclusion list is extensive (w/MNV) since elimination is difficult. Health status is carefully monitored by prescreening, quarantine, and ongoing health surveillance. After animal shipments are prescreened by review of customized questionnaire and past year's health reports, a shipment is accepted or rejected. Incoming shipments are directly quarantined for a 6- to 8-wk period. Negative animals are moved to Step-Down and retested after 6 to 8 more weeks before being transferred to existing colonies. Positive animals are either treated or eliminated depending on the agent. Using a uniquely designed rodent sentinel and health monitoring program, extensive testing is performed (serology, bacteriology, parasitology, molecular techniques (PCR), and necropsy/histopathology). Problems are detected, contained, tracked, treated, and eliminated before an outbreak occurs. Since establishing the "No Tolerance" standard, this facility has maintained MPV/RPV-negative rodent colonies; survived a worldwide MHV outbreak; dealt with the MNV dilemma; detected/eliminated a novel Reovirus; eliminated *CARbacillus* (rats/rabbits), *E. cuniculi, Pasteurella*, and *Coccidia* (rabbits); dealt with pinworm and Rotavirus (rabbits); prevented *Helicobacter* introduction; and characterized lesions in acute deaths. Ninety-two positives/30,000 tests/5 y were detected, contained, and eliminated. Currently, all rodent colonies at this institution are negative for 25 mouse and 15 rat viruses (including MNV) and *Helicobacter*. Key factors for successfully maintaining the "No Tolerance" standard include commitment, cooperation, containment, consistency, compliance, and contingency. Although this standard is higher than at most institutions, for this institution it is the most cost-effectively, scientifically, and ethically appropriate standard.

PS7 Expansion Mania: Effectively Training a Large Increase in Staffing in an Animal Facility

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Our animal program has exponentially expanded over the course of a year. Active animal housing rooms increased from 12 to 35 and the staff increased from 43 to 74 employees, including myself as the training coordinator. This kind of growth is hard on any facility and can be a huge pill to swallow for the training department. Most facilities may never see this kind of expansion but may experience a large intake of new employees due to turnover. In either case, it makes provision of training and proficiency challenging, especially with only one dedicated trainer. However, we found effective ways to meet this challenge. First, a three-way split in initial training was established by coordinating peer-shadowing, direct training by management, and instruction with a trainer. This strategy requires collaborative efforts from the whole staff, but promotes an atmosphere of support and quickly integrates new employees into the workplace. The initial demonstration of standard operating procedures (SOPs) was done by either the trainer or the trainee's direct supervisor. The trainee was then matched with an established level 2 employee within his/her department for shadowing. Shadowing reinforces the initial training and allows the new employee to gain knowledge from peers that perform the task on a daily basis. Proficiency is then evaluated by the management/training department in a 3-check system. First, the new employee must properly perform the task or be able to correctly answer questions on the SOP. Then, trainees must pass a written exam with a minimum of 80%; and finally, be evaluated by a staff veterinarian. This system prevented any one person from being overwhelmed with the increase of new staff while effectively staying on track with a 90-d training period. We were also able to complete training for up to 7 employees per month.

PS8 Intermittent Mock Box Programs Improve Technicians' Ability to Locate Rodent Cages with Missing Food and Water

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To improve animal care technicians' ability to locate rodent cages with missing food and water, we implemented a mock box program once a year, beginning in 2012. The first program occurred over a 6-mo period. In the first 3 mo, at half of the facilities, technicians who located boxes were recognized on a public board. During the same time period, staff at the other facilities received only verbal feedback. Conditions were reversed for the latter 3 mo. The recognition board did not affect staff performance, but it did improve staff engagement. At one facility, staff independently started an art hour to decorate their recognition stars. Cagewash staff asked to be involved in the program and requested to shadow care technicians. Managers also became more engaged. One placed cards within transfer stations and toy mice in mouse traps, all of which were returned by the employee to confirm that tasks were completed. Over the course of the 2012

program, time to find mock boxes decreased from 1.2 to 0.65 d. The 2013 program was conducted for 12 wk, during which time a "race" poster was posted within each facility. Facilities were given a point for each box identified within 24 h, and at the conclusion of the program, the winning facility was awarded a pizza party. As a result, the number of mock boxes found within 3 d increase from 82% in 2012, to 97% in 2013. Moreover, no occurrences of missed food or water resulted in death during the 6 mo following the program. Finally, in 2014, supervisors asked staff to find the mock box in the room while they were present, and provided real-time performance feedback. The mock boxes contained 2 flash cards with images of health conditions and for each condition correctly described, the employee was entered in a gift card drawing. Staff were later surveyed and reported that the training was a good opportunity to communicate with the supervisor. As a result of these programs, technicians' reporting of missing food and water incidents increased, while incidents affecting animal welfare simultaneously decreased. These observations suggest that staff are identifying issues more quickly, before the animals are compromised.

PS9 High-Throughput Identification of Animals Using Barcoded Ear Tags

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Herein we report the results of an evaluation of barcoded metal ear tags for the rapid identification of standard rodent species. Commonly used methods for identification of animals include conventional metal ear tags, tattoos, ear punch or toe notch, but none of these methods are compatible with automated data entry into a research database. An evaluation study was conducted to determine the safety and durability of barcoded metal tags, ease of compatibility with our research database, and its application in animal identification. In our evaluation, we showed the tags to be safe, humane, easy to attach and scan. Barcoded ear tags also offer an efficient option to directly enter animal IDs into the database using a Bluetooth scanner. Animal IDs are captured using an iPad and this information is used to identify animals and to print labels for sample collection, such as blood, serum, tissues, etc. Label printing is done directly from a tablet computer. A combination of automated data entry and label printing has resulted in significant time saving (approximately 40%) and has helped us to efficiently manage daily processing of bleeds, serum, and tissue samples. In summary, barcoded metal ear tags offer a safe, humane, and efficient way for rapid identification of laboratory animals.

PS10 Of Mice, Rats, and Men: Operating a Facility with Disposable Caging

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In August of 2013, we completed construction of a 19,000-ft^2 facility. At full capacity, it is designed to house over 3,500 cages of mice, and 1,300 cages of rats. All of the cages, bedding, food, and water are sterile. All of this in itself is unremarkable; however, it is the unique nature of this facility's design that is noteworthy. This facility was designed solely around the use of disposable, independently ventilated caging and has no cage washer, no automatic water, and the autoclave is only large enough to run a dozen mouse cage set-ups at a time. The disposable caging and components are all recyclable and 70% of the material is recycled, including the dirty bedding. The concept was simple, and there would be no need to wash cages or water bottles, no waste to dispose of or bedding to dump and most of the materials would be recycled. However, because of these unique features there was a distinct learning curve to its initial set-up and continuing maintenance. We quickly became aware of issues that had not been fully flushed out during the design phase, such as investigator needs for specialized caging or bedding, cage wash needs, waste disposal, room efficacy, and technician time demand. Over the course of 6 mo, we developed a specialized workflow and ordering system to maximize efficiency, developed specialized SOPs to deal with the unique nature of the facility, and modified our methodologies to accommodate the new caging. In the end, this facility has evolved into a model of eco-friendly disposable caging use and flexibility.

PS11 Pharmacokinetics of Ceftiofur Crystalline Free Acid (CCFA) in Rhesus Macaques (*Macaca mulatta*) after Subcutaneous Administration

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Rhesus macaques (*Macaca mulatta*) live in social groups with a strict behavioral code of conduct and social hierarchy to ensure stability. Trauma is a common sequela to maintaining order, often necessitating antibiotic therapy. First-generation cephalosporins are commonly used twice daily minimally for 5 d. This routine may have both animal welfare implications (increased stress, distress, and time away from the social group) and management implications (increased time, supplies, and occupational risk). Ceftiofur crystalline free acid (CCFA) is a long-acting, single-dose, injectable third-generation cephalosporin that delivers ≥ 7 d of therapeutic plasma concentrations in swine *(Sus scrofa domesticus)*. We hypothesized CCFA would provide ≥ 7 d of therapeutic plasma concentrations in rhesus macaques as compared with swine. Thus, we sought to describe the pharmacokinetic profile of CCFA in healthy, adult male rhesus macaques (n = 6) in this 2-period, 2-treatment crossover study at 5 and 20 mg/kg SC administered once. Plasma ceftiofur metabolite concentrations were determined by tandem liquid chromatography-mass spectrometry prior to drug administration and for up to 21 d post. Noncompartmental pharmacokinetic analysis was performed. For each dose (5 and 20 mg/kg, respectively), maximum plasma concentration was 2.24 ± 0.525 and 9.18 ± 4.90 µg/mL, occurring at 2.59 ± 1.63 and 1.82 ± 1.29 h. The area under the curve was 46.9 ± 17.6 and 331.2 ± 84.4 h/µg/mL and the terminal elimination half-life was 56.5 ± 21.7 and 69.7 ± 8.86 h. No adverse effects were noted after drug administration at either dose. Results suggest that for macaque bacterial isolates with mean inhibitory concentrations ≤ 0.2 µg/mL, a single injection of CCFA at 5 and 20 mg/kg SC provides therapeutic plasma concentrations similar to swine for at least 3 and 9 d, respectively, and can significantly reduce stress and risk to personnel and animals as well as time and financial costs.

PS12 Evaluation of Analgesic Efficacy in Piglets Using a Novel Pig Grimace Scale

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There is a critical lack of information surrounding methods to improve the wellbeing of piglets undergoing painful procedures. It is not uncommon for piglets to undergo potentially painful procedures without anesthesia or analgesia, particularly for agricultural research projects. The objectives of this study were to develop and validate a Pig Grimace Scale (PGS) in association with behavioral scoring techniques to assess analgesic efficacy in piglets undergoing castration. Castration was performed on 4 litters of 5-d-old pigs (n = 19) with treatments randomized across litters: no treatment, meloxicam +

EMLA, meloxicam + unmedicated cream, saline + EMLA, and saline + unmedicated cream (4 to 5 pigs per group). Pens were videorecorded for 1 h the day prior to castration, immediately after castration for 7 h, and for 1 h at 24 h postprocedure. Thirty behaviors or postures were scored continuously for the first 15 min at −24, 0, 1, 2, 3, 4, 5, 6, 7, and 24 h by an observer blinded to treatment. For PGS scoring, 627 facial images were captured across the 9 time points. Facial action units and an associated scale were developed, including ear position, orbital tightening, and cheek bulge. Three individuals blinded to treatment scored each photo separately. Baseline PGS scores from −24h pigs were subtracted from scores obtained after castration. Data was analyzed using a linear model ANOVA with post hoc Bonferroni tests. Pigs demonstrated significant behavioral changes up to 7 h after castration and the use of meloxicam and EMLA were not associated with a reduction in painful behaviors or postures. No litter-associated differences were noted in behavioral or PGS data and data was combined across litters. There were no treatment differences in PGS scores and PGS scores at 0, 3, 4, and 5 were significantly higher than those at 7 h after castration ($F_{4,15} > 3.06$, $P < 0.05$). These findings indicate that the PGS may be useful for evaluating pain in piglets.

PS13 Production of Polyclonal Antibodies in Chickens with No Interference with the Animals

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Production of polyclonal antibodies in the EU is a regulated license requiring procedure associated with injections (immunizations) of the animals with antigen (immunogen), often in combination with a more or less aggressive adjuvant, and subsequent blood sampling regimes through a period of time. The overall object of the projects presented here was to develop alternative noninvasive methods to produce polyclonal antibodies. Chickens are an attractive alternative to mammals and in large comparative immunization studies of chickens and rabbits we have demonstrated that approximately 10 times more antibody can be harvested from chicken egg yolk compared with what can be obtained from rabbit serum. The methods chosen to eliminate parenteral antigen injections included oral voluntary ingestion of antigen-adjuvant, which, combined with purification of antibodies from the egg yolk, eliminates all animal handling, restraint, and stress. The model antigens included human IgG and bovine serum albumin (BSA). The nontoxic cholera toxin B subunit (CTB) and pegylated C8/C10 mono/di-glyceride were both found to be effective adjuvants stimulating not only a mucosal, but also a peripheral immune response to an antigen following oral administration by gavage of 15-d-old chickens on days 0, 14, 28 and 42. A positive correlation ($P < 0.001$) was found between the adjuvanticity and immunogenicity of CTB. However, an anamnestic memory response is difficult to induce through oral immunizations rendering booster administrations of little effect. Oral immunization of 45 50%-inbred White Leghorn layers with antigen (BSA) and pegylated C8/C10 mono/di-glyceride administered not as single doses, but as doses divided over 3 to 5 d, followed by 2 monthly single booster administrations, effectively introduced immunologic memory. However, the resulting antibody concentration was only about 12% of the antibody concentrations obtained after classic subcutaneous immunization of chickens. Nonparametric statistics were applied due to nonnormal distribution of data. Most recently we have combined administration of antigen with respiratory virus vaccines against which the chickens are routinely aerosol vaccinated as day-old chickens, and this approach for polyclonal antibody production eliminates any handling and interference with the animals. In conclusion, the use of this methodology results in polyclonal immunospecific antibody production systems, which can be applied at commercial egg-producing plants with no added stress for the chickens used for this purpose compared with other egg-laying chickens.

PS14 Pharmacokinetics of Daily Subcutaneous Injection of Meloxicam over a 72-Hour Period in C57BL6 Mice

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At this institution, it is recommended that analgesics be administered for a minimum of 72 h following all major, invasive procedures. Meloxicam is a common analgesic agent used by the investigators to meet this recommendation. However, pharmacokinetic information for meloxicam in mice is scarce, and pharmacokinetic information for repeated subcutaneous administration is currently not available. C57BL6 mice are a widely used strain at this institution, and 1 to 2 mg/kg SC every 24 h is the most frequently chosen method of administration for meloxicam. To determine if this dose maintains a sufficient plasma concentration, the pharmacokinetics of meloxicam were characterized. Meloxicam at 1.6 mg/kg SC was administered to 4-wk-old male and female C57BL6 mice every 24 h for 3 d. Blood samples were collected at time points from 1 to 72 h from the first injection. Reverse phase liquid chromatography-tandem mass spectrometry analysis was employed to quantitate the plasma drug concentrations in organic solvent-precipitated plasma using a meloxicam standard curve. The pharmacokinetics of meloxicam in C57BL6 mice were compared with the reported plasma concentration values that would inhibit 50% (IC_{50}) of Cyclooxygenase-1 (COX-1) and Cyclooxygenase 2 (COX-2) activity. The mean plasma concentration at 24, 48, and 72 h was found to be above the reported COX-1 and COX-2 IC_{50}. Because skin lesions at the meloxicam injection site have occasionally been observed in mice at this institution, skin samples from the injection site at 72 h were harvested for histopathologic evaluation, and only mild inflammation was identified. These results demonstrated that 1.6 mg/kg SC of meloxicam in C57BL6 mice will maintain a plasma concentration above the IC_{50} of COX-1 and COX-2 for 24 h. Given these findings, this study will contribute information to improve evidence-based decisions for meloxicam use in mice.

PS15 Utility and Limitations of Real-Time PCR of Zebrafish Embryos for Pathogen Detection

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Quarantine for zebrafish colonies presents distinct challenges. Many zebrafish colonies only allow entry of surface-disinfected embryos to limit the introduction of new pathogens. However, zebrafish embryos are typically too old for surface disinfection by the receiving institution at the time of arrival. Receiving institutions must therefore decide whether to trust the health status report and surface disinfection practices of the submitting institution or raise the fish to maturity, spawn them in quarantine, surface-disinfect the next generation, and submit the parents for diagnostic evaluation. Diagnostic evaluation of a subset of the embryos received could provide useful information about the infection status or surface-disinfection practices of the submitting institution if there is a high likelihood of detecting the pathogens in only the subset of embryos evaluated. Complicating this process are the pH-dependent chlorine resistance of *Pseudoloma neurophilia* spores, intraovum transmission of *P. neurophilia*, and the unknown chlorine susceptibility of other agents. In order to prospectively investigate the likelihood of pathogen detection in a subset of treated or untreated embryos from subclinically infected parents, adult pairs of clinically unaffected zebrafish were spawned from established zebrafish colonies with multiple pathogens. Embryos from each clutch were divided into unrinsed, rinsed, and surface-disinfected treatment groups. From each cross, the 3 groups of embryos and each parent were independently evaluated by real-time PCR for 3 parasites and 6 *Mycobacterium* spp. Parent(s) were often infected with pathogen(s) that were not detected in their

embryos. Fewer pathogens were identified in surface-disinfected or rinsed embryos than in unrinsed embryos. *P. hyphessobryconis* was detected in all embryo treatment groups. Environmental mycobacteria were detected in unrinsed and rinsed embryos, but not in surface-disinfected embryos. *M. chelonae* and *P. tomentosa* were detected in unrinsed embryos only, and *P. neurophilia* was not detected in embryos. Thus, while unrinsed embryos provide an antemortem diagnostic sample in quarantine, negative results obtained from a subset of embryos often do not reflect the infection status of the parents.

PS16 Comparison of Exhaust Air Dust PCR Testing and Sentinel Screening for Rodent Infectious Agents on Individually Ventilated Caging Racks at Multiple Rodent Vivariums

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The availability of PCR panels has led to development of alternative screening methods for infectious agents. Exhaust air dust (EAD) testing of individually ventilated caging (IVC) racks by PCR has been previously reported for small panels of assays. To further investigate EAD testing as an alternative to traditional nonPCR screening of soiled bedding sentinels (SBS), we initiated a multifacility study to investigate its advantages and challenges. Real-time PCR panels included agents commonly screened during routine health monitoring. The data for this project currently represent 29 IVC racks among 6 facilities screened at one or more time points. Traditional SBS screening for each rack was compared with PCR screening of EAD samples (plenums plus an optional postplenum site (EAD other)) and with PCR from SBS or from SBS plus 8 colony cages (SBS+8). Not all samples were taken from all racks at all time points. In aggregate, 19 organisms were detected. Eighteen (306 positives among 29 racks) were detected using EAD plenum, 15 (153 positives among 15 racks) were detected using EAD-other, 15 (215 positives among 29 racks) were detected using PCR of SBS/SBS+8, and 5 (39 positives among 19 racks) were detected using traditional SBS. EAD plenum PCR detected more organisms compared with EAD other. EAD samples from racks with cage level filtration (n = 4 racks) did not consistently detect organisms, including MPV, found by traditional and PCR SBS testing. In contrast, EAD samples from racks without cage-level filters (n = 25 racks), consistently detected organisms found via traditional and PCR SBS testing. However, among racks reporting MNV positives, all methods did not agree at all time points. Organisms detected by EAD plenum PCR, but inconsistently or undetected by traditional SBS or SBS/SBS+ PCR included Beta strep group B, *C. bovis*, *Helicobacter*, *K. pneumoniae*, *P. pneumotropica*, *S. xylosus*, *Cryptosporidium*, and *Entamoeba*. In conclusion, EAD PCR testing may be hampered by IVC type and sampling location, and all agents, save MNV, were best detected using EAD plenum samples.

PS17 Dust Sampling Compared with Sentinel Testing for Murine Norovirus in Individually Ventilated Caging Racks

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Health monitoring of rodents in individually ventilated cage (IVC) systems is a challenging task. Each single cage represents a separate hygienic unit. Usually, used bedding sentinels (UBS) are examined every 3 mo, but limitations of this method are obvious: IVCs prevent transmission of airborne pathogens, transmission of unwanted organisms by used bedding is uncertain and susceptibility of UBS to some pathogens may be low. In order to improve health monitoring in IVC systems, we screened for excreted nucleic acids of unwanted organisms present in the exhaust air prefilter of an IVC rack. Murine norovirus (MNV) was used as a representative unwanted organism in our tests. In an experimental colony kept in IVCs with a defined prevalence of MNV, dust in the exhaust air prefilters was tested weekly for MNV nucleic acids with RT-qPCR and results were compared with the outcome of UBS serology. Several 12-wk testing periods showed clear superiority of exhaust air prefilter PCR in comparison to UBS serology. UBS serology did not detect MNV at low prevalence. In contrast, dust PCR detected MNV in each testing period. Further, as few as one cage with 5 naturally MNV infected mice in a rack (prevalence < 5%) were detected after only 1 wk of dust sampling and PCR testing. We recommend dust PCR as a screening method in addition to sentinel monitoring for those pathogens that are difficult to detect by UBS serology.

PS18 Animal Facility Exposure to Light at Night Suppresses Circadian Melatonin Production and Drives Intrinsic Resistance to Doxorubicin Therapy in Female Nude Rats Bearing Human Breast Cancer Xenografts

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Light exposure at night, as experienced by night shift work and/or in response to perturbed sleep-wake cycles, disrupts circadian time structure and may lead to a markedly increased risk of breast cancer. Currently, development of intrinsic drug resistance is a major impediment to the successful treatment of breast cancer with either hormonal or chemotherapy. Previous studies in our laboratory demonstrated that dim light exposure at night (dLEN), as often occurs in animal facilities, suppresses the normal nighttime production of the major circadian neurohormone melatonin. Melatonin's inhibition of human breast cancer metabolism and growth functions to reestablish the tamoxifen sensitivity of breast cancer, that is lost in response to dLEN, and drive tumor regression. In this study tissue-isolated MCF-7 (ERα+) human breast cancer xenografts were grown in female nude rats (Crl:NIH-*Foxn1*^{rnu}; n = 6 per group) maintained on a 12:12dLEN-h (0.2 lx; suppressed endogenous nocturnal melatonin) light:dark cycle and were subjected to the following treatment regimens: Groups A, controls (no treatment; vehicle); B, Doxorubicin (Dox) (an anthracycline antibiotic/chemotherapeutic drug) (6 mg/kg/d body weight, IP; initiated when tumor reached 2 g); C, Dox + melatonin (2.73 ± 0.34 µg/d in nighttime drinking water to reestablish elevated nocturnal levels); and D, melatonin alone. Results revealed a significant decrease (P < 0.001) in tumor latency-to-onset, increased tumor metabolism and growth in groups A and B compared with C and D, and complete intrinsic resistance to Dox therapy (group B), compared with all other groups. Human breast tumor xenografts in group C regressed at a rate of −0.18 ± 0.06 g/d, compared with tumors from groups A and B (0.68 ± 0.01 g/d) and D (0.30 ± 0.04 g/d), respectively, which continued to grow. The study shows that dLEN-induced circadian disruption/suppression of nocturnal melatonin production leads to a complete loss of human breast cancer sensitivity to Dox that is reestablished by melatonin supplementation leading to tumor regression. These results also show that the presence of LEN in animal facilities can compromise experimental outcomes directed at testing the efficacy of anticancer drugs in preclinical animal models of cancer therapy.

PS19 Animal Facility Light Exposure at Night-Induced Disruption of Aerobic Glycolysis (Warburg Effect) and Fatty Acid Metabolic Signaling in Human Prostate Cancer Xenografts in Nude Rats

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Light is arguably one of the most potent biologic forces on the planet, which entrains circadian rhythms of all mammals, including laboratory animals. In previous studies we showed that light and lighting cycles, as outlined in the *Guide,* as well as spectral transmittance (color) of light, influence laboratory animal health and wellbeing and scientific outcomes. We demonstrated that animal facility dim light exposure at night (dLEN) with as little as 0.20 lx suppressed production of the circadian neurohormone melatonin and stimulated human breast tumor growth and metabolism. Here, in conjunction with our GLAS-supported studies, we examined whether suppression of the nocturnal melatonin signal alters normal nighttime tumor total fatty acid (TFA) uptake, aerobic glycolysis (Warburg effect), 13-HODE production, and [^{3}H]thymidine incorporation into tumor DNA in tissue-isolated VCap human prostate cancer xenografts. Male nude rats (Crl:NIH*Foxn1*^{rnu}; n = 4 per group) bearing VCap prostate cancer xenografts were maintained on either a control (C) 12:12-h light:dark cycle (300 lux; 123 mW/cm^2 light phase intensity) or experimental (E) 12:12dLEN-h (0.2 lux; 0.08 µW/cm^2 dark phase intensity) light:dark cycle (lights on 0600) in an AAALAC-accredited facility. Plasma melatonin levels in C were high in the dark phase (183.8 ± 12.8 pg/mL) and low (2.5 ± 0.3 pg/mL) in the light phase, and low in E throughout the 24-h period. Diurnal plasma TFA was similar for C and E (high during night; low during day). Tumors harvested during dark phase (2400) revealed cAMP levels, TFA uptake, and DNA [^{3}H]thymidine incorporation, and aerobic glycolysis (Warburg effect) elevated significantly ($P < 0.001$) by over 350%, 2000%, 1200%, and 5000%, respectively, in E, compared with C. Activation of signaling pathways such as ERK 1/2, Akt, and glycogen synthase kinase-3β (GSK3β), involved in energy metabolism, and PCNA (DNA replication and cell cycle regulation) were markedly elevated in E, compared with C ($P < 0.001$). These findings show in vivo that the integrated circadian rhythms of signaling, metabolism, physiology and proliferation underlying human prostate cancer growth can be disrupted by dLEN, as may occur in animal facilities that are contaminated by ambient light sources during the dark phase.

PS20 Impact of Gestational Nicotine Exposure on Intrauterine and Fetal Infection in a Rodent Model

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Nicotine exposure from cigarette smoking and other forms of tobacco use in pregnant women continues to occur, even though the risks to the fetus during pregnancy are well established. Intrauterine infection is another major risk factor to perinatal health; however, unlike nicotine exposure, intrauterine infections are not associated with a modifiable behavior of the mother. We investigated the interaction between prenatal nicotine exposure and intrauterine infection using an established rodent model in order to determine if the preventable risk factor of nicotine exposure may contribute to intrauterine infections. Timed-pregnant Sprague–Dawley rats were implanted with an osmotic minipump at gestation day (GD) 6; received intravenous injections at GD 14; and were necropsied at GD 18. Groups evaluated included: dams that received saline infusion and sterile broth (control); 6 mg/kg/d nicotine infusion and sterile broth (Nic only); saline infusion and 10^7 CFU *Mycoplasma pulmonis* (MP only); or nicotine infusion and 10^7 CFU *M. pulmonis* (Nic+MP). Maternal and fetal tissues were cultured for presence of *M. pulmonis* and processed for evidence of histologic lesions. Nicotine exposure did not impact colonization rates of maternal sites but significantly increased ($P \leq 0.02$) the percentage of amniotic fluids and fetuses that were infected. Although the percentage of placentas infected did not significantly differ between MP only and MP+Nic groups, the microbial load was significantly higher in MP+Nic rats ($P = 0.04$). The threshold of placental microbial load associated with ≥ 10^4 CCU *M. pulmonis* in the amniotic fluid was significantly lower in Nic+MP compared with M*P* only rats ($P < 0.01$). Fetal inflammation was most extensive in the Nic+MP group ($P < 0.05$), with an increased occurrence of both chorionic vasculitis ($P < 0.001$) and umbilical arteritis ($P < 0.004$). Prenatal exposure to nicotine increased the risk for intrauterine infection, lowered the bacterial load required to breach the placental barrier and infect the amniotic fluid and fetus, and altered the pathology associated with maternal and fetal sites.

PS31 Reducing the Ergonomic Challenges Associated with Rodent Caging Filter Top Maintenance Using Lean Management's Problem-Solving Tool

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In our department, laboratory animal technicians are expected to find opportunities to reduce the physical demand and improve the safety associated with the tasks that they performed. One of the tasks highlighted during our routine problem identification process is the physical nature of disassembling filter tops in order to replace damaged filters. At our largest rodent facility we replace over 300 filters per month. Standard practice was to collect damaged filter tops and replace them once a week or when there is extra time which created a batched, repetitive, potentially unsafe process. The methods for removing damaged filter material from the lids varied from manual removal with significant pressure on the staff member's thumbs to the use of different tools to pry off the top. Broken cage tops and potential safety concerns with slip and cut "near misses" were reported. The team was challenged to find potential improvements using the lean problem solving process called the PDCA (plan do check act) Cycle. Several issues with the current process were identified and several improvement options were tested. Ultimately the team defined the requirements for a specially designed tool and partnered with the hospital's metal shop to have 2 simple presses made that disassembled and reassembled the cage lids. By employing the mechanical advantage of this new tool, the amount of physical force on a staff member's thumbs and the risk of damaging the lids from various tools were eliminated completely.

PS32 Ensuring Quality and Consistency of Husbandry in a Large Academic Program

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Ensuring that an animal facility program consistently maintains compliance with all laws, regulations, policies, guidelines, and standard operating procedures can be a daunting, time consuming, and expensive process. This task is further complicated in large, decentralized animal programs in which technicians are not always assigned to the same room every day. A program was created to leverage the husbandry technicians that were already present in the facility, in a way that was commensurate with existing job descriptions, to perform regularly scheduled quality control assessments of animal rooms and associated support areas. The selected technicians were called Area Leaders, to denote their responsibility for the assigned space. Initially, the Area Leaders participated in a training regimen detailing the animal program's standards and the processes for rectifying self-identified deficiencies and a pilot program was instituted to work out the operational issues. Specifically, the Area Leaders were assigned specific "areas" consisting of animal rooms and support space. Every 2 wk, they inspect their designated areas of responsibility to verify that standards are met through the use of established criteria that are summarized and documented using checklists. The Area Leaders are responsible for identifying any noncompliant issues. The entire checklist is submitted to their manager for review and follow-up. Some items are the Area Leaders responsibility to take corrective action and track, such as submitting repair

request for facility maintenance problems, hazard or specialized service requests from investigators, and outdated, incorrect, or omitted room signage. Other deficiencies require corrective actions that are addressed by management, including performance of room technicians and investigator compliance issues. As each facility adopted the new system for biweekly quality control assessments, the number of IACUC deficiencies decreased and the staff's comfort with inspection readiness increased. IACUC findings for our animal facilities have decreased by approximately 50% since instituting this program and the consistency of practices and performance across 18 unique facilities was a commendation from our most recent AAALAC site visit.

PS33 Small Ruminant Parasite Management in Biomedical Research

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Small ruminants are common animal models utilized in biomedical research. Housing sheep and goats in an outdoor setting is cost-efficient and provides a naturally enriching environment for the animals. Parasite management in small ruminants on pasture is challenging, as a limited number of anthelmintics are approved for use in sheep and goats and parasite resistance to those anthelmintics continues to increase. Preliminary data obtained from commercial sheep flocks in the state of Michigan indicate mild to moderate parasite resistance to benzimidazoles and select macrocyclic lactones, representing 2 of the 3 major chemical classes of anthelmintics approved for sheep in the United States. Implemented at our institution, an integrated parasite management program is key to minimizing anthelmintic resistance without compromising the welfare of a flock. The central theme of this management scheme is the maintenance of pasture refugia, a parasite population naïve to anthelmintics. Program success is only possible through proper utilization of effective anthelmintic therapies, infection monitoring to minimize anthelmintic treatment, understanding infection risk, and proper quarantine procedures. Flock parasite burdens may be effectively monitored by scheduled quantitative fecal egg counts and clinical scoring via physical examination and the FAMACHA system. Fecal egg count reduction tests allow monitoring of anthelmintic treatment efficacy and provide information on the incidence of anthelmintic resistant parasites in the herd. Caretakers and veterinarians responsible for the care of outdoor housed small ruminants in research should remain cognizant of the impact unrestrained anthelmintic use can have on the environment and animal welfare and consider implementation of an integrated parasite management program.

PS34 Management Challenges of a Complex and Continuously Growing Animal Care and Use Program

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The Department of Experimental Medicine at our institution is the AAALAC-accredited centralized laboratory animal facility of the University and the University Hospitals. It has grown and been in constant development over the past 10 y. More than US$25 million have been invested in upgrading and refurbishing the facilities and lean projects have been applied to streamline organization and services. It is an ongoing process to meet the demands of the scientists for new relevant core facilities and in particular to combine these to make them available in BSL and GMO-classified areas. A change to IVC housing systems in all our units has created new challenges regarding health monitoring and handling of increased activity in a confined space automatically creating challenges with respect to the working environment, in particular in relation to ergonomics and allergen exposure. The 6 units and the Department's constant effort to create a common team spirit, uniform work processes, and maintain all units live in compliance with the AAALAC accreditation, creates a continuous demand for developing and applying tools and strategies for an efficient and transparent management of this large and complex animal care and use program. In addition, the ambition to operate the Department as a professional business with the researchers as our customers, which we strive to provide a world-class service, often brings challenges when the researchers' wishes collide with things like health and safety issues, animal welfare issues, our strict subdivisions according to animal health status, or rules and regulations from the national authorities. We will give an overview of how we have dealt with management challenges of our complex and continuously growing animal care and use program.

PS35 A Committee Approach to Environmental Enrichment

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The Department of Comparative Medicine has established an enrichment program committee to provide assessment of animal enrichment. To improve the quality of this committee the members have developed and implemented an effective strategy using good laboratory procedures and the use of the *Guide for the Care and Use of Laboratory Animals* as ground building components. The continual improvement process of this committee is comprised of 5 lab animal technicians and one departmental supervisor. Committee members are drawn from a pool of volunteers. Each member provides a mechanism of quality to develop, review, implement, organize, and structure the consistency of the program with observation and individual research on the enrichment of specific species. In order to achieve new ideas and continual improvement, committee members rotate one at a time every 3 mo. Rotating committee members allow for continuity as well as new ideas. The organizational structure of this committee has allowed for necessary changes to be more easily and effectively initiated when changing or adding species-specific enrichment. The committee has successfully and quickly aided the PIs to remain in compliance with IACUC policies regarding enrichment. In addition we have assured that animals are provided with adequate, effective and consistent enrichment that not only benefit the animal but the safety, ease, and accuracy of administration. The efficacy of this program hinges on the ability to make immediate changes when necessary, maximize the ability to make long- and short-term goals as a committee and improve the availability of enrichment for the animals to thrive in the various environments. We will describe the program, the process of development, the maintenance and the best way to overall encompass improvement standards set forth by the committee members and *the Guide for the Care and Use of Laboratory Animals*.

PS36 The Summer VETS Program: Introducing High School and College Students to Laboratory Animal Science and Medicine

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The American Association for Laboratory Animal Science (AALAS) has long held the belief that the future of biomedical science depends upon promoting the benefits of animal research through public outreach. At our institution, the Summer Veterinary Exploration through Science (VETS) program was initiated in 2010 as a means to introduce students interested in veterinary medicine to aspects of the career. For groups of high school and college students, VETS was designed to encompass a full week of tours, lectures, and in-person sessions at the School of Veterinary Medicine (PennVet). In 2011, the administration requested that a laboratory animal medicine component be incorporated into the existing VETS structure. As a result, we developed a hands-on session for the VETS students to work with laboratory mice and rats in order to learn the key role these species hold as animal models of human disease. Working collaboratively

with our clinical laboratory animal veterinarians, residents, training specialists, and staff, students collectively received a short lecture and handout, and then dispersed in small groups led by a designated laboratory animal specialist to assist with handling, restraining, and sexing of animals. VETS graduates rated the program on a 1 (low) to 5 (high) scale; average ratings were between 4.26 to 4.36 over the years that the laboratory animal medicine course has been involved. By 2014, 144 high school and 166 college students will have taken the lab animal session. Many graduates of the college Summer VETS program have matriculated into veterinary programs, including a total of almost 40 enrolled at PennVet, exemplifying the successful outreach efforts of this program for bringing interested individuals into the profession. We feel that this exposure to laboratory animal science prior to veterinary school will promote appreciation for animal research and enable students to pursue additional experiences in laboratory animal medicine during veterinary school.

PS37 Postapproval Monitoring: Why Be a Monitor When You Can Be a PAL?

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The Animal Welfare Act and the Public Health Service Policy require IACUC oversight of animal activities. Ongoing protocol assessment ensures the wellbeing of the animals, regulatory compliance and is a factor in maintaining AAALAC accreditation. Postapproval monitoring programs are increasingly used to meet this need. Our Postapproval Monitoring Program uses an educational approach in a collegial partnership with 650 animal researchers working on 350 protocols. Two full-time Protocol Advisors and Liaisons (PALs) report directly to the Institutional Official and act as liaisons between animal research laboratories, the IACUC and the Animal Resource Center. Prior to visiting research staff, our PALs read the laboratory's protocol(s) and prepare folders containing animal program information as well as protocol-specific policies and guidance. Protocol meetings allow for candid discussions of protocol procedures as well as create familiarity between PALs and research staff, important when observing protocol procedures. Once a laboratory visit is complete, our PALs discuss concerns with appropriate animal program subject experts and conduct debriefing meetings with the research staff. PALs train, or involve the appropriate campus resource in researcher training and compliance. An increase in submission of protocol amendments, as well as refinement of research procedures through standard animal training classes, and development of laboratory-specific training modules have resulted from PAL visits. The PALs have become a trusted resource to our animal research community.

PS38 Challenges of Managing an Animal Facility During the Economic Downturn

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After years of downturn, the US economy is showing signs of recovery but financial conditions in biomedical research remain challenging. The impact on endowments and grant funding continue to impose enormous budgetary constraints on institutions. Many have implemented cuts in operating budgets/subsidies to core resources to cope with these uncertainties. At our Institution, the Animal Resources Department (ARD) was mandated to change its business model from partial to full cost recovery while minimizing the impact on per diems. Our total operating budget decreased by 6.6% from FY08-09 to FY12-13. Subsidies decreased from 25% of the operating budget to 3.1% in FY12-13. This loss was not offset by an increasing rodent census or modest 3% per diem adjustment over the period. Costs were also expected to increase due to inflation, maintenance of aging facilities/equipment and programmatic changes. As a response, ARD implemented several cost control measures. First, all facility, equipment and health monitoring contracts were renegotiated. Expenditures exceeding US$500 were subject to mandatory review. Work processes were reevaluated to increase efficiency and productivity (for example, 10-d mouse cage change cycle, modification of sanitation intervals), decrease costs (for example, recycling/autoclaving of PPE) while other services normally outsourced (for example, valve and cage refurbishing, laboratory diagnostics) were performed inhouse. Considering that payroll represented 75% of the budget, new hires were frozen and total FTE decreased 8% through attrition. The Institute also mandated OT reduction, which was met by switching from a Mon through Fri schedule with paid weekend OT to 3 split shifts working Mon through Fri, Tue through Sat or Sun through Thu. As a result, average yearly OT decreased by 89%. Overall, these measures led to a 7% decrease in payroll despite increasing wages and benefits. An unanticipated effect of these cost saving initiatives was a significant increase (133%) in workers compensation claims due to increase demand and workload. Together with our insurance underwriter and administration, we revised the occupational health and safety program to include enhanced ergonomic equipment, processes and training to begin addressing these challenges while maintaining budgetary goals.

PS39 Fit To Work: Interview Skills

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Hiring in the context of a laboratory animal science setting has added complexities related to the contention of the work conducted in this field combined with the negative consequences of poor performance or mistakes that can adversely impact research and compromise the health and welfare of the animals under our care. Typical Human Resources benchmarks focus on qualifications, previous experience, levels of education, and use such measures as pretesting of computer skills which all add value but may not be relevant to performing well in a laboratory animal setting. Moreover, HR professionals may not have a fundamental understanding of the operations of a laboratory animal facility or the work performed by technicians. Consequently, they may in fact impede the hiring process rather than support it. A framework with appropriate objective tools and measures for facilitating the selection process is important. The framework involves developing candidate profiles based on vivarium operational needs rather than generic roles. The interview is conducted using a descriptive scoring grid and a weighted metric when appropriate. Interview questions are specifically structured for each position and circumstance. The interview reveals the nature and environmental conditions of working in a vivarium and some of the truths about working in our industry with the goal of educating the potential candidates when possible. Additionally, we use real vivarium scenarios as questions for internal promotions. Lastly, we set tangible and measurable objectives during the interview process for internal promotions to be achieved within a prescribed period of time. This framework was formulated over a number of years through general research on the topic of interviews, organic ideas developed over time and through exposure to vivarium operational needs, feedback from supervisors and staff and through a certain level of trial and revision. The underlying result has been a reduction of unforeseen turnover, a more engaged workforce interested in vivarium operations, and a large increase in requests for long-term employment opportunities from our pool of student and casual employees. However, the process must still integrate some level of subjectivity to capture the intangible assets of the prospective candidate, be focused on selecting a long-term employee to maintain a marginal turnover rate, which, if high, can be costly to the organization, compromise the level of care given to the animals and service provided to the clients.

PS40 Program Implementation of the new European Animal Welfare Directive: An AAAALAC-Accredited Organization Expands the Use of PAM Internationally

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Postapproval monitoring (PAM) is a required element of laboratory animal care and use programs. As an AAAALAC-accredited international organization, we recently leveraged an effective PAM program element to extend local program compliance to an international scope. With the implementation of the new European Union Animal Welfare Directive (DIRECTIVE 2010/63/EU) transfers of animals to and between institutions within the European Union (EU) are associated with increased legal requirements, but most international organizations collaborating with EU institutions are not yet in full compliance. The new EU regulations govern the maintenance of genetically altered animals. The maintenance of colonies of genetically altered, established lines, with a likely harmful phenotype, requires a project authorization, whereas the maintenance of lines without a likely harmful phenotype does not. To categorize each line, an animal welfare assessment scheme determining all relevant data is needed. The documentation is necessary in order to determine the applicable legal requirements of each line. This increased regulatory burden is responsible for increased administrative data collection prior to import and maintenance. Our company addresses this challenge by using an element of its PAM program that includes welfare assessment data needed to be in compliance with the EU legal requirements. A global effort was undertaken to harmonize the data collection and corresponding welfare assessment. To ensure consistent documentation among our programs worldwide, our proprietary database was adapted to serve multiple objectives. Examples of metrics captured include essential welfare indices that enable veterinary, regulatory, IACUC/Animal Welfare Oversight, and colony management to perform animal welfare assessments to fulfill PAM requirements as well as EU regulations, including: numbers of animals, occurrence of abnormalities, and mortality observations. We will describe the implementation of the PAM data to support compliance with the new EU requirements concerning harmful phenotypes and to support seamless transfer of genetically modified animal models between institutions, ensuring continuation of responsible animal care and use to fulfill valuable research objectives.

PS41 Evaluation of Analgesic Efficacy of Firocoxib, a Selective COX-2 Inhibitor in the Mouse Model of Incisional Pain

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Pain management in laboratory animals is generally performed using opioids and/or NSAID. However, opioid use is hindered by controlled substance requirements and a relatively short duration of action. In this study, we evaluated the analgesic efficacy of firocoxib (a COX-2 selective NSAID) relative to buprenorphine in the mouse model of plantar incisional pain by objective measurement of mechanical allodynia and thermal hyperalgesia using electronic von Frey and Hargreaves equipment, respectively. Our experimental design included 5 groups (n = 10 mice per group): 1) firocoxib 10 mg/kg (F10) IP every 24 h, 2) firocoxib 20 mg/kg (F20) IP every 24 h, 3) buprenorphine 0.2 mg/kg (Bup) SC every 8 h (positive control), 4) normal saline intraperitoneally every 24 h (negative control), and 5) sham group (anesthesia, no incision) treated with firocoxib 20 mg/kg IP once every 24 h. All mice underwent nociceptive response assays examining mechanical allodynia and thermal hyperalgesia at −24 (baseline), 4, 24, 48, and 72 h postsurgery. All drugs were administered preemptively and continued up to 72 h postsurgery. Buprenorphine provided alleviation ($P < 0.05$) from allodynia at all time points postincision. F10 alleviated allodynia at 4, 24, and 48 h postincision while F20 alleviated allodynia at 24, 48, and 72 h. For thermal hyperalgesia, none of the drug groups provided alleviation at 4 h. With the exception of F10 at 24 h ($P = 0.19$), thermal hyperalgesia was alleviated for all drug groups at 24, 48, and 72 h. Thus, our results indicate that once daily firocoxib administration alleviates pain resulting from soft tissue injury in mice and may be a suitable alternative to buprenorphine dosing at every 8 h.

PS42 The Role of *Fni* in Skeletal Muscle Metabolism, Fiber Type Specification and Muscular Dystrophy

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Mammalian skeletal muscle is composed of a mosaic of myofibers, which differentially contribute to strength and endurance dictated by their cytoskeletal structure and metabolic strategy. Slow twitch "red" muscle fibers are rich in mitochondria, use predominantly oxidative phosphorylation for energy production, and are resistant to fatigue. In contrast, fast twitch "white" fibers are generally low in mitochondria, rely heavily on glycolysis for energy production, and are susceptible to fatigue. Although the biochemical differences between skeletal muscle fiber types are well characterized, the molecules that control fiber type specification are incompletely defined. Using a chemical mutagenesis strategy in mice, we previously identified a unique pedigree that lacks mature B lymphocytes due to a deletion in the *Fnip1* (Folliculin Interacting Protein 1) gene. Surprisingly, skeletal muscle from *Fnip1*-null mice appeared deeper red in color when compared with wildtype (WT) mice. We hypothesized that the loss of *Fnip1* shifts skeletal muscle from predominantly type II fast twitch to type I slow twitch myofibers. To address this hypothesis, we performed biochemical, molecular, and functional analyses on skeletal muscle from *Fnip1*-null and WT mice (n = 4 to 6 mice per group). Using real-time PCR, immunoblotting, and electron microscopy, we found that loss of Fnip1 results in increased myoglobin content and mitochondrial mass, as well as enhanced expression of genes and proteins characteristic of type I fibers. *Fnip1*-null skeletal muscles sustained more prolonged contraction, and faster postcontraction recovery when compared with WT skeletal muscle. Remarkably, by breeding *Fnip1*-null mice to mdx mice, we found that loss of *Fnip1* significantly reduced muscle damage in a murine model of Duchenne muscular dystrophy. We conclude that Fnip1 plays an essential role in skeletal muscle fiber type specification, and suggest that inhibition of Fnip1 could be utilized as a potential therapeutic strategy to increase mitochondrial biogenesis and muscle endurance in patients with muscular dystrophy disorders, which are typified by defective mitochondrial function. Funded in part by NIH grants K26RR024462, P30 CA015704, R56AI092093, P30-DK035816 to BMI.

PS43 Mammalian Target of Rapamycin Signaling is Essential for B-cell Development

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Mammalian target of rapamycin (mTOR) is a protein kinase that controls protein synthesis, cell growth, and proliferation. Inhibition of mTOR activity with the immunosuppresent rapamycin is a major mode of preventing transplant rejection in humans. However, the cellular and molecular mechanisms of how inhibiting mTOR suppresses the immune system are still poorly understood. Previous studies have elucidated essential roles of mTOR signaling in T-cell activation, proliferation, and differentiation. We hypothesized that the mTOR pathway is also integral for B-cell development and survival. To test this hypothesis, we disrupted mTOR signaling specifically in mouse pro-B-cells in vivo using tissue-specific gene targeting techniques. Bone marrow and spleen cells were collected from 6- to 8-wk-old mTOR-signaling deficient mice and wildtype controls (n = 6 to 10 mice per group). Disruption of mTOR signaling was confirmed

by immunoblotting. B-cell development was analyzed using flow cytometry to evaluate expression of cell surface and intracellular proteins that are differentially expressed during B-cell development. Statistical differences between groups were determined by a 2-tailed Student *t* test. Loss of mTOR signaling in the B-cell lineage resulted in a block in B-cell development at the pro B-cell to pre B-cell stage, with a corresponding lack of peripheral B-cells. B-cell survival, as measured by annexin V staining, and B-cell proliferation, as measured by BrdU incorporation, were also significantly reduced in B-cells from mTOR-signaling deficient mice, while mitochondrial superoxide, measured by red fluorogenic staining, was increased. To determine if inhibiting B-cell apoptosis could rescue the B-cell development phenotype, we crossed our mTOR-signaling deficient mouse with a transgenic mouse expressing the antiapoptotic protein Bcl-x_L specifically in the B-cell lineage. However, while apoptosis was decreased in these mice, B-cell development was not rescued. Our data reveal a critical role of mTOR signaling in B-cell development and survival, and reveal an additional mechanism for how mTOR inhibitors suppress immune function. These studies also suggest that mTOR inhibitors may have clinical efficacy in B-cell-mediated autoimmune disease or B-cell lymphoma.

PS44 The Search for an Earlier Endpoint: Clinical Parameters in Guinea Pigs Experimentally Infected with *Mycobacterium tuberculosis*

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Guinea pigs are a common model used to evaluate vaccines for *Mycobacterium tuberculosis* (TB). As a prey species, they tend to hide clinical signs of disease until late in the course of the condition. Currently, visual detection is limited to increased respiratory effort, decreased activity, and cyanosis—all of which can be highly subjective parameters and are only apparent in late stages of infection. Our hypothesis is that guinea pigs have detectable markers in the blood or urine that indicate severity of disease, and these markers can be a tool for identifying humane endpoints prior to the onset of clinical disease. Guinea pigs were either BCG (Bacillus Calmette–Guerin)-vaccinated or sham-vaccinated, then infected via aerosol with *M. tuberculosis* H37Rv. Blood and urine were sampled from the guinea pigs every 30 d. Diagnostic tests included complete blood counts, serum chemistry analyses, urinalyses, and venous blood gasses. A full necropsy complete with blood and tissue culture and histology was performed on each animal. There were significant differences between the BCG-vaccinated and sham-vaccinated groups; most notably in the complete blood count and blood gas data, as well as histologic lesions. Although both groups demonstrated a leukocytosis early in response to infection, sham-vaccinated guinea pigs had a greater degree of sustained neutrophilia than the BCG-vaccinated group. Both groups of animals became acidemic within 30 d of infection as evident by increases in blood pH and lactate, and remained acidemic for the duration of infection. There were no differences in serum chemistry or urine assessments. Sham-vaccinated guinea pigs had more severe and disseminated histologic lesions than BCG-vaccinated guinea pigs. These results suggest leukocytosis with severe neutrophilia at days 90 and 270 for sham-vaccinated and BCG-vaccinated guinea pigs, respectively, may be used as early endpoints for TB studies in guinea pigs. Additional studies to evaluate other early endpoints and biomarkers are not only needed to provide a practical and fast patient-side diagnostic tool for detection of severe disease in guinea pigs, but also to translate to human medicine.

PS45 Murine Norovirus Influences Outcomes in Adoptive Transfer Models of Inflammatory Bowel Disease

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Murine norovirus (MNV) infection is highly prevalent in laboratory mice. Although MNV infection does not induce clinical disease in most strains of mice, infection may nonetheless impact mouse models of disease by altering immune responses. Given prior evidence that MNV infection alters disease in a bacterial-induced colitis model in Mdr1a$^{-/-}$ mice, we investigated the hypothesis that MNV infection would exacerbate colitis in an adoptive transfer (AT) model of inflammatory bowel disease (IBD) with and without *Helicobacter bilis* infection used to drive colitis development. Eight-week-old, female, B6.129S7-Rag1^{tm1Mom}/J (Rag1$^{-/-}$) mice were infected with *H. bilis*, given CD4+CD45RB^{HI} T cells 5 d later to induce colitis, and then a subset were infected with MNV (n = 16 to 17 per group). Mice rapidly developed signs of IBD including diarrhea and weight loss and were necropsied four weeks post transfer. When compared with uninfected mice, no changes in IBD were found due to MNV infection using histologic scoring of the cecum, colon, and mesenteric lymph node. In the more mild, slowly developing model of IBD lacking *H. bilis*, Rag1$^{-/-}$ mice were given CD4+CD45RB^{HI} T cells, and infected with MNV either 3 d before transfer (n = 12), 7 d after transfer (n = 10), or remained uninfected (n = 8). Animals were evaluated 25 wk later and, in addition to IBD, had histologic evidence of severe inflammatory disease in numerous nonintestinal tissues including multifocal dermatitis, stomatitis, granulomatous mesenteric steatitis, delamination of the nonglandular stomach, and pneumonitis. Both extra-intestinal autoimmune lesions and IBD varied with the timing of MNV infection. Mice infected with MNV following AT had significantly higher IBD (P = 0.03) as well as higher extra-intestinal inflammation scores; mice infected with MNV following AT also had greater inflammation of the glandular stomach (P = 0.006) and greater inflammation of their lungs (P = 0.002) compared with those infected with MNV prior to AT suggesting that MNV modulates development of disseminated autoimmune disease in this model.

PS46 Raise a Glass: Verification of Mouse Euthanasia by Ethanol Injection

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Compassion, professional ethics, and public sensitivity require that animal euthanasia be conducted in a manner that is humane and appropriate. Research staff and veterinarians rely upon the expertise provided in the American Veterinary Medical Association (AVMA) Guidelines for the Euthanasia of Animals. In the 2013 edition, intraperitoneal injection of ethanol was added to the "acceptable with conditions" category. As this method is documented by only one paper, we sought to better evaluate it with the benefit of advanced monitoring equipment, including ECG and high-definition video recordings. Only mice that were designated for euthanasia from existing rodent colonies were used in this study. All animals were clinically normal with no prior history of intraperitoneal injection. Mice (n = 91) were grouped by gender and strain, then randomly assigned to treatment with ethanol (70% or 100%, 0.5 mL IP), a positive control agent (pentobarbital/phenytoin combination, 0.5 mL IP), or a negative control agent (saline solution, 0.5 mL IP). Following injections, mice were assessed for time to loss of consciousness, time to respiratory arrest, heart rate, and behavioral responses. Most mice (55%) responded to the injection itself and fewer mice (20%) showed signs of altered behavior after injection regardless of treatment group. Mice that received an intraperitoneal injection of 70% or 100% ethanol lost consciousness at 48.75 ± 14.5 s and 45 ± 9.5 s, experienced respiratory arrest at 4.4 ± 4.7 min and 2.5 ± 0.6 min and cardiac arrest at 9 ± 6 min and 9 ± 12.9 min, respectively. Mice that received pentobarbital/phenytoin lost consciousness at 49.5 ± 10.1 s, experienced respiratory arrest at 2.6 ± 0.5 min and cardiac arrest at 4.6 ± 1.4 min, while mice that received saline injection did not lose consciousness. No mice in the groups that received ethanol or pentobarbital/phenytoin regained consciousness. Overall, intraperitoneal ethanol injection resulted in rapid and irreversible loss of consciousness followed by death and appears to meet the euthanasia criteria outlined by the Panel on Euthanasia, as described in the AVMA Guidelines.

PS47 Gastric Wash as a Noninvasive and Sensitive Assay for Detec-

tion of Gastric *Helicobacter* spp. Infection in Rhesus Macaques (*Macaca mulatta*)

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Over half of the world's human population is infected with the class 1 gastric carcinogen *Helicobacter pylori*. Diagnostic modalities to detect *H. pylori* commonly used in humans include histologic and molecular detection of *Helicobacter* spp. in gastric biopsy, fecal *H. pylori* antigen tests, and urea breath test (UBT). Nonhuman primates (NHPs) used in biomedical research are naturally and more commonly infected with zoonotic *Helicobacter suis.* Associations between gastric *Helicobacter* spp. infection and gastritis have been reported in rhesus and cynomologus macaques. While the UBT has been validated as a noninvasive test to detect *Helicobacter* spp. in experimentally infected rhesus macaques (*Macaca mulatta*), this technique requires endotracheal intubation and does not provide specific *Helicobacter* spp. information. Previous studies in our laboratory have successfully utilized gastric wash from NHPs as a method of diagnosing *Helicobacter* spp. infection; however, we have not been able to determine the sensitivity of this assay due to inability to perform gastric biopsies on the subject animals. In this study, we demonstrate the sensitivity of this assay on research rhesus macaques. At subject necropsy, gastric wash and gastric tissues were collected from 13 NHPs from 2 colonies and were evaluated by 16s rRNA PCR and sequence analysis for *Helicobacter* genus and species. Formalin-fixed paraffin embedded tissues were evaluated using hematoxylin and eosin, Warthin–Starry, and fluorescent in situ hybridization (FISH). Histologically, all 13 NHPs had mild to moderate lymphoplasmacytic gastric inflammation. All 13 NHPs were considered positive for *Helicobacter* spp. infection based on the presence of gastric spiral organisms noted on gastric tissue histology and/or FISH. Of these, one NHP was negative for *Helicobacter* spp. infection via gastric wash PCR, demonstrating an assay sensitivity of 92%. 16s rRNA PCR and sequence of gastric wash and gastric tissues identified four NHPs coinfected with both *H. pylori* and *H. suis,* and 4 and 5 NHPs were solely infected with *H. pylori* and *H. suis,* respectively. Given that PCR is ubiquitous and gastric wash is a sensitive, quick, nontechnical procedure, this technique can be easily incorporated into routine exams of captive NHPs to evaluate gastric *Helicobacter* spp. infection.

PS48 Validation of a Novel Behavioral Ethogram for Identification of Postoperative Pain in the Guinea Pig (*Cavia porcellus*)

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Although the guinea pig (*Cavia porcellus*) has been used in research for over a century and remains one of the most prevalent AWA-covered species today, very little has been elucidated in the literature about recognition of clinical pain or analgesic efficacy in this species. Difficulty in recognizing pain in guinea pigs is primarily attributed to their innate "freeze" behavior in the presence of an observer. Classically, methods of evaluating pain in the guinea pig focus on measures of nociception (hyperalgesia and allodynia), but these lack practicality for clinical evaluation of spontaneous pain. We sought to assess pain in the guinea pig using a newer, potentially more clinically relevant, method that has been validated in other rodent species, the behavioral ethogram. In this study, 10 male guinea pigs were acclimated to novel behavioral observation cages where they were assessed by electronic von Frey measurements and their behavior recorded by remote video across 3 conditions (baseline, anesthesia only, and castration surgery) and at 3 different time points (2, 8, and 24 h post condition). The anesthesia-only condition served to control for the nonpainful, but potentially distressing components of the surgical experience. We hypothesized that at all 3 postsurgery time points there would be an increase in pain-associated behaviors via ethogram, corresponding to a decrease in nociceptive threshold measured by von Frey. When comparing postsurgery to post anesthesia-only conditions, behaviors associated with pain were significantly increased while nociceptive thresholds were significantly decreased at the 2- and 8-h time points. By 24 h, neither pain-associated behaviors nor nociceptive thresholds differed between the 2 conditions. By correlating ethogram scores with measures of nociception using electronic von Frey, we were able to validate behaviors as pain specific in the guinea pig. Thus, our novel ethogram may be used as a potential postsurgical pain assessment tool and serve as a platform for direct clinical application and future analgesia studies in this species.

PS49 Increased Dietary Vitamin D Decreases Inflammation and Subsequent Colitis-Associated Colon Cancer in 129-Smad3^{tm1Par}/J (*Smad3*$^{-/-}$) Mice

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Epidemiologic data suggest that low serum vitamin D levels are associated with an increased risk for developing colon cancer and inflammatory diseases such as inflammatory bowel disease (IBD). As vitamin D has been shown to decrease inflammation as well as tumor formation in various animal models, we hypothesize that increased dietary vitamin D is an effective therapy against inflammation-associated colon cancer. To test this, we used 129-Smad3^{tm1Par}/J (*Smad3*$^{-/-}$) mice which have defective transforming growth factor β-signaling and develop colon cancer in response to an inflammatory insult such as infection with *Helicobacter bilis*. Mice were fed purified diets containing either maintenance (1 IU vitamin D/g diet; control) or increased levels of vitamin D (5 to 10 IU vitamin D/g diet; High Vit D) and were infected with *H. bilis*. Animals were necropsied at several time points to assess inflammation, dysplasia, and neoplasia incidence. *Smad3*$^{-/-}$ mice fed High Vit D had a significantly decreased incidence of colon cancer compared with those fed control diet (11% compared with 41%, $P = 0.0121$). At an early time point in disease development (1 wk postinfection), *Smad3*$^{-/-}$ mice fed High Vit D had decreased MAPK (p-p38 and p-JNK) activation in lamina propria leukocytes and decreased NFκB activation in colonic epithelial cells. Reduction in MAPK and NFκB activation correlated with decreased IBD scores (2.7 compared with 15.5, $P < 0.0001$), decreased inflammatory cell infiltrates (T cells and macrophages), and reduced expression of proinflammatory cytokines in cecal tissue. To determine which cells were mediating protection, we created *Smad3*$^{-/-}$ mice deficient in T and B cells by crossing them with 129S6/SvEvTac- *Rag2* ^{tm1Fwa} mice. Interestingly, High Vit D diet did not protect these animals from *H. bilis*-induced tumors or dysplasia suggesting that the protective effects of vitamin D are elicited at least in part through adaptive immune responses. These findings suggest that increased dietary vitamin D may be beneficial in preventing inflammation-associated colon cancer by decreasing MAPK activation in lamina propria cells, resulting in suppression of inflammatory responses during neoplasia initiation or early stage carcinogenesis. Funded by R21 CA149995-01A1.

PS50 Evaluation of Preoperative Skin Preparation Agents in the African Clawed Frog (*Xenopus laevis*)

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Scientific research with African clawed frogs (*Xenopus laevis*) commonly involves surgical collection of oocytes. Despite routine use of this procedure, there have been few studies evaluating the method by which to prepare *Xenopus* skin prior to surgery. We evaluated the use of 3 preoperative skin preparatory agents by examining their antibacterial properties and the gross and microscopic appearance of

Xenopus skin following exposure to these substances. Frogs (n = 14) were sedated and underwent treatment with 0.9% sterile NaCl (as the control) on one half of their ventrum, and either 0.5% povidone iodine, or 0.75% chlorhexidine, each with 10 min of contact time, on the other half. Bacterial cultures were obtained before and after skin treatment; bacteria were identified via the novel diagnostic method of MALDI-TOF mass spectrometry. Gross photographs and biopsies of the incision sites were taken at 0, 1, and 7 d following surgery to evaluate skin for inflammation and degenerative change. We isolated over 22 genera of bacteria from the skin of our frog population, with a mean of 5.21 ± 0.82 genera isolated from each frog. Iodine and chlorhexidine both had significantly greater antimicrobial activity than saline with means of 2.00 ± 0.44 and 0.29 ± 0.76 genera isolated per frog, respectively. Grossly, skin erythema did not correlate with treatment group. Microscopically, chlorhexidine-treated skin had greater histologic evidence of epidermal degeneration and necrosis than iodine- or saline-treated skin at both day 1 and day 7. Additionally, frogs that were treated with chlorhexidine had a higher incidence of clinical illness associated with the site of chlorhexidine exposure. In summary, while chlorhexidine appears to have superior antimicrobial activity, treatment with this agent also leads to skin damage and subsequent clinical complications to the frog. As such, we do not recommend using chlorhexidine as a preoperative skin prep agent in this species.

PS51 Positive Reinforcement Training in a Research Setting: An Introduction to Sling Training the Ossabaw Pig

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Over the past year, the large animal care staff at our institution was introduced to an unfamiliar breed of pig, the Ossabaw Island pig. Not only were they tasked with adapting to this unique research model in their facility, but also with training them to undergo restrained medical procedures. The task was to train the animals to willingly travel from the housing room into a sling. Positive reinforcement training techniques were employed to assist in this process and to find the best way to accomplish our training goals with the Ossabaw pigs, as their behavior tends to be slightly different than your typical research pig. The overall training process proved challenging for many reasons, including: finding the proper equipment to restrain the animals, the overall size and personality differences of the pigs, and finding their primary motivator, which at times was not food. While the training process was difficult, what started out as a fairly labor-intensive and time-consuming process became manageable with just a few people and very little effort. We will discuss the use of positive reinforcement training for pigs in a research setting, with a brief overview of the mechanics and science behind this type of training. Challenges associated with our specific project will be discussed, as well as an overview of the various training methods that were employed to get the desired results. Lastly, we will provide some advice to those wanting to implement a positive reinforcement training program for the animals at their facility.

PS52 Nonhuman Primates Enrichment Room Planning, Design, and Use

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Princeton University continually makes efforts to improve and enhance the quality of life of our NHPs used in research. A part of this effort is to continue to improve our environmental enrichment program, promoting the expression of species-typical behaviors. Having had the privilege to be part of a move to a new facility, we were able to facility new innovative techniques in building an European-style NHP enrichment room and face this balancing act first hand. The new facility was built specifically as a vivarium to house primates, rodents, and aquatics. Due to this, we were able to create an enrichment room for the primates that would allow natural sunlight, species-specific behaviors, and increase the likelihood of pair housing older, singly housed macaques. Some of the issues we faced were cleaning schedules, room maintenance, scheduling the use of enrichment rooms, regression of learned behaviors, staff training inconsistencies, and enrichment room item conflicts. Each monkey had been target trained prior to the move so that when they were introduced to the enrichment room they would return to their home cage via target. If the target training was learned and the animal responded to the target within a latency of 1 s, they were "Okayed" to be released into the enrichment room. A few unexpected snags were the permanent and nonpermanent structures within the enrichment room. With monkeys being monkeys, they found weaknesses within the room we were not aware of, such as the drain covers and paint chips. Also, things like the play sets we had placed in the rooms held water for hours after cleaning, which posed conflicts with the research staff that had animals on water restriction. With each obstacle faced, we were able to work through it or find a solution and we continue to work for and test novel, interactive enrichment items in this space that are conducive with research needs.

PS53 Behavioral Management Approach to Facilitate Use of Pen-Housed Nonhuman Primates for Toxicology Studies

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Housing laboratory primates in large pens can improve welfare by providing ample opportunity to express normal social and locomotor behaviors. This type of housing, however, can present great challenges in terms of manipulating animals for study procedures. The use of positive reinforcement training (PRT) has been used successfully to allow for cooperation with study techniques. PRT requires much time be spent on implementation of training plans tailored to each animal. However, toxicology studies typically afford minimal time for training and little opportunity to design and apply individualized training plans. In preparation for movement to a new facility, we implemented a comprehensive behavioral management program to facilitate the use of a pen setting for toxicology studies with aggressive timelines between receipt of nonhuman primates (NHPs) and initiation of study. Our first phase of preparation required training our technical staff on the basic tenants of operant conditioning and natural behaviors of cynomolgus macaques. Staff then applied those lessons by teaching NHPs to elicit behaviors on command. The second phase required that we take a behavioral management approach to both pen design and evaluation. Using knowledge of cynomolgus macaque natural behavior, we ensured the area where NHPs would be handled for study was also the most desirable in terms of structure and enrichment offered. Our last phase of planning involved receipt of animals into our pen prototype, as we defined how quarantine, general husbandry, and study procedures would occur applying some basic tenets of operant conditioning. The defined set of practices that accompany the pen design has resulted in a highly functional caging system that will facilitate efficient and safe handling of NHPs for toxicology studies.

PS54 Use of Visual Barriers by Breeding Groups of Long-Tailed Macaques (*Macaca fascicularis*): An Indication of Color or Location Preference?

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Visual barriers have been shown to be valuable environmental components of nonhuman primate housing to break visual contact between conspecifics as well as between primates and their human keepers. The effect of visual barriers can be to reduce aggression and stress in complex social housing contexts. At our institution, visual barriers are an important component of the environmental

enrichment program. They are provided as items within the cage (for example, 'tents' and barrels) and as panels on exterior and dividing cage sides. This investigation aimed to determine if animals had any preference for different visual barriers based on their location or color. The distribution of individuals was examined in relation to the visual barrier panels in 12 breeding groups of long-tailed macaques with an average of 1.5 adult males, 28.81 adult females, and 32.03 unweaned infants per group. Measures were taken to control color/location variables across the different cages. The distribution of individuals was recorded using scan samples at 2-min intervals during 31 20-min sampling periods (total: 620 min, 310 scans). Analyses indicate a preference among adults for the orientation of visual barrier panels ($P = 0.001$), a preference for panels located next to corner perches ($P = 0.002$), and no clear preference for green, orange, or yellow visual barriers ($P = 0.094$). Bright colors in primate housing provide valuable sensory stimulation. This study indicates that which particular colors are used on visual barriers is likely to be less important than locating them adjacent to other key behavioral features, such as rest areas.

PS55 Construction of a Rodent In Vivo Research Facility to Study Cellular Stress Responses in Aging-Associated Diseases

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Our institution is conducting research into molecular mechanisms behind the aging process. we seek to identify points for possible therapeutic intervention for the whole spectrum of aging-associated diseases such as cancer, diabetes, and neurodegenerative disorders. 'Model organisms' such as the mouse allow us to gain knowledge about functions and processes which could be transferable to humans. The German federal and state Excellence Initiative allowed funding of the research center harboring 6 core research areas. An integral part of this building is a state-of-the-art animal research facility (in vivo Research Facility, ivRF) fulfilling needs for researchers, animal care technicians, and animal welfare. The 32,000 ft^2 facility comprises 5 independent SPF barriers each equipped for 4,000 mouse IVC cages. The core of the facility is a central fully redundant automated cage processing unit, where robots handle up to 20,000 cages per week during regular working hours. Attached to the animal facilities is a large transgenic core facility servicing assisted reproductive techniques, cryoconservation of embryos and sperm, as well as the production of transgenic mice via embryonic stem cells, DNA, or endonuclease mediated RNA. Planning, design, launch, and operation will be presented, as well as conceptual integration of the ivRF into the institutional research platforms.

PS56 Use of Cuttlebones to Reduce the Incidence of Feather Pecking in Chickens

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Feather pecking is a maladaptive behavior where chickens (and other poultry species) peck the feathers of conspecifics and damage the plumage and frequently the skin. It is one of the most common behavioral problems in chickens today and can lead to considerable welfare concerns. Numerous preventative measures have been discussed, with none eliminating or alleviating the problem to a significant degree. Here we present data suggesting that the addition of cuttlebones to the cage at an early age can potentially reduce or eliminate this behavior in laboratory housed chickens. Four groups of day-old chicks were received into our facility over a period of approximately 1 y. The first 3 groups experienced an incidence of feather pecking of 2.6 % ($n = 38$), 15.4 % ($n = 26$), and 55.6 % ($n = 45$). At this point the decision was made to add cuttlebones to the cages 5 to 7 d after their arrival. The incidence of feather pecking dropped to zero percent (group 4, $n = 35$) after cuttlebones were introduced. While cuttlebones have been used as a source of calcium for pet birds, to our knowledge this is the first time they have been reported as enrichment in a laboratory setting to alter pecking behavior in chickens. Cuttlebones will be given to subsequent groups of incoming chickens to gather additional data and verify or disprove these findings. Further testing should also be done to see if this nonexperimental variable alters the chicken's physiology in a way that might affect data and results.

PS57 From the Kennel to the Couch: The Transition of Dogs From Research to Home

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Adoption of research dogs to private homes is increasingly common; however, the transition can be stressful. Our teaching dog program prepares former laboratory dogs for adoption by introducing challenges experienced in a home setting. All dogs were donated from other research institutions and were purpose-bred for research from Class A vendors. An integral part of the teaching dog program is designed to enable a hands-on experience for veterinary students to learn important clinical skills while preparing the dogs for successful adoption to a home. Although the dogs generally have gentle dispositions and are accustomed to handling because of their previous research experience, they are unfamiliar with leash walking, house-training, and the use of stairs. Our program includes daily outside group play among the dogs for socialization, leash walking by students and staff, and frequent handling by our animal care staff. First year veterinary students are required to work with the dogs 3 to 5 d/wk as part of the Human Animal Relationships course. The students leash walk the dogs and train them in basic commands such as sit, stay, and lie down. While on walks, the dogs are encouraged to eliminate on leash, which is found to be one of the most difficult challenges in the transition to an adoption home. In an effort to evaluate the success of adoption, we created a survey that was sent out to more than 20 owners of adopted teaching dogs. In the survey, 56% of owners said it took less than 2 mo to house-train their adopted dog while 25% said they are still not fully house trained. We believe that the teaching dog program has greatly assisted the new owners with house-training. We will continue to gather feedback from owners and will modify the program to improve the adoption process for future dogs. Our adoption program has been around for more than 15 y and more than 80 teaching dogs have been successfully placed in forever homes.

PS58 Effect of Red Light Exposure at Night on Circadian Metabolism and Physiology in Rats

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Early studies on rodent species showed that short-term exposure to high-intensity (above 70 lx), long-wavelength light (red-appearing) influences circadian neuroendocrine and metabolic physiology. Since experimentation with light wavelengths above 600 nm is uncommon, particularly during the animal room dark phase, researchers and animal care personnel assumed there is little to no impact of long-term red light exposure at night (rLEN), such as that passing through red-tinted animal room observation windows or that of nighttime safety lighting. In the present study and in conjunction with our GLAS-supported investigations we examined how transmission of low intensity, rLEN affects rodents' daily rhythmic nocturnal melatonin signal, thereby altering temporal coordination of normal metabolic and physiologic functions. Male Sprague–Dawley rats (Crl:SD[SAS]; n = 12 per group) were maintained in an AAALAC-accredited facil-

ity on either control (C), 12:12-h light:dark (300 lux; 123.0 mW/cm^2; lights on 0600 to 1800 or experimental (E), 12:12rLEN-h light:dark (8.6 lux; 3.5 mW/cm^2 [within cage at eye level] during the dark phase; red safety light) lighting regimens. The red safety light was adjusted to simulate conditions imposed by red-tinted observation windows. After 1 wk, animals underwent a series of 6 low-volume blood draws via cardiocentesis (0400, 0800, 1200, 1600, 2000, and 2400) over a 4-wk period to assess arterial plasma melatonin, total fatty acid (TFA), glucose, lactic acid, pO_2, pCO_2, insulin, leptin, and corticosterone concentrations. Results revealed plasma melatonin levels (mean ± 1 SD) were high in the dark phase (197.5 ± 4.6 pg/mL) and low in the light phase (2.6 ± 1.2 pg/mL) in C, and low in E throughout the 24-h period ($P < 0.001$). Circadian rhythms of plasma levels of TFA, glucose, lactic acid, pO_2, pCO_2, insulin, leptin, and corticosterone were significantly disrupted in E as compared with the corresponding entrained rhythms in C ($P < 0.05$). The present findings indicate that long-term exposure to low-intensity rLEN can profoundly impact the circadian regulation of neuroendocrine, metabolic, and physiologic parameters that influence laboratory animal health and wellbeing, and ultimately the outcome of scientific investigations.

PS59 Establishing Long-Term Sustainable Solutions in a Laboratory Animal Facility Housing USDA-Regulated Species

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Problem solving using Lean Management approaches has gained popularity in the laboratory animal science field for optimizing workflow and adding value to the research customer. Lean Management aims to empower employees to reduce waste and implement sustainable improvements to continuously enhance their contribution to the organization's mission. The problem solving success is highly dependent on the direct support and mentoring of animal care staff by the Animal Facility Manager. Animal care staff within the Center for Comparative Medicine (CCM) are empowered to not only identify operational deficiencies "on the floor," but as a team, thoroughly analyze the root cause to a problem prior to proposing solutions. By using a plan-do-check-act (PDCA) cycle, the Animal Facility Manager is able to guide the animal care staff in a comprehensive root cause analysis that: 1) defines the current state, 2) establishes metrics to validate the improvement, 3) develops countermeasures, and 4) tracks the implementation of a sustainable solution. In 1 y of using the PCDA Cycle, a team of 8 animal care staff identified 200 opportunities for improvement in the areas of animal welfare, safety, operational workflow, training, and cage wash practices within a facility housing nonhuman primates, swine, sheep, and canines. These opportunities have resulted to-date in 13 long-standing solutions and over 150 small-scale process improvements. These small-scale improvements or Just-do-its (JDIs) are completed immediately upon identification by the team. By establishing goals, collecting metrics and analyzing outcomes through Lean's PDCA Cycle, these long-term sustainable solutions have improved the quality of care provided to the USDA regulated species housed in our animal facility.

PS60 Improving the Weighing Process of Young Swine Using Lean Problem Solving Tools

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At our facility, livestock are weighed within 24 h of arrival to record initial body weight. To weigh young swine, 7 to 11 kg, animal care staff carry them to a platform scale located at the opposite end of a long corridor from their housing room. During this process, they often vocalize, defecate, and urinate while being carried and it is difficult to keep the piglets centered on the scale during weighing. Once piglets are returned to the animal housing room, any urine or feces deposited in the hallway has to be cleaned. The piglet weighing process was determined to be time-consuming, inefficient, and stressful on the newly arrived animals. In order to improve this process, all staff required to perform this task met and listed all the issues with the current process. They determined possible improvements using a lean problem-solving sheet to explore all the issues experienced during weighing. The goal was to decrease handling time and minimize stress. The staff tested the solutions one-by-one using the plan-do-check-act (PDCA) method to determine the effectiveness of each solution. Two solutions were trialed that proved ineffective. The third and most effective solution was a custom made portable weighing station set up directly in the animal's pen. The piglets are placed in a large canvas support and then placed on the hook of a hanging scale that is attached to a metal rod resting on the top of the pen. Piglets do not vocalize or struggle when in the support. The new weighing process, has decreased piglet handling time, the number of staff required to weigh piglets, and most importantly, improved animal welfare by decreasing the stress on the newly arrived animals. The PDCA method led the staff through planning, executing, checking, and improving or rejecting possible solutions.

PS61 Refinement of the Rabbit (*Orytolagus cuniculi*) Paracentesis Model of Acute Ocular Inflammation

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The rabbit paracentesis model of acute ocular inflammation is well established and is a standard testing paradigm to evaluate the ocular antiinflammatory effects of drug candidates. In our model, aqueous humor levels of total protein and prostaglandin E2 (PGE2) are measured as indicators of acute inflammation. Investigators typically perform paracentesis in conscious rabbits after application of a topical anesthetic choosing not to employ general anesthesia or preemptive systemic analgesics out of a concern the inflammatory response will be altered. Literature addressing this concern is limited. We investigated the effects of 2 common general anesthetics, ketamine/xylazine and isoflurane and a systemic analgesic, buprenorphine on this model. Two studies were performed using 3 groups of 4 adult New Zealand white rabbits. In the first study, group 1 consisted of topical anesthetic only, group 2 received ketamine/xylazine in addition to topical anesthetic and the third group was administered isoflurane in addition to the topical anesthetic. A second study was performed to further evaluate results of the first study and to test the effect of preemptive buprenorphine. In the second study, groups of 4 rabbits were placed into a topical anesthetic only group, a ketamine/xylazine group and a preemptive buprenorphine group. Results indicated that there was no significant difference in total protein or PGE2 levels between isoflurane, buprenorphine or topical anesthetic only groups. Ketamine/xylazine results were inconclusive. Results show that neither isoflurane nor preemptive buprenorphine when used in conjunction with a topical anesthetic affected the acute inflammatory response in this model.

PS62 The Art of Artificial Seduction: Training New Zealand White Bucks to Mount a Phantom and Development of an Inexpensive Artificial Vagina

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An animal use protocol was approved by the IACUC for a semen collection procedure on New Zealand white bucks. Current methods of semen collection include mounting a teaser animal (anesthetized or awake) and diverting the buck into an artificial vagina or electroejaculation under general anesthesia. These techniques, however, come with risks to the animals; complications from general anesthesia range from hypothermia to respiratory distress and possible cardiac arrest. During procedures using teaser animals, there is an

increased risk of fighting between animals and a risk of impregnating the doe if the buck is not diverted correctly. The objective of the study was 2-fold; to train the bucks to mount an inanimate object (phantom) and to develop an inexpensive artificial vagina fashioned with laboratory materials. Knowing that bucks are scent driven and often exhibit sexual excitement when females are present, we placed rolled towels in the cages of does; they enjoyed playing with the towels and would mark their scent with face rubbing and urination. The towels were then placed in the cages of the bucks. The scent of the females initiated the mounting behavior which was rewarded with treat enrichment. Once we determined it was feasible to collect semen in this manner, we were able to develop a device. The device was fashioned using a conical tube, a microcentrifuge tube, fingers of size 8.5 surgical gloves as the liner, and nonspermicidal lubricant was used to fill the gap between the liner and the conical tube. The device was heated to 40 to 45 °C prior to collection; confirmed via infrared thermometer. Initially, the phantom was too unstable and was difficult to position properly, leading to unsuccessful collection attempts. We altered the technique and draped the phantom over a para-aramid synthetic fiber sleeve-protected–arm for easier manipulation of the device, which led to successful sample collections. We were able to collect from bucks daily, greatly reducing the number of animals needed to supply the investigator with fresh semen. Furthermore, the quality of the semen was great, and there were large numbers of sperm with high motility. In conclusion, the development of the semen collection procedure and artificial vagina was successful.

PS63 Early Pregnancy Determination and Daily Observations of Fetal Development via Ultrasonography in Dutch Belted Rabbits

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Manual palpation has been the predominant method of pregnancy determination in rabbits, typically practiced 10 to 14 d post copulation (PC). With the modern technique of ultrasonography, it allows producers to detect pregnancy significantly sooner. A sample size of $n = 21$ does were evaluated from a Dutch Belted breeding colony to determine the earliest date of pregnancy detection by ultrasound and to chronicle fetal development. The does were naturally bred 14 d apart in 2 breeding groups: group 1, $n = 11$ and group 2, $n = 10$. A linear array 13-6 MHz transducer was placed in the area between the pubic bone and most caudal teat line. As gestation progressed, the positions of the developing fetuses were observed to move more cranial. Group 1 was evaluated daily from day 7 PC through day 28. Group 2 was evaluated from day 5 PC through day 28, with 3 does in the group being evaluated on day 5 and 6 PC. Day 5 PC images did not accurately confirm pregnancy in all does, but 2 does presented clearly visible vesicles. For all does evaluated on day 6, there was 100% pregnancy confirmation. Day 6 PC vesicles measured an average size of 0.40 cm in diameter, increasing to an average of 0.55 cm in diameter by day 7 PC. Images were captured daily until nest boxes were placed into their enclosures on day 28 PC. Clear images and detailed guidelines of ultrasound techniques were documented in an attempt to make ultrasonography a more easily accessible method of pregnancy determination for rabbit producers. Daily observation of fetal development allowed for examination of key aspects that could be useful for future producers to determine gestational age and evaluate fetal health. This report demonstrates that pregnancy can be determined as early as day 5 PC, and reliably at day 6 PC, as opposed to earlier reports that stated the earliest date for pregnancy detection via ultrasound was day 7 PC.

PS64 Ultrasound-Guided Vessel Catheterization as a Refinement Technique for Swine under Prolonged Anesthesia for Extracorporeal Life Support Device Evaluation

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Swine are often used for evaluation of biomedical devices due to their anatomic and physiologic similarities to humans. Female Yorkshire-cross farm pigs (75 to 90 kg) were used to compare pulsatile versus nonpulsatile flow of a novel extracorporeal life support (ECLS) device in anesthetized swine over a 24-h period. A challenge for this study was establishing and maintaining reliable venous and arterial access for delivery of continuous intravenous anesthesia, drug and fluids, and monitoring of blood gases, central venous (CVP) and arterial pressures over 24 h. Auricular vein catheters could not be maintained for 24 h and were unsuitable for measuring CVP. Central venous and arterial catheterization in large swine is often performed by surgical incision and dissection due to the deep location of the jugular vein and femoral artery. Surgical isolation of the vessels was problematic in this study due to continued bleeding from the surgical incision and catheter sites secondary to the anticoagulation required for the study. In addition, surgical dissection results in increased tissue damage and activation of inflammatory pathways which introduced a confounding variable for the research goals. Previously described blind percutaneous catheterization techniques in pigs were not practical due to the large size of the animals. A published method for percutaneous catheterization of pigs using ultrasound guidance suggested it may not be applicable to swine with body weight greater than 50 kg. Despite the reported limitations, we were able to successfully catheterize the jugular vein and femoral artery via the Seldinger technique using ultrasound guidance. Ultrasound-guided catheterization avoided a surgical procedure and associated complications, and consistently yielded venous and arterial access with catheter patency maintained over the 24-h study period. Based on our experience, ultrasound-guided catheterization is an important refinement technique that should be considered in all large animals where target vessels are not easily accessible.

PS65 Development for a Cotton Rat Model for Influenza and *S. aureus* Coinfection

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In 2009, a novel H1N1 virus caused the first influenza pandemic in 40 y, killing an estimated 284,400 people worldwide. The exact effect of secondary bacterial disease is not clear, but in severe cases, bacterial pneumonia was involved in 25% to 50%. *Staphylococcus aureus* was one of the most common etiologies of secondary bacterial pneumonia. A rise in the incidence of methicillin-resistant *Staphylococcus aureus* (MRSA) has increased the mortality rate of influenza coinfection. Of the many animal models available to study this disease, we chose the cotton rat (*Sigmodon hispidus*) for its similarity to human pathophysiology, and for its ability to replicate both influenza and MRSA. Cotton rats (6 to 8 wk old) were intranasally challenged with different concentrations of MRSA SF8300 or influenza A/CA07/2009 H1N1 and sublethal concentrations were determined: MRSA (1×10^9 CFU/rat) and influenza (7×10^5 $TCID_{50}$/rat). In the next study, groups of cotton rats were intranasally challenged with either influenza followed 1 d by MRSA infection, influenza alone or MRSA alone. Survival rate, weight loss, and clinical signs were monitored. MRSA and influenza titers were measured in lung homogenates at day 2. Survival in the influenza and MRSA coinfection group (5 of 6) was comparable to influenza alone (6 of 6) or MRSA alone (6 of 6). Coinfected cotton rats exhibited significantly greater weight loss (15%) compared with influenza alone (5%) or MRSA alone (8%). Clinical signs including lethargy unbalanced gait and labored breathing were increased in the coinfected group. Lung bacterial titers (6.3×10^6 CFU/lung) on day 2 were equivalent in both MRSA alone and coinfection groups. Lung viral titers (4.9×10^4 gene copies/ng lung

RNA) on day 2 were also similar in both flu alone and coinfection groups. We have established a novel sequential influenza and MRSA coinfection cotton rat model. We will use this model to evaluate therapeutic efficacy of monoclonal antibodies and vaccines.

PS66 Spontaneous Cholelithiasis in a Squirrel Monkey (*Saimiri sciureus*)

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A pair-housed, 14-y-old, 600-g female squirrel monkey (*Saimiri sciureus*) was noted to have moderate weight loss and thinning of the haircoat, but no other abnormalities, during a routine semiannual examination. Blood collected for a serum chemistry panel revealed elevated liver enzymes. Repeat physical examination and serum chemistry panel 6 mo later showed progressive weight loss, marked increases in liver chemistry values and hypoalbuminemia: ALP 602 IU/L, ALT 301 IU/L, AST 233 IU/L, GGT 830 IU/L, cholesterol 216 mg/dL, and albumin 2.6 g/dL. An abdominal ultrasound revealed echogenic, shadowing debris in the gallbladder, consistent with cholelithiasis. No mineral opacity in the cranial abdomen was visible on abdominal radiographs, which suggested the choleliths were predominantly composed of cholesterol. The squirrel monkey was placed on a daily oral supplement containing 90 mg S-adenosylmethionine/9 mg silybin for hepatic antioxidant support. While squirrel monkeys on a high-fat diet have been used as a model for cholesterol gallstones, the squirrel monkeys at our institution are fed a commercially available biscuit diet containing 5% to 6% fat and 75 ppm cholesterol. As this diet is well below the fat and cholesterol levels previously reported to induce cholesterol cholelithiasis, this is the first report of spontaneous cholelithiasis in a squirrel monkey, to our knowledge. Fecal samples from the squirrel monkey were PCR positive for *Helicobacter* spp., and 16S rRNA sequencing revealed 98% similarity to the enterohepatic strain *H. macacae* first isolated from a rhesus macaque. Although enterohepatic *Helicobacter* spp. have previously been associated with cholesterol gallstone formation in C57/LJ mice, we believe the positive *H. macacae* status requires further investigation, as follow-up diagnostic testing has found other *Helicobacter* spp. positive squirrel monkeys in our colony. At this time, the etiology of the cholelithiasis is unknown and the squirrel monkey remains stable with medical management.

PS67 The Use of Budesonide in Marmoset Wasting Syndrome

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Marmoset wasting syndrome (MWS) is one of the leading causes of morbidity and mortality in captive marmosets. Antemortem clinical signs include weight loss, muscle atrophy, alopecia (especially at the tail base), and sometimes diarrhea. Postmortem changes most often include lymphoplasmacytic infiltration. Recent reports indicate that weight loss and low albumin levels are very sensitive and specific predictors of MWS. Thus far there has not yet been a reliable treatment for MWS. Glucocorticoids are arguably the most effective treatment for inducing remission in humans with inflammatory bowel disease, which also is histologically characterized by lymphoplasmacytic inflammation in the small and large intestines. Budesonide is a glucocorticoid with few reported side effects due to the majority of it being metabolized into inactive compounds by the liver before entering the systemic circulation. After presenting with hypoalbuminemia and/or weight loss without evidence of infectious etiology, marmosets in our colony ($n = 13$) demonstrated a significant ($P < 0.05$) increase in both weight and albumin levels (relative to pretreatment values) after once per day treatment with oral budesonide (0.5 mg) for 8 wk. Treatment was discontinued after 8 wk, and at that time animals were weaned to the lowest budesonide dose that maintained remission. We conclude that budesonide is an ideal therapy to ameliorate the clinical signs associated with MWS with minimal side effects.

PS68 Self-Injurious Behavior Secondary to Cytomegalovirus Neuropathy in an SIV Infected Rhesus Macaque (*Macaca mulatta*)

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A 3.5-y-old, female, rhesus macaque (*Macaca mulatta*) was inoculated in March 2013 with Simian Immunodeficiency Virus (SIV) mac239 and presented in November 2013 for inappetance and facial bruising. Physical exam revealed a superficial skin abrasion below the left eye, bruising below the left brow and epistaxis of the left nostril. There were no significant findings on complete blood count or urinalysis. Serum chemistry showed a mild hypoproteinemia (6.5 g/dL). Skull radiographs showed no abnormalities. Urine and blood cultures were negative and a nasal flush cultured light normal flora. Differential diagnoses included infectious etiologies, self-injurious behavior, immune-mediated dermatitis and neoplasia. Antibiotic and analgesic therapy was initiated. Lack of response to treatment and observations of the animal made it apparent that the skin lesions were self-inflicted. The excoriations rapidly progressed to extend over the nose and the left palpebrae became edematous. Because the animal appeared to be experiencing significant discomfort despite analgesic therapy, euthanasia was elected. Histopathologic examination revealed systemic cytomegalovirus (CMV) infection involving the facial nerves, periocular nerves, meninges and perimesenteric lymph nodes. CMV is a common infection in macaques with adult seroprevalence close to 100% in most colonies. Infection in immunocompetent animals is usually asymptomatic, but it can cause significant clinical disease in immunodeficient hosts. CMV is associated with a painful peripheral neuropathy in human AIDS patients and analgesic treatment is often unsatisfactory. Peripheral neuropathy secondary to CMV should be considered as a possible underlying cause of self-injurious behavior in SIV infected macaques.

PS69 Acute Subcutaneous Swelling in a Playpen-Socialized Male New Zealand White Rabbit

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An 18-mo-old, intact, male New Zealand white rabbit presented acutely with a firm, 10-cm subcutaneous swelling over the ventral aspect of the left thorax. Approximately 12 mo prior to presentation, the rabbit had received an ultrasound-guided percutaneous intrahepatic injection of 10^6 cells from a Shope papillomavirus-associated VX2 carcinoma cell line to establish hepatic neoplasia for evaluation of a novel therapeutic nanoparticle. Serial radiographic evaluation conducted by the investigator following the initial injection had revealed no evidence of primary hepatic neoplasia or metastases prior to our involvement. At presentation, evaluation by ultrasonography and radiography revealed a subcutaneous, fluid-filled structure with no body cavity communication that was most consistent with abscess formation. Concurrent aspiration of the mass yielded over 200 cc of brown to red, turbid fluid that was culture positive for *Staphylococcus* spp. The mass was excised surgically and subsequent evaluation through histopathology and immunohistochemical staining with cytokeratin characterized the mass as an epithelial carcinoma. The entire cohort of rabbits was euthanized at 18 mo postinjection for necropsy with no other individuals exhibiting clinical signs associated with neoplasia. At necropsy, it was found that a second rabbit possessed a large abdominal mass in the region of the pancreas that was also characterized as an epithelial carcinoma. No additional

lesions were observed in the remaining rabbits. VX2 carcinoma cell lines have previously been reported to harbor papillomavirus genome and support viral replication. Typically, VX2 injection results in malignant tumor involvement in an average of 2 to 4 wk, commonly with metastasis to the lungs. The tumors found in these rabbits are phenotypically similar to the VX2 carcinoma cell line and may represent 2 cases of abnormal manifestations of the VX2 tumor injection due to the prolonged latency to tumor development.

PS70 Neurologically Induced Self-Injurious Behavior (SIB) in a Rhesus Macaque (*Macaca mulatta*)

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Self-injurious behavior (SIB) is a significant problem both in human and animal health. There is no apparent singular factor that causes the disease, and no clear reason for the varying severity of the disease. A 10-y-old male vasectomized rhesus macaque (*Macaca mulatta*) presented to clinic with a 5-y history of intermittent bouts of SIB related mostly to times when capture for routine health processing was imminent. He also had a 6-y history of hand-eye incoordination, and slow, but deliberate movements. The monkey's SIB and motor skill abnormalities were unresponsive to SSRIs (selective serotonin reuptake inhibitors - fluoxetine 2 mg/kg PO) and various sedative agents (acepromazine 0.5 mg/kg PO, diazepam 0.5 mg/kg PO or IM, midazolam 0.1 mg/kg IM). Due to the decline in his condition as exhibited by more frequent occurrence and severity of SIB, euthanasia was elected. Necropsy revealed a large (5 cm × 2.5 cm × est. 3 cm) fluid filled cyst occupying the left caudal aspect of the brain and a small (1 cm diameter) cyst noted to the right midbrain. Histologically, the cysts represented dilated lateral ventricles causing compression and distortion of the temporal lobe. Chronic internal hydrocephalus was also seen. It is suspected these brain lesions were at least partially, if not wholly, responsible for the disease severity. Studies have shown that in captive rhesus monkeys, SIBs can be related to malfunctions in the basal ganglia, which may be caused by chronic hydrocephalus, but no reports have been found describing large cranial lesions like the one seen in this case.

PS76 Characterization of Porcine Vascular Tissue and Gold Nanoparticles as a Vascular Repair Material

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Synthetic and biologic patches; the standard for cardiac and vascular reconstruction, have problems with rupture, calcification, and restenosis. Our aim was to perform an in vivo study of the feasibility, remodeling, and biologic effects of a nanostructured vascular patch to improve the effectiveness of repair materials. A porcine vascular tissue patch was conjugated with gold nanoparticles (AuNP) and evaluated to determine if enhanced integration occurred while avoiding rupture, calcification, and neointimal hyperplasia when compared with a currently used biologic patch material. Adult female Large White swine underwent a bilateral patch angioplasty of the carotids with the experimental patch on the right and a control of bovine pericardium on the left. Evans blue dye was administered before euthanasia. The patency of the arteries was checked using ultrasound and the vessels harvested. The carotid was examined grossly, with Evans blue for neoendothelial targeting, and with microscopy using trichrome and hematoxylin and eosin stains. Doppler ultrasound was performed every 3 wk to evaluate the flow rates of the blood through the carotid arteries at the site of patch implant. There was a 100% success rate of implantation and a 0% mortality rate in survival animals. All patches were patent on ultrasound. At 3 wk, regenerating endothelial cell growth was noted on the experimental patches. Histology showed normal inflammatory and healing response in all the experimental and control groups. At 9 wk, the experimental groups showed better integration with the host tissue grossly. Histology showed cellular ingrowth into the experimental patches, particularly the carotid patch and no major foreign body reactions. We demonstrate the feasibility of a novel nanomaterial vascular patch for aortic, vascular and cardiac reconstructions. There was no evidence of rupture, pseudo aneurysm, or rejection. Superior reintegration and equivalent patency was demonstrated by the use of nanoparticle cross linking. Longer studies will be conducted to further evaluate the biologic reactions to the patch material and durability.

PS77 Composition of Mouse Gut Microbiota Differs between Vendors and Host Genetic Backgrounds

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The development of next-generation sequencing techniques has revolutionized the investigation of microbial communities found on and within host organisms. As the costs associated with this methodology have decreased and throughput has increased, research on the role of the gut microbiota (GM) in host health and disease susceptibility has emerged as a rapidly expanding area of discovery. Considering the diverse range of human conditions associated with differences in the GM, it follows that animal models of disease may also be impacted by differences in their GM. To determine the composition and variability of the GM in mice of different genetic backgrounds, we performed next-generation sequencing of the gut microbial DNA collected from C57BL/6, A/J, and BALB/c mice. In addition, we surveyed mice of each genetic background from more than one vendor to determine whether host genetic background would influence bacteria toward a uniform composition at different institutions. Principal component analysis revealed that the GM of mice clusters tightly in vendor-specific profiles, and then by genetic background within each vendor cluster. In general, the GM of mice from vendor 1 comprises fewer microbial operation taxonomic units (OTUs) than the GM of mice from vendor 2. Specifically, we detected certain physiologically relevant bacterial species, including segmented filamentous bacteria (SFB), endemically in mice from certain vendor colonies but never in others. Ex vivo stimulation of splenocytes collected from isogenic mice from different vendors resulted in consistent differences in the peripheral immune tone which correlated with differences in the GM. Lastly, by rederiving genetically identical mice in surrogate dams harboring different complex GM, we demonstrated that model phenotypes may be significantly altered by the GM. These findings emphasize the need to consider the GM as a variable in animal models of disease, and rederivation as a potential cause of changes in the GM.

PS78 Reducing Stress during Carbon Dioxide Euthanasia in Rodents

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Euthanasia is by definition intended to result in a 'good,' and by extension, a minimally stressful death. Here, the primary objective for this study was to measure the stress response during CO_2 euthanasia under 6 different conditions: low light, bright light, anesthesia with isofluorane prior to euthanasia, no anesthesia, CO_2 instillation flow at 30% chamber volume per minute, and instillation at 70% chamber volume per minute. The hypothesis was that CO_2 euthanasia in a dark environment, with preanesthesia, and a 30% rate would result in the least stress response prior to death. A total of 108 animals (n = 6 per treatment group replicated 3 times) were used. During euthanasia, behavioral responses were measured, and blood was collected immediately following euthanasia for serum corticosterone measurement. An elevated activity level during introduction of isofluorane compared with CO_2 alone was observed, and fewer stress-related behaviors (digging, pawing at face, quick movement,

etc.) were observed with high-flow CO_2 protocols compared with low-flow. There was a slight increase in serum corticosterone levels in animals euthanized under the high CO_2 flow rate (average 45.3 ng/mL increase), and levels in animals euthanized under low-light conditions were lower, by an average of 32.1 ng/mL, than animals euthanized under bright light conditions. The data indicate that high-flow CO_2 protocols may result in increased stress despite animals showing fewer stress-related behaviors and decreasing the interval to unconsciousness. In addition, euthanizing rodents in low-light conditions may help decrease stress experienced with CO_2 euthanasia.

PS79 Natural History of Gastrointestinal Acute Radiation Syndrome in Gottingen Minipig: An Exploratory Study

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In the absence of supportive care, lethality from the hematopoietic acute radiation syndrome (H-ARS) in the Gottingen minipig is achieved at gamma radiation doses below 2 Gy. Additionally, in the dose range between 2 and 5 Gy, an accelerated hematopoietic syndrome occurs, characterized by villus blunting and fusion, the beginning of sepsis, and a mild, transient reduction in plasma citrulline concentration. At doses up to 5 Gy, though, the classic signs of gastrointestinal (GI) damage, characterized by vomiting, diarrhea, loss of crypts, bacterial translocation, and a drop in plasma citrulline levels, are absent. To test whether minipigs would exhibit classic gastrointestinal acute radiation syndrome (GI-ARS), prior to lethality related to cardiovascular and respiratory complications observed at H-ARS doses, we tested doses of 5 Gy and above. In this exploratory study, we followed the natural history of the minipig to evaluate the potential of this model for GI-ARS studies, and to compare the minipig response to what is known concerning human GI-ARS. Thirty-two male Gottingen minipigs, approximately 5 mo old, 9 to 11 kg, were irradiated bilaterally using gamma radiation (total body, Co-60, 0.6 Gy/min) in the dose range 5 to 12 Gy. Following exposure, the animals were evaluated for up to 10 d through observation of clinical signs, CBC counts and parameters associated with the development of the GI syndrome (including vomiting, diarrhea, bacteria translocation, loss of crypts, shortening of villi, and decline in plasma citrulline). A dose-dependent occurrence of all classic parameters associated with acute GI-ARS takes place in the Gottingen minipig exposed to gamma radiation in the dose range between 5 and 12 Gy. These results suggest that the Gottingen minipig may be a suitable model to study GI-ARS. Knowledge of the natural history, as well as determination of reliable endpoints for the different ARS sequelae, are required for drug testing. This study is the first step toward determining such requirements and toward evaluating the feasibility of using the Gottingen minipig for drug efficacy testing to treat GI-ARS. Additional studies are necessary to test radiation doses bracketing the GI syndrome and to confirm validity of endpoints for euthanasia.

PS80 Validation of Dried Blood Spot Test Collection Method for Routine Serosurveillance of Rabbit and Hamster Colonies

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The dried blood spot test (DBST) utilizes dried whole blood spot sample collection technique instead of serum for routine serology. Immune and nonimmune DBST-serum sample pairs from rabbits and hamsters were tested by using routine species-specific multiplex fluorescent immunoassay (MFIA) bead panels. The immune (known positive) whole blood or sera samples were prepared from naturally or experimentally infected rabbits and hamsters with one or more pathogens including CAR bacillus, *C. piliforme*, *E. cuniculi*, lymphocytic choriomeningitis virus, pneumonia virus of mice, reovirus-3, rotavirus-A, toxoplasma, simian virus-5, and Sendai virus. The nonimmune (known negative) whole blood or sera samples were collected from historically known negative SPF colonies. Eight positive and 8 negative whole blood samples were spotted on DBST cards and corresponding sera samples from the same animals were collected for each species. Elution of serum IgG from the individual card spots was performed 3 separate times. Each of these 3 eluted DBST-serum paired samples was run separately by MFIA with a total of 3 runs for each species. DBST MFIA data from triplicate runs was compared with the serum MFIA data to evaluate diagnostic sensitivity and specificity, reproducibility, and ruggedness. A total of 576 rabbit and 480 hamster assays were performed and analytical performance of the DBST MFIA assay including selectivity and limit of detection was found to be comparable to those obtained by serum MFIA. Diagnostic specificity of rabbit as well as hamster assays was 100% showing complete agreement between DBST and serum samples. The diagnostic sensitivity of individual infectious agents was 98% and 100% for rabbit and hamster DBST, respectively. The data from the validation study proves that DBST MFIA results are analytically and diagnostically equivalent to those with serum indicating that DBST is a suitable alternative to serum for routine serologic testing.

PS81 Metabolic Effects of Duodenojejunal Bypass in Diet-Induced Obesity Mice

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Obese diabetic patients after gastric bypass surgery show a rapid improvement of glucose control independent of body weight loss. The underlying mechanisms are not fully understood. We have developed and characterized a mouse duodenojejunal bypass (DJB) surgical model to study the effects and mechanisms of bariatric surgery on glucose homeostasis. Diet-induced obese (DIO) C57BL/6N mice underwent DJB or sham surgery and were compared with a nonsurgery control group. Mouse gastrointestinal bypass surgery is known to be technically challenging, as reported in the literature by a few groups with the survival rate typically no more than 50%. We achieved an 83% survival rate in DIO mice with our optimized procedure. DJB mice exhibited continued body weight loss of 25% to 30% for 2 wk postoperatively and then sustained the body weight up to 6 wk. The sham-operated group showed reduced body weight for 4 d, but by 2 wk postsurgery there was no significant difference in body weight between the nonsurgery and sham groups. The weight loss in DJB mice was due to fat mass loss with minimal lean mass loss. DJB mice also demonstrated increased glucose tolerance during oral glucose tolerance test (OGTT) and improved insulin sensitivity as measured by homeostatic model assessment-insulin resistance (HOMA-IR). Moreover, their plasma total bile acid pool was increased by 4-fold as compared with sham and nonsurgery groups. Primary bile acids—cholic acid and chenodeoxycholic acid (CA and UDCA)—and some taurine conjugated bile acids—taurocholic acid, tauroursodeoxycholic acid and taurochenodeoxycholic acid (TCA, TUDCA, and TCDCA)—were significantly increased, whereas secondary bile acids and other conjugated bile acids remained unchanged. Collectively, our results indicated that DJB mice displayed significant and sustained body weight loss and improved glucose homeostasis, which mimicked findings in obese diabetic patients following bariatric surgery. The data also suggested that the beneficial effects of the DJB procedure on normalizing glucose homeostasis may involve changes in circulating gut hormones and bile acids.

PS82 Characterization of Grooming Behavior in Outbred P-Rats as a Model of Trichotillomania and Dermatotillomania

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Trichotillomania (TTM) and Dermatotillomania (DTM) are body focused repetitive diseases (hair pulling and skin picking) affecting as much as 4% of the population and causing impairment in daily

function and significant distress. Women are 4 times more likely to be affected than men. The diagnostic criteria for TTM have been detailed in the Diagnostic and Statistical Manual of Mental Disorders (DSM-IV), but DTM is not as well described. The outbred alcohol preferring P-rat displays the clinical signs and behavioral characteristics associated with TTM and DTM, so we hypothesized that this outbred stock could be a valuable animal model. In this study, 172 female rats were recorded on digital media for 15 min after being sprayed with a mist of water and assessed for grooming patterns-oral, manual and scratching. Oral grooming showed significance (P = 0.0078) as a predictor of which animals would develop skin lesions. This study suggests that P-rats may be a preferred model to study TTM and DTM due to the outbred rats increasing genetic variation which mirrors the human population affected by TTM/DTM.

PS83 A Porcine Model of Dual Exchange Allogeneic Kidney Transplantation

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Porcine models of kidney transplantation (PKTx) are essential for advancing therapies for human disease, but are expensive and complicated, requiring a large team, infrastructure, and a high degree of clinical skill. In the swine, the proteins of the Major Histocompatibility Complex are determined by the swine leucocyte antigens (SLA), and SLA determines rejection or acceptance of transplanted tissues. The objective of this work was to develop a porcine model of dual exchange allogeneic kidney transplant including SLA in the criteria to ensure rejection. This model could allow researchers to test new treatment modalities to enhance kidney transplant success. The study was approved by the IACUC, and it included (n = 6) Yorkshire (YS) pigs and (n = 4) Yucatan mini swine (MS), 30 kg of body weight. Orthotopic auto-Tx (OAT) with unilateral nephrectomy was done in (n = 6) pigs. Four animals (2 YS and 2 MS) underwent dual exchange orthotopic (DEO) PKTx. Surgeries were performed via midline under isoflurane anesthesia. The left kidney was resected and ex vivo perfused (10,000 U heparin, 22.3 meq HCO_3 and 25 g mannitol) in an ice bath until the venous effluent was clear. The kidney was reimplanted in OAT. For PKTx, 2 pigs at a time had DEO-PKTx. After one organ was excised and perfused, it was stored on ice. The second kidney was then removed, perfused and stored on ice while the first kidney was Tx'd. All vascular anastomoses used 7-0 suture. Renal function was monitored with serum creatinine (cre, mg/dL). Results showed successful OAT techniques and surgery routines. For PKTx, 1 YS pair (pigs 7 and 8) and 1 MS pair (pigs 9 and 10, precre = 0.7) were performed. At 48 hr, pig 7 demonstrated hyperacute rejection and pig 8 vascular rejection. Genotyping showed nonA and weak A blood types, respectively. For MS, pig 9 showed normal kidney histology after 9 d (cre = 2.3), and pig 10 died of uremia on day 6 (cre = 19.8). Both kidneys were grossly normal with intact blood supplies. SLA genotypes were XY for pig 9 and XX for pig 10. Both were blood type A. Mean ischemia time = 75 min for 4 kidneys with SD = 13 min. In conclusion, DEO-PKTx is a viable approach with acceptable ischemia times, and OAT helped to define pitfalls. Genotyping for SLA is essential for predicting rejection.

PS84 Generation of a Coat Color Double-Mutant–Albino C57BL/6N Mouse as Embryo Donor for Efficient Chimera Generation and Germline Transmission of C57BL/6 Embryonic Stem Cells

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C57BL/6 is the preferred inbred mouse strain for the generation of genetically modified mouse models. Consequently, members of the International Knockout Mouse Consortium (IKMC) aimed to provide mutated mouse embryonic stem (ES) cells in every protein-coding gene, decided on the use C57BL/6N ES cells. However, there are limitations in the production of such mutant mice with regard to availability of suitable embryo donor strains. Donors should allow efficient embryo harvest and optimal participation of introduced ES cells in embryogenesis, as well as simple recognition of ES cell contribution to chimeras and germline offspring, ideally by coat color. Currently employed donors such as BALB/c strains, for example, display poor superovulation response, whereas spontaneous C57BL/6 albino mutants do not allow recognition of germline transmission via coat color. Aiming for process optimization and at the same time reduction of animals used in research according to the ‚3Rs of animal welfare, we developed a new phenotypic albino mouse model. We introduced, by targeted mutation in C57BL/6NTac ES cells, a point mutation (G to C) at nucleotide position 308 of the *tyrosinase* gene analogous to the mutation causing Albinism in BALB/c. In a second, independent gene targeting approach in C57BL6NTac ES cells, we deleted the 14.7 kb retrotransposon insertion in the *nonagouti* locus of C57BL/6, thereby restoring the function of this locus to produce the agouti-signaling protein causing agouti coat color in mice. Chimeras were generated by independent injection of tyrosinase- and nonagouti-mutated ES cells into BALB/c donor blastocysts and, upon germline transmission, both mutations were intercrossed to produce phenotypically albino C57BL/6Ntac-$A^{tm1.1Arte}Tyr^{tm1Arte}$ (referred to as Albino A^{++}). The superovulation response between Albino A^{++} and BALB/cJBomTac was compared by harvest of 3.5 dpc embryos. We confirmed the superior superovulation response typical for C57BL/6 strains (12.6 embryos per female). Chimeras generated by introduction of different targeted C57BL/6NTac ES cells into Albino A^{++} blastocyst host embryos could be produced at high frequency (77%), and germline transmission was readily obtained (82%). As anticipated, chimeras (black/agouti/white) and germline transmission upon mating to C57BL/6 partners (black or agouti) could be identified by coat color. Taken together, Albino A^{++} combines advantages of different embryo donor strains and therefore provides an ideal embryo host for C57BL/6 ES cells.

PS85 Aesthetics of Carbon Dioxide Euthanasia of Mice and Rats

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The 2013 AVMA Panel on Euthanasia utilized 14 criteria in their evaluation of methods of euthanasia for animals. Although most of these criteria are centered around the animal, safety of personnel and consideration of the documented emotional effect on observers or operators should also be considered. In this study, we evaluated the aesthetics of rat and mouse euthanasia using pentobarbital administered intraperitoneally or CO_2 inhaled using volume per minute displacement rates of 10%, 30%, 50%, 70%, and 90%. Volunteers were solicited from the biomedical research community to observe a random selection of 3 euthanasia methods. Each volunteer was blinded to the method of euthanasia that was depicted in their 3 recordings. They were asked to complete a survey that provided demographic information, personal experience performing euthanasia, their evaluation of the potential distress experienced by the animal, and whether they would agree to perform the depicted method of euthanasia. Over 250 people completed the survey for both rats and mice, with a minimum of 100 people evaluating each method of euthanasia for each species. Overall, respondents indicated that the use of the 10% volume per minute displacement rate resulted in increased apparent distress (P < 0.0001) as compared with the other euthanasia methods. Pentobarbital euthanasia had the least apparent distress (P < 0.0001). Most individuals reported being more likely to refuse to perform the CO_2 euthanasia with 10% volume per minute displacement rate (P < 0.0001), though there were significant differences associated with job title (leadership and research staff more likely to refuse to perform this method of euthanasia) and gender (males less

likely to refuse to perform this method of euthanasia). The comments provided by the participants offered insight into the perceived distress experienced by the animals and how that affected the observer. This study provides insight into the consideration of the effect of CO_2 euthanasia on the human observer that can guide future refinements to guidelines for euthanasia of laboratory rodents.

PS86 Simple Sample Collection for Health Monitoring

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Timely and accurate diagnosis of infectious disease in rodent colonies is critical to assuring the integrity of biomedical research. To this end, institutional veterinarians closely monitor the health of research animals through periodic systematic examination of sample groups of animals against a predetermined list of infectious agents. Traditional approaches for sample collection require euthanasia of the animal, collection of numerous tissues, expertise in sample collection, and extensive sample processing prior to shipment of the samples to a diagnostic laboratory for testing. Technological advances in diagnostic testing, most notably dried blood spot analysis and real-time PCR, have resulted in a paradigm shift in animal health monitoring by dramatically simplifying sample collection and processing. The data presented herein demonstrates that all agents of concern in rodent colonies can be detected through a combination of serological evaluation of dried blood spot samples and real-time PCR testing of oral swabs, pelt/cage swabs, and feces. Further, evaluation of exhaust debris (plenum) samples from individually ventilated caging systems provides an excellent adjunct sample for detection of colony contamination. This new diagnostic testing paradigm provides data of the same high quality as traditional approaches, allows the collection of all samples from live animals, simplifies the collection process for all health monitoring samples, and detects both current and past infection.

PS87 Successful Eradication of Pinworms from Mice with a 4-Week Fenbendazole Treatment Regimen

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A study was performed to evaluate effectiveness of a 4-wk fenbendazole treatment regimen in eradicating mouse pinworm infections. Eight weeks of continuous treatment is currently recommended for pinworm eradication. However, a 4-wk regimen would offer significant cost savings in terms of supplies, labor and research productivity. Three groups of female mice, 15 per group, that were naturally infected with pinworms were housed 3 per cage in a ventilated microisolation rack. Group 1 mice were treated with fenbendazole for 8 wk, group 2 mice were treated for 4 wk and group 3 mice were untreated controls. Fenbendazole (at a concentration of 150 ppm) was administered continuously to mice via a stable medicated product delivered via water bottle. Fecal samples and perianal tape tests were collected prior to onset of the study, at 2-wk intervals throughout treatment, and at 4-wk intervals afterward for a total of 24 wk. At each time point, feces and perianal tape tests were subjected to nonmolecular diagnostic examinations, and feces were tested by pinworm PCR assays. Additionally, cage swabs were collected and tested by pinworm PCR to determine if environmental monitoring was useful for measuring treatment efficacy. Tape tests were negative at 2 wk and fecal floats were negative at 4 wk after treatment. No pinworm DNA was amplified by PCR from feces from either treatment group at 4 wk to study end. Cage swab PCR data closely mirrored fecal PCR data. At study end, mice were euthanized for examination of the large intestinal contents for parasites. All treated mice were parasite free. Results of the study verified that 4 wk of continuous fenbendazole treatment via water bottle successfully eliminated pinworm infections. Testing feces by PCR provided the most sensitive indicator of pinworm infection followed by environmental swabs and lastly by traditional parasitologic tests.

PS88 Direct Sampling of Quarantined Mice Accurately Reflects Their Health Status and Shortens Quarantine Time

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Quarantine programs for imported mice may last up to 12 wk when the method of soiled bedding transfer to sentinel mice is used to screen for pathogens. Decreased quarantine time may result in failure to detect pathogens and placement of diseased mice into the vivarium. A 4-wk study was performed to determine if results of testing samples directly from quarantined mice would accurately reflect their health status and allow for decreased quarantine time. Female 4- to 5-wk-old outbred mice naturally infected with MHV, TMEV, MNV, MPV, *Helicobacter*, *Pasteurella pneumotropica*, furmites, and pinworms were divided among 10 cages, 2 per cage. During the second week of quarantine, samples including feces and swabs were collected from quarantined mice for testing. In a subset of the quarantined mice, blood was also collected. Sentinels were 4- to 5-wk-old CD1 female mice housed in 5 cages, 2 per cage. Each sentinel cage received soiled bedding from 2 quarantine cages weekly for 3 wk. At study end, samples from sentinel and quarantined mice were collected for comprehensive testing. Results from samples collected from quarantined mice at week 2 of quarantine correlated 100% to the infection status determined at the end of the study. Results from samples collected from sentinels revealed a lack of transfer of MNV, *Helicobacter*, fur mites, and *Pasteurella pneumotropica* and ineffective transfer of pinworms. In conclusion, testing samples directly from quarantined mice can provide an accurate reflection of their health and can be used to dramatically shorten quarantine.

PS89 Transgene Interference Leading to a False Positive Lymphocytic Choriomeningitis Virus Test Result on PCR of Exhaust Plenum Dust

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Rodent sentinel detection programs relying on dirty bedding to transfer pathogens have limited reliability to detect organisms not readily transferred in bedding. Sentinel programs are costly in animal lives, labor, cage space, and money. In considering whether to move to a new detection method, we decided to compare the results of our sentinel program with PCR of exhaust air samples taken from ventilated cage exhaust plenums. These samples provide a comprehensive testing of all cages in the rack without relying on disease transfer to sentinels. We swabbed rack exhaust plenum dust and sent the samples to a diagnostic laboratory for real-time PCR testing for a battery of rodent pathogens. The PCR detected *Helicobacter* and norovirus, consistent with the results of our dirty bedding sentinel program. The PCR also detected lymphocytic choriomeningitis virus (LCMV), localized to 2 racks in our mouse breeding colony. Although LCMV may not transfer efficiently to bedding sentinels, we had no prior LCMV-positive test results. Researchers at our facility had conducted a study in which mice were infected with LCMV to create a model of type 1 diabetes, but neither of the LCMV-positive racks had been used in the study. However, one of the strains used to create the model, the RIP-GP mouse (B6.C-Tg(Ins2-GP)34-20Olds/MvhJ), expresses the LCMV glycoprotein under the control of the rat insulin promoter, and has the DNA to encode this glycoprotein in all its cells. The starting region of the LCMV glycoprotein gene sequence is highly conserved among LCMV strains, and the real- time PCR for LCMV targets this region. We confirmed that the RIP-GP breeding colony animals were housed on the LCMV-positive racks. Buccal swab samples taken from RIP-GP mice from these racks tested posi-

tive for LCMV in the PCR assay performed on the exhaust plenum samples. Cells shed from the mice comigrated with other detected organisms into the ventilated rack exhaust plenums. If there had been actual LCMV infection present in the racks, the RIP-GP animals would have become diabetic, but no diabetic animals were observed in these racks. This scenario serves as a reminder that unexpected positive findings, especially for rare agents, should be investigated before culling rodents.

PS90 Detection of Infectious Agent Nucleic Acids on Bedding Disposal Cabinet Filters

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The ability to reliably detect infectious agents from a site where soiled bedding debris is aerosolized and concentrated could provide a timely, efficient, and convenient adjunct to traditional soiled bedding sentinel testing. We hypothesized that monitoring by PCR of debris collected from the bedding disposal cabinet prefilter (BDCP) could be used to determine if infectious agents are present within the animal facility. In the first study, 4 samples of debris were collected from the BDCP prior to the dumping of 36 soiled cages that had housed female SW mice (3 mice per cage) experimentally infected with mouse parvovirus (MPV) and mouse hepatitis virus (MHV) 9 d postinfection and 4 samples of debris were collected after soiled bedding was dumped. All predump samples were negative for MPV and MHV and all postdump samples were positive for MPV and MHV by PCR. In the second study, 3 samples of debris were collected from the BDCP prior to the dumping of soiled bedding. Five samples of debris were collected after soiled bedding was dumped from 39 cages that had housed female SW mice (2 mice per cage) naturally infected with MPV, *Mycoplasma pulmonis*, *Syphacia obvelata*, and *Myocoptes musculinus* via contact with naturally infected pet store mice for 21 d. All predump samples were negative for with MPV, *M. pulmonis*, *S. obvelata*, and *M. musculinus* and all postdump samples were positive for MPV, *M. pulmonis*, *S. obvelata*, and *M. musculinus* by PCR. Additionally, in both studies *Helicobacter* spp. DNA and murine norovirus RNA were detected in both pre- and postdump samples, as both agents are endemic in our facilities. These studies show that BDCP testing can be used to screen for a wide range of infectious agents using a single sample to monitor the whole facility. Detection of infectious agents on the BDCP could provide an early warning of contamination by excluded pathogens within a facility and prompt rapid, location-specific testing to pinpoint the extent of the infection.

PS91 Computational Flow Dynamics of Commonly Used Configurations of Euthanasia Chambers for the Delivery of Carbon Dioxide

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The 2013 AVMA Panel on Euthanasia has established a recommendation that CO_2 gas should be applied using a rate of 10% to 30% volume displacement per minute for rodent species to minimize potential pain associated with this euthanasia process. However, these low-flow rates have the potential to result on prolonged distress for the rodents as they are unable to escape from prolonged exposure to CO_2 concentrations associated with air hunger and panic. The configuration of the euthanasia chamber and the properties of the tubing used to deliver the gas to the chamber could have a significant effect on the concentrations of CO_2 in different locations of the chamber over time. We used computational flow dynamics to model CO_2 administered at the recommended flow rates (1) with or without a diffuser on the tubing, (2) directly into a euthanasia chamber or into a euthanasia chamber containing cages, and (3) with the gas inlet located on the top of the chamber or on the side of the chamber. We concluded that locating the gas inlet at the top of the chamber, with a diffuser, provides the most rapid and consistent distribution of gas. Additionally, we concluded that the use of a euthanasia chamber into which cages are placed can result in inconsistent gas concentrations within the cages, potentially increasing the time that the animals are exposed to distressful concentrations of CO_2 by more than 30 s when compared with the situation where the gas is administered directly into the chamber (or home cage) with no cages. Moreover, the animal size (rat compared with mouse) must be considered in these evaluations. We concluded that simply setting a flow meter to administer carbon dioxide at a rate of 10% to 30% volume displacement per minute will not minimize potential pain and distress. Consideration must be provided to the geometry of the euthanasia chamber, the delivery system used to administer the gas to the chamber (including location of the gas inlet), and the species that is being euthanized.

PS92 PCR Monitoring of Rack Exhaust Dust for Mouse Parasites in a 400-Rack Facility: Problems, Solutions, and Results

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PCR of exhaust from ventilated racks is a novel and sensitive method for parasite diagnosis in rodent colonies. Indeed, rack testing has the potential to replace sentinels for monitoring not only parasites but also overall colony health status. Here we report on instituting a program for rack monitoring in a large (400 rack) rodent facility. In operation for 12 y, the facility had a history of sporadic fur mite and pinworm infestations that had not been completely eradicated despite treatment of all known affected colonies. We suspected that ongoing parasite detections were related to infested colonies that had remained undiagnosed via sentinel testing. Problems encountered in establishing rack monitoring included difficulties in maintaining the regular rack washing and testing schedule needed to generate periodic health reports, ensuring that PCR-positive tests were not related to dust from previous infestations, removing old PCR-positive dust from inside racks and connecting ductwork, establishing the appropriate interval between population of sanitized racks and PCR testing, and confirming the existence of current infestations on animals from PCR-positive racks. The first 4 mo of testing targeted 114 racks from high-risk laboratories (those that collaborated widely or had previously been treated for parasites) and their contacts, and resulted in the discovery of 14 racks positive for fur mites and 3 racks positive for *Aspiculuris* pinworms. Sentinel tests from those racks had been negative for several years. Treatment of all racks associated with those laboratories followed. To date an additional 255 racks have tested negative by PCR over a 7-mo period. We conclude that sentinels do not efficiently detect rodent parasites, and a regular program of rack testing offers the possibility of complete parasite eradication using targeted treatments that minimize impact on other laboratories.

PS93 Effects of Commercial Breeding Diets on Reproductive Success and General Health

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Improved rodent production is essential to researchers requiring very specific models. “Breeding diets” higher in fat and/or protein are a common strategy to enhance reproductive success of mice. We designed a controlled study to evaluate commonly used “breeder diets” and supplements to determine if these resulted in the production of more weaned offspring. Further, we monitored and evaluated the produced offspring for 6 mo to see if there were detrimental consequences of a high-fat maternal diet later in life. We established 33 breeder pairs of C57Bl/6 mice fed one of 3 dietary strategies: our standard diet (control), a higher fat diet recommended by the manufacturer as a breeder diet, and standard diet supplemented as directed with a commercial high-fat treat. Breeders produced over

200 litters. Using several methods of evaluation, we have found that all 3 groups weaned approximately 82% of their pups with a pup production index averaging 0.16 ± 0.01 (statistically the same). Offspring from each group were imaged at day P21 and P60 using 3T MRI to determine body composition. To permit direct comparisons with treatment, gender, and litter effects with time, data were expressed as the change in percent body fat (DBF) of the animals' total body volume, and were statistically analyzed using a 3-way ANOVA. Overall, there were no statistical differences with treatment ($P = 0.285$) or gender ($P = 0.075$) in DBF. Combined, these data suggest that diet does not significantly impact body composition when accounting for gender and/or litter. However, final body weights of breeders showed a significant increase in males supplemented with the high-fat supplement ($P = 0.036$, females were increased with $P = 0.0505$). We found no significant biologic differences on CBC from breeders or offspring. Our data suggests these diets are probably a waste of valuable resources including money, personnel time, and reproduction time.

PS94 Effect of Cage Wash Temperature on Removal of Infectious Agents from Caging

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The use of evidence-based standard operating procedures in Animal Resource Centers is critical to cost containment and sustainable energy use. Cage wash facilities are historically the largest utility consumer in any animal facility. We hypothesized that the volume and force of the wash water alone would dilute infectious agents to levels below those necessary for transmission of infection, irrespective of the wash temperature. The efficacy of cage washing at 110 °F (standard building hot water) and 180 °F (steam boosted hot water) on preventing the transmission of viruses, bacteria, and parasites was compared. In the first study, 16 soiled cages that had housed mice infected with mouse parvovirus (MPV) and mouse hepatitis virus (MHV) were washed at 110 °F, 16 soiled cages were washed at 180 °F, and 4 positive control cages were not washed. All 36 cages tested positive for MPV DNA and MHV RNA prior to dumping. Little to no residual waste was present, as determined by ATP monitoring, on the washed cages indicating the lower temperature provided equivalent sanitation. More importantly, all female SW sentinel mice (1 per cage) housed in the 32 washed cages did not become seropositive for MPV or MHV, while sentinel mice in positive control cages seroconverted to both agents. In the second study, 16 soiled cages that had housed mice infected with MPV, *Helicobacter* spp., *Mycoplasma pulmonis*, *Syphacia obvelata*, and *Myocoptes musculinus* were washed at 110 °F, 16 soiled cages were washed at 180 °F, and 7 positive control cages were not washed. Infection rates in these 39 cages ranged from 61% to 97%. Female SW sentinel mice housed in the 32 washed cages did not become infected with any of the infectious agents while 71% of sentinel mice in the positive-control cages became infected with MPV and *S. obvelata* but did not become infected with *Helicobacter* spp., *M. pulmonis*, and *M. musculinus.* These 2 studies showed that cage washing at either 110 °F or 180 °F is sufficient to remove a wide range of infectious agents from caging and prevent transmission of these agents. Further, since 110 °F is often within the range of building-supplied hot water, there could be a significant energy savings if boosting house hot water to a higher temperature is not needed for effective cage sanitation.

PS95 Stability of Antibiotic Trimethoprim-Sulfamethoxazole in 3 Different Rodent Water Bottle Formulations

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The administration of antibiotics, and in general medications, in drinking water is desirable as it results in decreased handling and less stress to the animal while also saving time for research and animal care staff. However, specific guidelines regarding dosages and appropriate formulation preparations are scarce. This preliminary study determined the stability of pharmaceutical-grade trimethoprim sulfamethoxazole (TMP-sulfa) in 3 different rodent water bottle formulations: reverse osmosis (RO) water, acidified water (AW), and sweetened water gel (SWG) suspension. Water bottles containing 490 mL of RO water ($n = 3$) or AW ($n = 3$) or 495 mL of SWG ($n = 3$) were prepared with 10 mL of TMP-sulfa (200 mg of sulfamethoxazole and 40 mg of trimethoprim per 5 mL) per RO or AW bottle and 5 mL of the antibiotic per SWG bottle for a total of 500 mL per bottle. They were then hung from wire racks in standard conventional mouse cages for 1 wk. The volume used in the SWG suspension was halved due to the product's indicated ability to maintain an additive in homogeneous suspension. Sample aliquots of 5 mL were collected from each water bottle automatic watering system at different time points (0, 4, 8, 24, 48, and 72 h and 7 d) for analysis by high performance liquid chromatography. Our results showed that the concentration of trimethoprim remained steady throughout the experimental period in all 3 groups (RO water: 0 h, 19.80 ± 0.42 µg/mL; 7 d, 21.92 ± 3.32 µg /mL; AW: 0 h, 50.90 ± 18.21 µg /mL; 7 d, 44.60 ± 3.85 µg /mL; and SWG: 0 h, 93.01 ± 1.15 µg /mL; 7 d, 93.36 ± 0.31 µg /mL). However, sulfamethoxazole precipitated out in the RO water group (0 h, 59.50 ± 10.76 µg /mL; 7 d, 37.47 ± 4.52 µg /mL) and the AW group (0 h, 116.43 ± 15.62 µg /mL; 7 d, 84.66 ± 10.47 µg /mL), whereas levels were steady in the SWG group (0 h, 336.39 ± 1.32 µg /mL; 7 d, 344.69 ± 10.44 µg /mL). The results indicate that the antibiotic TMP-sulfa concentration available to the animal is influenced by compounding and can vary in different rodent water bottle formulations depending on the characteristics of the solvent.

Poster Sessions

P1 Spontaneous Development of Rheumatoid Arthritis in huTNFα Tg Mice

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Rheumatoid arthritis is a chronic inflammatory disease that affects multiple peripheral joints. A transgenic mouse with deregulated expression of human TNFα (huTNFa Tg mice) develops polyarthritis with many characteristics observed in rheumatoid arthritis patients, which supports the theory that TNFα plays a central role in development of the disease. In this study we evaluated the kinetics of arthritis development in male and female huTNFa Tg mice from weaning (4 wk old) until age of 22 wk. The progression of arthritis was assessed by clinical scoring and measuring paw swelling and body weight. Histopathologic analysis of paw and knee joints and mesoscale measurements of tissue proinflammatory cytokines were performed at 3 time points. We established that both male and female huTNFa Tg mice exhibit the onset of progressive spontaneous inflammatory arthritis at the age of 6 to 7 wk. Paw and knee joint lesions consistent with inflammatory arthritis were observed in 12-, 16-, and 20-wk-old transgenic male and female mice but not in age-matched control mice. Proinflammatory cytokines, such as IL1b, KC/GRO, and IL6 were significantly elevated in paw joints of all the transgenic mice in comparison to the wildtype controls. While the levels of IL1b, KC/GRO, and IL6 in wildtype mice were under assay detection level (< 2 to 5 pg/mL), these proinflammatory cytokines in huTNFa Tg mice were detected at 1,000 to 2,000 pg/mL for IL1b, 50 to 150 pg/mL for IL6, and 100 to 200 pg/mL for KC/GRO. The results of our study will be instrumental in designing experiments for efficacy evaluation of novel antiTNFa therapeutics for rheumatoid arthritis.

P2 Chronic Unilateral Ear Droop in a Male Yucatan Miniature Swine

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A 10-mo-old male Yucatan miniature swine (*Sus scrofa domesticus*) presented with asymmetric ear placement and a decreased appetite. Physical examination revealed a right sided ear droop, the animal tended to lean to the right side, but there was no head tilt or circling. Vital signs were normal and examination of the oral cavity and ear canals under anesthesia revealed no significant findings. The remaining physical examination findings were normal. CBC and serum chemistry analysis revealed a neutrophilic ($31.9 \times 10^3/\mu L$) leukocytosis ($37.5 \times 10^3/\mu L$) and hypoglycemia. Treatment was initiated for a presumptive ear infection with multiple daily cleanings of the external ear canal with chlorhexidine, systemic antibiotics, and analgesics. Additional food supplements and hand feedings were instituted along with gastric protectants. Although appetite increased over the next week, the ear droop did not resolve. Follow-up CBC 12 d later revealed a mild neutrophilic ($13 \times 10^3/\mu L$) leukocytosis ($20.6 \times 10^3/\mu L$). The animal was maintained on antibiotics until the study endpoint, approximately 2 mo later. On postmortem analysis, the right external ear canal was normal and the tympanic membrane was intact. On further investigation the middle ear and tympanic bulla were filled with thick, opaque, yellow purulent exudate. The abscess cultured positive for *Arcanobacterium pyogenes*. Otitis media can result from an extension of organisms from the external ear canal, from the oral cavity via the Eustachian tube, or hematogenous and has been associated with several different bacteria. Although some clinical signs were present that would indicate otitis externa/media, many of the more common associated clinical signs were not consistently seen, such as discharge, scratching, pain, and neurologic signs indicative of facial, vestibulocochlear, or sympathetic nerve involvement.

P3 Biochemical Reference Intervals of Aged *Xenopus laevis* in a Research Colony

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Xenopus laevis, the African clawed frog, is commonly used in developmental and toxicology research studies. Little information is available on aged *X. laevis*; however, with the complete mapping of the genome and availability of transgenic animal models, aged animals are increasing in research colonies. The goals of this study were to obtain biochemical parameters to establish reference intervals for aged *X. laevis* and to compare results to those from young *X. laevis*. Blood samples were collected from laboratory reared, female frogs ($n = 57$) between the ages of 10 to 14 y old. Reference intervals were generated for 30 biochemistry analytes. Data was compared with prior biochemistry results for young *X. laevis* from the same vendor. Parameters that were significantly higher in aged compared with young frogs included calcium, calcium:phosphorus ratio, total protein, albumin, HDL, amylase, potassium, CO_2, and uric acid. Parameters found to be significantly lower included glucose, AST, ALT, cholesterol, BUN, BUN:creatinine ratio, phosphorus, triglycerides, LDL, lipase, sodium, chloride, sodium:potassium ratio, and anion gap. These findings indicate that the use of previously reported chemistry reference intervals for young *X. laevis* may not be appropriate for use in aged animals.

P4 Pericardial Effusion in a 4-Week-Old Piglet (*Sus scrofa domesticus*)

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An apparently healthy 4-wk-old female Yorkshire cross piglet (*Sus scrofa domesticus*) arrived at our institution. During the 72-h quarantine holding period, she became depressed, bradycardic, tachypneic, and cyanotic with an elevated rectal temperature and weak femoral pulses. A blood culture taken at this time was negative for aerobic growth. Due to her rapid clinical decline, she was submitted for postmortem examination. On gross exam, the piglet was thin with severe, diffuse, fibrinosuppurative peritonitis, pleuritis, and pericarditis. These findings were confirmed histologically. Peritoneal, pleural, and pericardial swabs were submitted for bacterial culture and sensitivity and yielded no aerobic growth. Within the pericardial sac were approximately 2 mL of cloudy pink-yellow effusion. Cytologic examination of the pericardial effusion demonstrated a neutrophilic exudate with numerous organisms suggestive of *Mycoplasma* spp. PCR of the pericardial effusion was positive for *Mycoplasma hyorhinis*, a common cause of polyserositis and arthritis in young piglets. To our knowledge, this is the first report in which a presumptive diagnosis of *Mycoplasma hyorhinis* was made by visualizing *Mycoplasma* organisms on pericardial effusion cytology. *Mycoplasma hyorhinis* is ubiquitous in the respiratory tracts of healthy swine, and stress or concurrent illness are thought to predispose animals to developing severe systemic disease. In this case, shipping stress is the likely catalyst that led this piglet to develop clinical illness. This case underlies the importance of maintaining a strong quarantine program with close observation of animals during the quarantine holding period.

P5 Bilateral Ear Pinnae Thickening in a Rice Rat (*Oryzomys palustris*)

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An adult male rice rat (*Oryzomys palustris*) of unknown age presented for bilaterally enlarged ear pinnae. The rice rat was singly housed on pine shavings in a standard rat box with ad libitum access to irradiated rodent chow and reverse osmosis water and was part of an active breeding protocol. On physical examination, the rice rat was in good body condition but was hunched, lethargic, and dehydrated. Bilaterally, the ear pinnae were diffusely thickened and had a dark purple coloration. White, crusty debris was present along the medial aspect of both pinnae and obstructed the openings of the external ear canals. The skin on the medial and lateral aspects of both pinnae was ulcerated. Due to the severity of the clinical findings the rice rat was euthanized and submitted for necropsy. Gross examination confirmed the previous findings and white keratinaceous debris filled the external ear canal. Mild hepatosplenomegaly was also present. Histologic evaluation revealed diffuse dense infiltration of small mature lymphocytes into the dermis, epidermis, and adnexal structures of both pinnae with patchy ulceration of the skin. The spleen and liver also showed evidence of lymphocyte proliferation. Based on the histologic findings, a diagnosis of epitheliotropic lymphoma was made due to infiltrates into the epidermis and adnexal epithelium of the pinnae. Cutaneous lymphoma with epidermal tropism is an uncommon form of lymphoma which has been reported in humans and several other species. It is typically of T-cell origin and may be divided into 2 types: mycosis fungoides and Sézary syndrome. Prognosis and treatment differ between these types, highlighting the importance of differentiating between them for clinical cases. To our knowledge, this is the first reported case of spontaneous lymphoma with epithelial tropism in a rice rat.

P6 Isolation of *Trueperella pyogenes* in a Case of Thoracic and Abdominal Abscess in a Galago (*Otolemur garnettii*)

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A 9-y-old male galago (*Otolemur garnettii*) presented with fight wounds, lethargy, and weight loss following pairing for breeding. Physical examination revealed lesions consistent with bite wounds

on the right carpus, face, and chest. The wounds were cleaned and flushed with povidone-iodine solution and dilute chlorhexidine solution. Ceftiofur sodium was administered for 5 d, after which the animal was reevaluated. Due to continued evidence of infection, the wounds were cultured, flushed, and cleaned. The antibiotic was changed to cefpodoxime proxetil for 7 d; thus, clinical resolution preceded culture results. The facial wound cultures revealed growth of a pleomorphic beaded gram-positive bacilli, presumably identified as *Arcanobacterium* species, resistant to ceftiofur sodium. Culture results of the carpal wound revealed *Corynebacterium* species and unidentified mixed gram-negative and -positive bacilli. Forty-six days later, the animal presented acutely with lethargy and additional weight loss. CBC/serum chemistry panel showed an elevated WBC with monocytosis, eosinophilia, lymphopenia, elevated hematocrit, and markedly increased ALT and AST. Supportive care and antibiotic therapy enrofloxacin and penicillin G were initiated. Differential diagnoses included hepatitis or neoplasia. The animal died 3 d later and was submitted for necropsy (62 d after initial presentation). Necropsy and histopathology revealed a fistulous tract (exiting from the original site of the bite wound on the chest), which communicated with an abscess in both the abdominal and thoracic cavities. The abdominal abscess also encompassed a portion of the liver. Cultures taken of the abscess found heavy growth of *Arcanobacterium* species, most closely identified as *A. hippocolae*. Due to an unusual β-hemolysis pattern and CAMP test 16S RNA sequencing identified the organisms as as *Trueperella pyogenes.* To our knowledge this is the first case of a traumatic abscessation associated with *Trueperella pyogenes* in galagos.

P7 Efficacy of Drug Intervention in Murine Dystocia

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Dystocia, or difficult labor and delivery, is a common problem among most species. Mice involved in research are often susceptible to dystocia, which can be life-threatening to both the female and her offspring. Since many of the mice are transgenic and extremely valuable, this loss can be detrimental to a researcher. Current practice involves administering oxytocin to help promote the birthing process. However, the literature indicates that it would not prove effective because there are no oxytocin receptors in the mouse uterus. This study compares different treatment options and their effectiveness in treating dystocia, regardless of the cause. Components of the oxytocin cocktail (dextrose/ calcium and oxytocin) and PGF2α (prostaglandin F2 alpha) were our focus. Spontaneous cases of dystocia, regardless of strain, age, and cause were divided into the following groups: 1) control group, 74 mice; 2) dextrose and calcium, 34 mice; 3) oxytocin, 56 mice; 4) PGF2α, 34 mice. One-hundred and ninety-eight mice were qualified to participate in the study if one or more of these symptoms were observed: in labor for longer than 4 h, a lodged pup, a dam whose delivery was documented the previous day and still had intrauterine masses, a dam with a misshaped abdomen, or dilated but had not delivered. Each mouse received 2 to 3 injections a day until dystocia resolved or was euthanized. Regardless of the group, all mice received supportive care (LRS subcutaneously and moistened food on the cage floor) for dehydration. The dextrose/calcium group had the highest dam survival rate at 50% (17 of 34), while the PGF2α had a 41% (14 of 34) dam survival rate. The oxytocin group was the least effective treatment, and yielded a 37.5% (21 of 56) dam survival rate compared with the control group (no treatment) that had an 18.9% (14 of 74) dam survival rate. Females that received treatment were able to deliver again. Of 52 dams who resolved, 18 (35%) were documented as having a second, healthy litter. All treatments were statistically significant to the dam when compared with the control group. However, there was no significance when the treatments were compared with each other. Going forward, the use of both PGF2α and oxytocin with the combination of dextrose and calcium will be studied.

P8 A Series of Complications Following Vasectomy in an Adult Pigtailed Macaque (*Macaca nemestrina*)

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An 18-y-old male pigtailed macaque (*Macaca nemestrina*) was vasectomized in October 2012 to allow for social housing with an adult female. The vasectomy technique used in this animal involved bilateral vas deferens electrocauterization, with removal of a segment of vas deferens between cauterized ends. No postprocedure complications were noted during multiple physical exams from October 2012 to October 2013. During routine blood collection in October 2013, the animal presented with 2 cutaneous lesions of the inguinal region. The lesions were inflamed and involved the skin overlying both spermatic cords, approximately 4 cm cranial to testicles. The animal was otherwise healthy. Cytology of lesion discharge revealed erythrocytes, macrophages, cellular debris, and a large number of sperm. The animal was diagnosed with bilateral sperm granulomas. Therefore, the surrounding tissue was explored and debrided, and the animal was revasectomized using a ligation and excision technique. Biospy confirmed successful identification and removal of both testicular vas deferens segments. One week following surgery, the animal exhibited poor appetite and lethargy, and physical exam revealed bilateral firm, erythematous swellings within the region of the spermatic cords. The animal was placed on ceftriaxone and meloxicam for 1 wk, with no improvement of clinical signs. Following treatment failure, ultrasound examination of the swellings revealed hypoechoic regions surrounded by hyperechoic subcutaneous tissue, indicating possible cellulitis and abscess formation. Fluid aspirated from the hypoechoic regions contained a large number of sperm, degenerate neutrophils, cellular debris, and gram positive cocci. Aerobic culture of the fluid revealed bacteria growth. It was thus determined that the second vasectomy had failed, and postsurgical infection was also present. Bilateral orchiectomy and debridement was performed several days later, and antibiotic therapy was continued for 1 wk. Following debridement and antibiotic therapy, the infection resolved, and the animal was successfully sterilized. This case illustrates the possible complications associated with 2 vasectomy techniques used for male sterilization in captive macaque colonies.

P9 Detection of Acute *Corynebacterium bovis* Infection by Environmental Sampling of Individually Ventilated Caging Rack Exhaust Air Manifolds

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Environmental sampling for rodent pathogens is gaining momentum for the enhancement and potential replacement of traditional sentinel monitoring programs. *Corynebacterium bovis* is an opportunistic infection of nude (*Foxn1*, nu/nu) mice that spreads rapidly, and is not detected by traditional sentinel programs. We investigated how quickly postexposure *C. bovis* could be detected in nude mice by quantitative PCR swabs collected from the horizontal exhaust manifold (HEM) of an individually ventilated caging (IVC) system. We also determined if cage row position or animal housing density would have an effect on time to detection. Female nude mice were naturally infected by exposure to a soiled cage of a *C. bovis*-infected mouse. Exposed mice were then either housed singly or with 4 naïve nude mice in sterile caging. Cages of 1 or 5 nude mice were placed in the first or last cage position on the bottom row of 70-cage IVC racks. Daily sterile, dry swabs were used to serially sample the skin and oral cavity of exposed mice and the corresponding HEM for *C. bovis* detection. Rack ventilation remained consistent for the study with

supply airflow delivered at 12.3 ± 1.0 ft^3/min, exhaust airflow of 27.9 ± 2.4 ft^3/min, and 41.1 ± 1.8 air changes per hour at the cage level. Cage position on the row had a significant effect on the time required for *C. bovis* detection. The first cage position closest to the HEM required 6.7 ± 0.8 d (n = 6) as compared with 8.0 ± 1.1 d (n = 6) for the last position on the row ($P < 0.05$). The time required for mice to test positive for *C. bovis* postexposure was 4.0 ± 1.3 d (n = 12). The time to mouse infection postexposure and housing density did not have a significant effect on the time to *C. bovis* detection at the HEM. These findings suggest that HEM sampling can be used for routine surveillance of acute *C. bovis* infections in nude mouse colonies, irrespective of cage row position and housing density.

P10 Intranasal Dosing of Chemotherapeutic Agents in Rhesus Macaques

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Chemotherapeutic intranasal (IN) administration is a noninvasive drug delivery approach being evaluated for pediatric CNS solid tumors. Therapeutic drug levels to CNS tumors are reduced by the protective functions of the blood brain barrier (BBB), which limits CNS drug penetration via selectively low permeability. The interface between the CNS and the nasal mucosa provides a route, via the olfactory and trigeminal nerves, for drugs to bypass the BBB and any dose-limiting systemic toxicities associated with chemotherapy. A methodology for administration, dosing, and monitoring of IN administration in rhesus macaques using chemotherapeutic agents was explored for pharmacokinetic CNS penetration studies. Five adult male rhesus monkeys implanted with an indwelling, subcutaneous, fourth ventricular CSF reservoir system and central venous ports were utilized. Each animal was scheduled to receive, via IN administration, temolozomide (7.5 mg/kg), carboplatin (8.5 mg/kg), and perifosine (7.0 mg/kg) at systemic dose levels for pharmacokinetic analysis of CNS penetration. The macaques were anesthetized, intubated, and placed in the Mygind position. Each agent was administered in a volume of 0.5 mL per nare. The nasal epithelium was examined pre- and postadministration using a laparoscope or a flexible borescope endoscope. The positioning and procedure allowed for rapid and successful IN administration. The volume of 0.5 mL per nare is a dosing limitation only partially resolved by manipulation of the drug concentration. Perifosine was the only agent delivered IN at the total systemic dose. Measureable drugs levels were obtained in the CSF. The rigid and borescope endoscopes were both equally successful in monitoring the nasal epithelium. Moderate transient irritation was observed in the nasal epithelium without signs of clinical toxicity. IN administration and monitoring of chemotherapeutic agents in rhesus macaques was successfully accomplished. Delivery of a total systemic dose intranasally is limited by the volume and could require drug reformulation.

P11 An Electronic Mouse Dosage Calculator

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Calculating appropriate dilutions and doses for mouse injectable analgesics and anesthetics can be an intricate and time-consuming process. Determining the correct amount to administer to a mouse is dependent on several variables including animal weight, acceptable injection volume and simultaneous administration of multiple drug agents. An inappropriate dilution or a miscalculated dose can result in excessive anesthesia and or death of the animal. To simplify and streamline the process of mouse drug dilution and calculation, we have created an electronic dosage calculator in a spreadsheet for administering injectable anesthetics and analgesics to mice. By selecting mouse weight, number of animals to inject, and the desired drug or drug combination, the calculator instantly displays the necessary stock and diluent volumes to combine, along with the working concentration of the preparation. All calculations were standardized to produce a final injection volume of 0.3 mL. Users may modify the calculator to correct divergent stock concentrations and include additional drugs to the calculator. Within our Laboratory Animal Resource Center, the calculator has been field-tested by veterinary technicians on a range of mouse cases. We encourage laboratories or animal resource centers to adopt this calculator and significantly reduce calculation time and likelihood for error when administering injectable agents to mice.

P12 Retrospective Analysis of Success in Vasectomy Technique in Rhesus Macaques in a Research Facility

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Vasectomy is a simple, highly effective and minimally invasive method of contraception in primates. Various surgical approaches to occlude the vas have been employed in humans with few complications and low failure rate. Although vasectomy surgeries are not routinely practiced in veterinary medicine, this technique is more commonly performed in zoo and research settings to prevent breeding and facilitate social housing. We performed a retrospective analysis to determine the social housing outcome and surgical complications following vasectomy in a captive rhesus macaque population in a research facility. Adult male macaques can be challenging to pair due to their dominance-related behavior or aggression. At the authors' facility, one successful long-term pairing method for adult males is to pair them with an adult female. However, unwanted breeding should be prevented by sterilization such as vasectomy. Over a 7-y period, 14 male macaques in our facility were vasectomized. Of the vasectomized males, 85% (12 of 14) were successfully paired with a female. Two were unable to be paired due to aggression. The surgical technique employed in vasectomy in our institute is minimally invasive and has very few reported adverse effects. The most common method is to make a prescrotal incision to excise at least 1 cm of the vas deferens, cauterize the ends and ligate with suture material. Two males developed acute postsurgical infection: one with epididymitis and the other with dehiscence and incision site seromas. These resolved completely and without complication after antibiotic therapy. After surgery, 3 of the vasectomized males developed sperm granulomas, which is a common occurrence in humans and is a benign condition. Therefore, we conclude that surgical vasectomy can be used to facilitate social housing in adult rhesus macaques in research facilities with minimal side effects and complications.

P13 Morphologic Characterization of Bilateral Ectopic Ureters Mimicking Hydrometrocolpos in an Inbred Female BALB/c Mouse

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Congenital malformations constitute a rarity seldom diagnosed in rodent breeding colonies. This report characterizes a spontaneous case of bilateral ureteral ectopia draining into the grossly distended uteri of a female BALB/c N mouse diagnosed as term-pregnant and born from a small pedigreed stock of 30 breeding pairs (F 223). Failing to produce any offspring, the dam was clinically reassessed due to notable abdominal distension and lethargy. Careful abdominal

palpation of the uteri failed to identify any fetuses and induced vaginal urination, where skin irritation in the genital area from the dribbling urine was seen. The mouse was euthanized for necropsy to determine the cause of sterility and abdominal distention. The abdominal cavity was opened through a ventral midline incision revealing both uterine horns grossly distended. The left uterus appeared filled with hemorrhagic fluid while the right segment contained a yellow liquid confirmed as urine. The genitourinary system depicted an empty atrophic bladder lacking anatomic remnants of ureters. Both kidneys presented one tread-like duct connected ectopically to the fimbriae of the uteri consisting of a basal membrane coated with simple squamous epithelium. A discreetly congestive left kidney was also seen. Microscopically, the kidneys disclosed capillary dilatation, reflux data, and discrete tubular engorgement. The bladder architecture contained all its anatomic layers showing that the protruding mucosal lining was festooned to the lumen. The grossly dilated uterine bodies displayed atrophy of the epithelial lining and reactive changes; the layer of fibroconnective tissue was infiltrated by erythrocytes, illustrated capillary rupture and fibrinous thrombi. Also, abundant crystals consistent with urinary sediment were observed concomitantly with chronic morphologic changes leading to permanent sterility. This work may constitute the first characterization of bilateral ureteral ectopia associated to hydrometrocolpos in mice so far.

P14 More Than Meets the Eye: A Novel Congenital Anomaly in a Transgenic Rabbit Kit

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A 6-wk-old transgenic male New Zealand white rabbit kit was reported for "sunken eyes." On exam, both globes and corneas were subjectively small and pupils were dyscoric. The kit did not respond to menace nor would it navigate a novel environment; however, neither is unusual in prey species. It was clinically unclear whether or not the animal was visual. The animal was subjectively small but was otherwise clinically normal. The animal was deemed unsuitable for research use due to its heterozygous genotype and congenital ocular lesions, and a sedated ophthalmic exam was performed immediately before euthanasia. Direct and indirect pupillary light reflexes and dazzle responses were absent bilaterally. Intraocular pressures were 6 and 8 mm Hg. Direct ophthalmoscopy was unremarkable. At necropsy both ex situ globes were irregularly bilobed in shape due to marked, equatorial scleral thickening and stricture with posterior bulbous dilation/sacculation. The posterior sclera was remarkably attenuated. Gross and histologic lesions included microphthalmia, anterior segment dysgenesis, cataract; lenticular, scleral, and iridal colobomata; and severe retinal degeneration with multiple foci of lenticular metaplasia within the retina. Intraretinal formation of lentoid bodies is a rare lesion that has previously only been reported in traumatized or degenerate avian eyes. This is the first report of ocular lentoid bodies in mammals. There is no clear relationship between the lesions and the genotype; however, a role for genetic manipulation has not been ruled out.

P15 Evaluation of a Novel Nanoemulsion-Based *Feline herpesvirus* 1 Vaccine in Virally Challenged Specific Pathogen-Free Cats (*Felis catus*)

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A novel vaccine was investigated to determine the ability to effectively immunize against *Feline herpesvirus* 1 (FHV1). FHV1 is one of the most important viruses affecting cats worldwide. This virus causes an upper respiratory disease called feline viral rhinotracheitis with clinical signs including sneezing, ocular and nasal discharge, conjunctivitis, depression, and inappetence. Neonatal deaths are common. Infected cats may become latently infected, experience recrudescence of clinical signs, and subsequently shed virus. These cats act as life-long viral carriers that transmit FHV1 to other cats. Current commercial vaccines protect against clinical symptoms but do not prevent infection, and the vast majority of these vaccines are formulated using live virus. A nanoemulsion, an oil-in-water suspension, has been shown to be an effective vaccine adjuvant for a variety of human-associated pathogens. The vaccine used in this study is novel because it is formulated with a mixture of nanoemulsion and nanoemulsioni-nactivated FHV1, as opposed to live, replicating virus. The vaccine was administered via intranasal or intramuscular route to purpose-bred SPF cats every 3 wk for a total of 3 doses prior to FHV1 challenge. Each group consisted of seven cats with a third group (control) receiving saline only. We hypothesized that intranasal and intramuscular nanoemulsion FHV1 vaccine administration would be an effective means to attenuate clinical signs associated with FHV1 infection after challenge with FHV1. Clinical scoring and serum antibody neutralization titer was performed after each vaccination and after viral challenge. Animals in the intramuscular group had robust neutralizing serum antibody response prior to challenge. This was associated with significantly lower clinical scores compared with the control and intranasal group by Mann–Whitney analysis. We conclude that the nanoemulsion FHV1 vaccine may be an effective means to immunize cats against FHV1, but further optimization is warranted.

P16 Spontaneous Anesthetic Death in a Guinea Pig (*Cavia porcellus)*

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A clinically healthy, intact adult male guinea pig (*Cavia porcellus*) was anesthetized for a surgical head cap placement and cochlear implant procedure. During surgery, increased bleeding was observed, compared with other animals. Hemostasis was achieved and surgery continued. Later in the procedure, the animal's respirations ceased and resuscitation was unsuccessful. The animal was submitted for necropsy and histopathology. On gross necropsy, there was a 3.5-cm long rupture along the greater curvature of the stomach, with free blood clots and ingesta in the abdominal cavity. A focal, 2-cm nodular thickening was present in the pyloric region of the stomach. Histologic evaluation of the nodular thickening revealed marked infiltration of the wall of the pylorus with abundant adipose tissue, dissecting between smooth muscle bundles, causing focal myofiber degeneration and loss. Similar adipose infiltration was observed at the rupture site, in the colon, and small intestine. The histologic findings are consistent with gastrointestinal infiltrative lipomatosis. This is a rare lesion, not previously reported in the guinea pig, in which infiltration of normal fat within the wall of the gastrointestinal tract causes alterations in peristaltic function and compromises the integrity of the muscular wall. Loss of integrity of the stomach wall, coupled with expansion of the pyloric outflow tract, likely altered peristaltic function and/or decreased pyloric outflow in this case, leading to the gastric rupture seen in this animal.

P17 The Rabbit Fecal Microbiome: Antimicrobial Use and the Presence of Antimicrobial Resistance

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There is increasing interest in defining the impact of antimicrobial use on gut flora, especially as this relates to the development of antimicrobial resistance (AMR) over time. AMR may impact not only

animal health but also that of human handlers and caregivers. Rabbit populations are ideal for studying changes in the fecal microbiome as commercial meat rabbits are routinely fed antimicrobial supplements for enzootic enteric and respiratory conditions, while laboratory and pet rabbits are less frequently treated. This study is comparing the nature and stability over time of bacterial communities (fecal microbiome) in hard feces of laboratory, pet, shelter, and commercial meat rabbits, using high-throughput sequencing of the V4 region of the 16S rRNA gene. Pooled fecal samples from groups of weanling and adult female rabbits were collected from 25 healthy Ontario commercial meat rabbitries during both summer and winter months, as well as from 3 separate research laboratories and one animal shelter. Fecal samples were also collected from 54 healthy pet rabbits. Bacterial culture and AMR was also conducted to evaluate the presence of *E. coli* and *Salmonella* spp. isolates. Culture results demonstrated *E. coli* isolates in 92% of the samples collected, with no obvious differences in age or seasonality. However, moderate AMR was present only in samples collected from commercial farms at a level of 32%. *Salmonella* spp. isolates were identified in low numbers (5%), exclusively in commercial meat rabbits, and almost always in mature does. One *Salmonella* Kentucky isolate demonstrated moderate AMR. These results can be correlated to expression of fecal bacteria in the different rabbit populations and raise several questions regarding the role of asymptomatic rabbits as potential sources of enteric disease, how commercial farm practices contribute to development of AMR in rabbits, and whether there is the potential for cross-species and zoonotic transmission of agents to personnel working closely with rabbits.

P18 Skin Mass in an African Clawed Frog (*Xenopus laevis*)

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An adult female *Xenopus laevis* was reported for a skin mass. The frog was used for egg collection and was otherwise not experimentally manipulated. On physical exam, the frog was bright and active and had an approximately 1.5 × 0.5 cm firm, grey, multilobular, expansile mass on the left lateral abdomen. Fine needle aspirate of the mass yielded no cells and was nondiagnostic. The frog was sedated and an excisional biopsy was performed and the entire mass was removed. The frog was housed individually for observation during the postoperative period, and recovered uneventfully. The mass was submitted for histopathology and hematoxylin and eosin, trichrome, and Verhoeff–Van Gieson stains. The special stains revealed that the mass was a collagenoma, a collagenous connective tissue nevus. In humans, collagenomas show autosomal dominant inheritance and are very rare. The exact cause is unknown, but it is thought to be due to a genetic defect of the skin cells. In humans, associated conditions include cardiovascular disorders, hypogonadism, congenital exophthalmos, learning disabilities, hypertrichosis, nystagmus, café-au-lait macules, and acanthosis nigricans. Treatment is typically not indicated for the collagenoma itself, but they can be surgically excised for cosmetic reasons and usually do not recur. This frog had no signs of systemic illness, and because connective tissue naevi that are not associated with other diseases do not require any treatment, her prognosis is good. This is the first time a collagenoma has been reported in *Xenopus laevis*.

P19 Abnormal Postmortem Brain Morphology in Mice with Cranial Window Implants

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Mouse cranial window implantation surgeries are performed for longitudinal imaging studies. After a 2-wk recovery period that includes 2 d of prophylactic antibiotic treatment, mice undergo a regimen of behavior training that may last 2 to 4 wk followed by either 2-photon imaging or in vivo electrophysiology, which can last another 1 to 6 mo. Recently, it was discovered that mice euthanized 6 to 12 mo after initial surgery had abnormal brain morphology. Overall clinical health of these mice is assessed 5 d/wk by research staff and 7 d/wk by animal science staff for the entire postoperative period. Brains are collected when experiments are terminated either for research or monitoring purposes and morphologic findings are recorded for both instances. Typically, gross morphologic findings are within normal limits if implant surgery occurred less than 6 mo ago. However, if implant surgery occurred more than 6 mo, we found 46 of 48 mice had abnormal brain morphology, determined by gross examination alone, in areas that were not visible through the cranial window. No blood samples or cultures of affected areas were done at the time of collection. Only 40% of these mice had external signs of exudate surrounding the surgical site or decline in health status. Based on these findings, a recommendation is being made to not maintain these mice beyond 6 mo postsurgical implantation until further analysis is conducted (pathology, biomarkers) in order to minimize unpredictable research outcomes.

P20 Evaluating Pharmacokinetic Profile of Sustained-Release Buprenorphine in Mice Using Microcapillary Technique

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Three studies in female C57/BL6 mice were performed to evaluate the pharmacokinetic profile of sustained-release buprenorphine (SRB). A microsampling technique that collects 8 µL of whole blood in a capillary tube from the tail vein was used to collect samples. The method was used to assess the concentration of the primary metabolite of buprenorphine (BUP), norbuprenorphine (NBUP). BUP was detected at 0.5 h with at a concentration of 0.62 ng/mL and NBUP was detected at level of 0.178 ng/mL at the same time point in the first study with standard BUP dosed 0.06 mg/kg SC which established the method. BUP and NBUP pharmacokinetic profiles were evaluated for SRB dosed 1.2 mg/kg SC and both were detected at the 0.5-h time point at levels of 1.56 ng/mL of BUP and 0.055 ng/mL of NBUP. BUP was detected at each time points with a peak value of 2.68 ng/mL at 4 h after dosing. BUP was detected at the 72-h time point at a value of 0.084 ng/mL, which according to the literature is below levels that provide analgesia in other pain models. NBUP was detected out to the 48-h time point with a peak value of 0.054 ng/mL at 4 h and was detected at the 48-h time point at an average value of 0.04 ng/mL. The third study confirmed that the values detected in the whole blood samples for SRB were comparable for BUP but not for NBUP, which was not detected in plasma in this study. This data suggests that SRB should be dosed within an hour of surgery when using inhalation anesthetics and that analgesia should be closely monitored postsurgically after 48 h to ensure appropriate pain control since blood levels drop below what is considered to provide adequate analgesia.

P21 The Use of Shed Skin for Diagnosis of Cutaneous Disease in a Tentacled Snake (*Erpeton tentaculatus*)

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An adult, gravid, female tentacled snake (*Erpeton tentaculatus*) presented with swollen, discolored, yellow-white skin that diffusely involved the tentacles and extended onto the right side of the head and the right spectacle. Additionally, there were multifocal individual and small groups of dorsal scales which were raised, pale (yellow to white) and swollen. Since the snake was pregnant and had recently been shipped to the institution, a minimalistic handling approach

was instituted. The snake shed the skin within a couple of days and the skin was collected and processed for histology. Microscopic evaluation showed heterophilic dermatitis with an extensive serocellular crust including intralesional, PAS positive, septate and branching fungal organisms. A swab culture of the skin was submitted to the state diagnostic laboratory for bacterial and fungal culture. The fungal culture results were phenotypically and phylogenetically identified as *Chrysosporium longisporum* and placed in a proposed new family Nannizziopsiaceae. Postshedding, the number of lesions was markedly reduced and the snake's clinical condition significantly improved. The snake gave birth and was used in the study without treatment or complication. *Chrysosporium longisporum* has previously been known as a *Chrysosporium* anamorph of *Nannizziopsis vriesii.* It is an environmental opportunist, associated with stress, and clinically presents with fatal cutaneous mycosis in reptiles. In this report, the stress of shipment most likely precipitated the infection, but with appropriate husbandry and subsequent shedding, the infection markedly improved. The report shows the ability to use the shed skin for a noninvasive diagnostic evaluation of dermatitis in a snake.

P22 Prevalence of *Dirofilaria immitis*, *Borrelia burgdorferi*, *Ehrlichia canis*, and *Anaplasma phagocytophilum* Infection in Purpose-Bred Dogs Maintained in Sheltered Housing at a Commercial Production Facility in Central Virginia

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The vector-borne canine disease agents in dogs of most concern to the veterinary community in the United States are *Dirofilaria immitis*, *Borrelia burgdorferi*, *Ehrlichia canis*, and *Anaplasma phagocytophilum*. The risk of infection by these agents is increased in the purpose-bred dog when housed outdoors. Minimizing the exposure of dogs housed in sheltered housing to the vectors of these agents requires both environmental facility management as well as the use of antiparasitics. All colony animals are treated with ivermectin at least monthly to prevent heartworm disease. Further steps are taken to control vectors through management of water in and around animal housing. Routine serological screening is performed to evaluate the efficacy of such measures to mitigate vector exposure at this commercial production facility. Evaluation of 2,156 serological tests conducted over a 3-y period for antigen of *D. immitis* revealed no positive results (0 of 2,156). Similarly, of the 314 serological tests performed over the same time period for antibodies to *B. burgdorferi*, *E. canis*, and *A. phagocytophilium* no positive results were identified (0 of 314). The lack of disease in this closed production colony also correlates with the low prevalence of disease in this region of central Virginia. The reported number of dogs testing positive for *Ehrlichia* spp. was 361 of 1284; 12 of 1284 for *A. phagocytophilium*; 120 of 1284 for *B burgdorferi;* and 25 of 1284 for *D. immitis* in Cumberland county, Virginia for the same time period. These data suggest that a risk-based prevention plan can be effective to maintain a colony of sheltered-housed dogs free of vector-borne diseases.

P23 Cytokine Profiling by mRNA Multiplex Technology in the Cotton Rat Model of RSV Vaccine-Enhanced Disease

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The cotton rat *Sigmodon hispidus* is an important model of RSV vaccine-enhanced disease. Enhanced disease in the cotton rat presents with alveolitis, consisting primarily of neutrophil infiltrates. The effect of FI-RSV immunization is associated with an increase in Th1 and Th2 type cytokines. We wanted to determine whether a hybridization-based multiplexed mRNA assay, based on direct quantification of cytokine mRNA targets can be used to predict the occurrence of RSV vaccine enhanced disease in this model. Probes for the detection of the following cytokines were included: IL1b, IP10, IL6, MCP1, MIP1b, IFNγ, GROα, TNFα, IL4, and IL10. Female cotton rats (n = 4 per group), were immunized intramuscularly with FI-RSV or FI-mock control, and lungs were harvested and processed for histologic and cytokine analysis at 1, 2, 3, and 4 d postintranasal virus challenge with RSV. For cytokine analysis RNA was isolated from one lobe of the lung and the remaining tissue was homogenized to be tested in ELISA assays (IL6, IFNγ, IL4, and TNFα). For the histologic analysis (day 4), half of the lung was inflated with formalin and hematoxylin and eosin staining was performed. The other half of the lung was processed for virus titer determination via plaque assay. mRNA expression levels for IL6, IFNα, IFNγ, and IL4 were upregulated in the lungs of FI-RSV immunized animals compared with the FI-mock immunized cotton rats. IL6 mRNA expression was higher in the FI-RSV group at all time points tested. IFNα, IFNγ, and IL4 mRNA expression was primarily detectable at days 3 to 4 post challenge. These results correlated with increased cytokine levels measured by ELISA and enhanced cellular infiltrates in the alveolar space 4 d postinfection in FI-RSV immunized animals. Hybridization-based multiplexed mRNA analysis is therefore a quick and efficient method to complement histologic determination of disease enhancement when testing novel RSV vaccine candidates.

P24 Prevalence and Incidence of Cataracts in a Population of Alloxan-Induced Diabetic Yucatan Miniswine

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Cataracts as a consequence of chronic diabetes is considered a leading cause of legal blindness in humans in the United States and is also observed frequently in aged diabetic populations (> 65%). The objective here is to assess postinduction (PI) onset of clinical ocular cataract(s) in a colony of 266 castrated, male, diabetic, Yucatan miniature swine. Diabetic miniature swine were routinely screened by the veterinary staff for clinical ocular abnormalities including visible 'mature' cataracts. Over the course of a 6-mo period, the prevalence was 30% (80 positive of 266 animals). The most recent incidence (past 2.5 mo) was 20.4% (38 positive animals with 60 affected eyes from pool of 186 previously negative animals). Eighteen animals had bilateral and 20 animals had unilateral cataracts (OD: 31; OS: 29). Cataract onset ranged from 2 to 19 mo PI with an average of 11 mo PI. Cataracts were detected earlier in animals when euglycaemia was intentionally less controlled, which supports the current predominant theory of glycation-induced cataract development. Interestingly, swine unlike human are not capable of glycating their hemoglobin due to the lack of penetration of glucose into the red cells. Miniswine with cataracts appear to function acceptably well despite the assumed visual handicap by relying on other senses. Diabetic Yucatan miniature swine commonly manifest with cataracts on average at 11 mo postinduction. Insulin regimen and glucose control are strong factors in the prevalence and incidence of cataracts in diabetic miniswine. Our data also suggests that the glycation of swine eye lenses readily occurs due to the high incidence of cataracts in diabetic animals with nonoptimal glucose control. Diabetic miniswine would provide a good model for preventative or therapeutic cataract therapies.

P25 Multifactorial Morbidity and Mortality in an Aging Zebrafish Colony

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One hundred 24- to 36-mo-old EKW zebrafish (*Danio rerio*) were relocated to a new recirculating water rack system in a different

facility and 10% presented with ulcerative skin lesions approximately 10 d later. Fish were transported in their home tank, which was placed directly into the new rack. The water quality parameters of the new rack had been previously adjusted to match that of the old rack. Differential diagnoses included secondary bacterial or protozoal infection due to water quality abnormalities or other environmental changes, chemical irritation, and neoplasia. Two affected and 3 clinically unaffected fish were euthanized and evaluated by histology. All fish had histologic changes in the liver, ranging from a single adenomatous nodule and mild fibrosis to marked hepatobiliary adenocarcinoma. The neoplastic and preneoplastic liver changes are consistent with those previously reported in aged fish housed in similar types of recirculating systems using a fluidized bed for biologic filtration. Four of the 5 fish had evidence of eosinophilic coelomitis and granulomas within the liver, pancreas, and kidneys. This type of granulomatous response is highly suggestive of acid-fast bacterial infection (*Mycobacterium* spp.), though no organisms were identified on acid-fast stain of histologic sections. Moderate, focal, subacute ulcerative dermatitis was identified histologically on the clinically affected fish, with no intralesional organisms. The clinical decline of the colony is suspected to be due to a combination of several factors: age, the acute stress of moving, and underlying chronic liver disease, which all contributed to poor immune status and lead to focal and systemic secondary opportunistic infections. Younger replacement fish were acquired and integrated into the new system without similar morbidity, supporting this conclusion.

P26 Ranges of Commonly Evaluated Clinical Chemistry, Hematology, and Coagulation Parameters From Clinically Healthy Cynomolgus Monkeys of Asian Origin

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Cynomolgus macaques (*Macaca fascicularis*), also known as crab-eating macaques, are important species in the product development of both drugs and biologics. Historically, studies conducted in cynomolgus macaques were primarily for the determination of the safety of products. With the issuance of the FDA's final rule, 'New Drug and Biologic Drug Products; Evidence Needed to Demonstrate Effectiveness of New Drugs When Human Efficacy Studies Are Not Ethical or Feasible' (21 CFR 314.600 for drugs; 21 CFR 601 Subpart H for biologic products), nonhuman primates, including cynomolgus macaques, have also become essential animal models to address product efficacy. A comprehensive set of well-documented reference values for hematology, clinical chemistry and coagulation parameters can assist in interpreting clinical pathology data from cynomolgus macaques. These values provide data essential for the characterization of the cynomolgus macaque, as is required of an animal model in support of product development under the 'Animal Rule.' In addition, reference ranges assist in evaluating the overall health status of each animal as well as serving as a baseline for evaluation/interpretation of any changes that occur due to disease and/or toxicity. The values summarized and presented in this poster are intended to provide veterinary clinicians, researchers and toxicologists with reference ranges for hematology, clinical chemistry, and coagulation parameters commonly evaluated in toxicology and/or efficacy studies. Analyses were performed using samples collected from clinically normal, healthy, naïve, young adult, cynomolgus macaques of Asian origin. Methods of collection, sample handling and analysis are described. Samples were collected and analyzed as part of multiple studies across multiple sites sponsored by the National Institute of Allergy and Infectious Disease (NIAID).

P27 Enteropathogenic *Escherichia coli* Colitis and Membranoproliferative Glomerulonephritis in a Common Marmoset

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A 6.8-y-old female marmoset with recurrent colitis was euthanized due to poor body condition and abnormal renal function (BUN = 199 mg/dL, creatinine = 1.3 mg/dL). One month before euthanasia this animal responded to enrofloxacin treatment for acute hemorrhagic diarrhea. PCR for the intimin gene (*eae*) of *Escherichia coli* performed on fecal DNA was positive and suggested enteropathogenic *E. coli* (EPEC) as the cause of the colitis. Histopathologic lesions consistent with EPEC included a lymphoplasmacytic, neutrophilic typhlocolitis with apically adherent coccobacilli. Renal compromise was due to a severe membranoproliferative glomerulonephritis and tubulointerstitial nephritis. This case prompted a colony-wide review of the frequency of clinically observed colitis and the prevalence of renal lesions in marmosets undergoing pathologic examination. Cases of hemorrhagic diarrhea enumerated between June 2012 and September 2013 revealed a monthly incidence rate of 2.7 cases/100 marmosets. Fecal cultures were negative for *Salmonella*, *Shigella*, *Yersinia*, and *Campylobacter*; however, 48% were positive for *E. coli*. EPEC was detected in 89% of fecal samples using PCR. A review of necropsy records from years 2011 to 2013 revealed a 78% prevalence of renal lesions. Renal lesion severity in recent cases was significantly greater (48% characterized as moderate to severe) when compared with earlier cases (2003 to 2005; 16% characterized as moderate to severe; $P < 0.0001$). In an attempt to directly link *E. coli* colitis to renal pathology, PCR for Shiga toxin genes *Stx1* and *Stx2* was performed on a subset of *eae*-positive fecal samples. Negative results suggested that *E. coli* hemolytic uremic syndrome was not the cause of the renal pathology. While EPEC colitis is a well characterized syndrome in the marmoset, causal factors for the frequently observed glomerulonephritis have not been elucidated. Data from a plasma metabolomics screen of 50 marmosets will be presented and may suggest biomarkers of renal disease to guide research endeavors.

P28 Reproductive Pathology in Aged Female Rhesus Macaques Administered Depot Medroxyprogesterone Acetate (DMPA) and/or Leuprolide

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In nonhuman primates, hormone therapies such as depot medroxyprogesterone acetate (DMPA) and/or depot leuprolide have been used to treat endometriosis. This study examines the pathologic lesions seen in mature rhesus macaques after treatment with monthly DMPA (40 mg/animal) and/or depot leuprolide (1.75 mg/animal) over months to several years. Six rhesus macaques are included in this retrospective study, ranging from 19 to 31 y of age. Four primates were put on leuprolide therapy, 2 of which were transitioned to DMPA 5 y and 11 y later, respectively. Two primates were administered DMPA only. All presented with clinical signs associated with endometriosis before treatment such as heavy cycling, severe abdominal discomfort, as well as reproductive failure including abortions and failure to become pregnant. Gross and histopathologic examinations of uterus of each animal demonstrated a variety of common changes such as endometrial polyps, endometriosis, adenomyosis, and leiomyoma. All animals except for the youngest (19 y old) developed atrophic endometria characteristic of menopause. Of the 6 animals, 5 had marked decidualization of the stratum functionalis and of endometrial polyps. Decidualization was so marked in one case that gross distension of the uterus was evident. Four animals demonstrated extrauterine serosal decidualization, the severity of which in one case was evident on gross exam. These changes were present in both leuprolide and DMPA treated animals, but marked in animals treated with DMPA. The necessity of long-term administration of DMPA to control clinical signs of endometriosis should be reevaluated as animals approach menopause.

P29 Minimizing Symptoms and Stress in Seizure-Prone Mice by Using Acupressure During Routine Handling

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Many laboratory mice used in research experience spontaneous seizures during cage changing and routine handling. The triggers are usually the abrupt sound and sudden motion of the cage. These seizures can range from mild to severe, often resulting in uncontrollable convulsions and possibly leading to injury or death of the animal. Animal technicians and animal health technicians have no recourse other than to observe and handle the animals more gently, but this does not stop nor prevent seizures from occurring in the future. Such seizure episodes occur on at least a weekly basis, each time the cage is changed. In some cases of seizures in humans and canines, acupressure has been shown to halt and minimize seizure severity. This study tested acupressure techniques to minimize seizure symptoms in laboratory mice. Using a stopwatch, the seizures of individual seizure-prone mice housed in our facilities were timed to establish a baseline/control. Various anatomic locations including the tail, ears, and feet had acupressure applied manually during weekly cage changing, then timed and compared for effectiveness. Mice with spontaneous seizures of various strains were tested, using each of the aforementioned acupoints. The control seizures lasted an average of 34 s. The acupressure-assisted seizures lasted an average of 23 s. The most notable display of success occurred with a mouse whose control seizure of 34 s was reduced to 9 s when acupressure was applied to the right ear. Although there was not one particular anatomic location which proved most successful over all others, analysis did reveal that using this technique was an improvement over not using it. Statistical analysis of the data resulted in a $P \leq 0.0046$ indicating strong evidence against the null hypothesis. In some cases the seizure activity stopped altogether after several weeks of testing during cage changing. This is a simple and practical technique that can be implemented as a routine response to handling seizure-prone mice and reducing the detrimental effects of their seizure episodes.

P30 What's Your Diagnosis? A Prolapse in a Research African Clawed Frog (*Xenopus laevis*)

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An adult female research African clawed frog (*Xenopus laevis*) presented with a presumed prolapsed cloaca following administration of human chorionic gonadotropin (hCG) to induce ovulation. Three months prior, the frog produced eggs normally after hCG hormone priming. Aside from the tan, tubular structure projecting from the cloaca, physical examination was unremarkable; the patient was in good body condition and had no overt signs of septicemia. Attempts to reduce the prolapse with 0.4% sugar water immersion were unsuccessful and the animal was euthanized by an overdose of MS222 followed by pithing. Differentials for the cause of cloacal/rectal prolapse in *X. laevis* include a direct effect of exogenous hormone administration, trauma associated with massaging the frog for egg collection and secondary effects of gastric overload, neoplasia, parasites or ascites. Further diagnostics were pursued to rule out environmental or infectious causes that could affect other frogs in the colony; no additional recent prolapses were identified. Measured water quality parameters of the recirculating system were within normal limits. On postmortem examination, the lumen of the 4-cm loop of prolapsed tissue collapsed, revealing intraluminal eggs. Typical ovary/egg masses were observed at celiotomy. The prolapsed segment was found to be a continuation of the oviduct and was reducible by pulling the left intracoelomic oviduct cranially. All other coelomic viscera, including the gastrointestinal tract, were grossly normal. Histopathologically, the prolapsed oviduct was partially devitalized and had abundant bacteria diffusely within the surface and internal parenchyma, with no inflammation. The nonprolapsed segment was normal with no sign of bacterial extension. Moderate diffuse necrofibrinous, histiocytic, and granulocytic pneumonia was also detected, though no etiology could be assigned, along with mild multifocal colonic epithelial apoptosis/degeneration with intraluminal ciliated protozoa. Ziel–Neelson acid fast stains of the spleen and liver were negative for mycobacteria. Our final diagnosis was oviductal prolapse with possible bacterial septicemic insult. We speculate oviductal prolapse in this frog was due to exogenous hormone administration and associated handling.

P31 Poorly Differentiated Neoplasia Associated with Polyomavirus Infection in a Zebra Finch

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An adult female zebra finch (*Taeniopygia guttata*) used in neuroscience research presented with several subcutaneous nodules, unilateral periorbital swelling, and poor body condition. The finch was euthanized due to rapid clinical deterioration, and a necropsy was performed. Greater than 20 discrete subcutaneous nodules, concentrated in the neck, thorax and wings, were evident, whereas several nodules present near the tail base and cloaca were coalescing with overlying ulceration. The nodules were solid, cream-colored, and ranged in size from 2 to 10 mm in diameter. Internal organs appeared grossly normal. Histologic evaluation of the subcutaneous nodules revealed poorly differentiated, pleomorphic, round-to-polygonal and/or spindle-shaped neoplastic cells with frequent mitosis; some of the larger cells exhibited karyomegaly with faintly basophilic intranuclear inclusion bodies and chromatin margination. Mild heterophilic inflammation was also seen within the tumor tissues. Variable degrees of neoplastic infiltration with associated inflammatory cell populations were present in the crop, periorbital skin, skeletal and cardiac muscles, intestine and mesentery. On the basis of histologic evaluation, the lesions were diagnosed as a multisystemic, poorly differentiated neoplasm. Because of the intranuclear inclusions and overall morphology, a polyomavirus etiology was suspected. Other compatible viral etiologies included finch herpesvirus, canary circovirus, papillomavirus, and avian adenovirus. A nested PCR, using broad-spectrum consensus primers to detect polyomaviruses, was performed on frozen tissue and amplified a 403 base-pair fragment; the amplified sequence had 96% identity to a canary polyomavirus isolate. A quantitative PCR based on the fragment was developed to screen fecal samples from representative finch cages. Eighteen of 49 (36.7%) fecal samples were positive on qPCR, suggesting an endemic polyomavirus infection; however, signs of disease attributable to polyomavirus infection were not present in the birds surveyed. This case represents a rare manifestation of a polyomavirus infection, and underscores the importance of monitoring research zebra finch colonies for infectious diseases.

P32 Refining Methods for Mouse Hepatic Biopsy

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The mouse is a commonly used species in liver research such as oncology, toxicology, and disease models. The ability to collect specimens of liver is vital to such research. Such studies require either a major surgical procedure or euthanasia to obtain a liver biopsy. Ultrasound-guided biopsy proves to be a less invasive

method of obtaining histologic assessments of hepatocytes. In this study, 3 groups of mice were used, an ultrasound, surgical, and a terminal group with 10 mice per group. The use of ultrasound to obtain a liver biopsy is quicker, complete set up and biopsy via ultrasound averages 15 min as opposed to the surgical method which averaged 30 min. Ultrasound biopsy technique also proved to be safer; although no animals from either survival group showed complications in this study, the US group recovered quicker than the surgical group. Another benefit was the ability to view the liver prior to obtaining a biopsy. Although not a factor in our study, this can be useful for other studies in which the condition of the liver may be a factor in obtaining samples therefore reducing the number of animals needing surgery to assess the liver. Although ultrasound-guided liver biopsy is routinely performed in other species it can be a challenge to perform in mice. The smaller size of the mouse can make it a challenge to locate the liver lobe prior to introduction of the needle. Thermoregulation in mice is also a challenge when using the US gel for imaging. Mice must be monitored closely to ensure they are maintaining appropriate and consistent body temperature. Hemostatsis is also a challenge and is most successful by applying slight digital pressure. Mice from the survival groups were monitored for 8 h postprocedure as required by the protocol and any/all complications noted.

P33 Acute Aspiration Pneumonia and Septicemia in a Rhesus Macaque with Chronic Inflammatory Bowel Disease and Megaesophagus

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A 19-y-old, 8.5-kg male rhesus macaque presented with an acute history of partial anorexia and weight loss. This animal had been clinically managed for 8 y with 1 mg dexamethasone once weekly to treat chronic inflammatory bowel disease (IBD) diagnosed on intestinal biopsy. On physical examination, the only abnormalities were marked pyrexia and moderate dental disease. Abdominal ultrasound revealed marked gastric distention and hypomotile, fluid-filled loops of bowel with thickened walls. CBC demonstrated moderate anemia with a normal leukogram. Serum biochemistry demonstrated moderate hypoproteinemia and hypoalbuminemia. Hepatic and renal enzymes were elevated along with mild electrolyte imbalances. Intravenous fluid therapy was given during sedation, and broad spectrum antibiotic and NSAID treatment was initiated. The animal's attitude and appetite improved with therapy, but on the third day of treatment, the animal was found dead. The carcass was submitted to the pathology core for necropsy and histopathology. Significant findings included megaesophagus with ulcerative esophagitis, markedly distended stomach, lymphoplasmacytic gastritis with multifocal mucosal ulceration of the pyloric stomach and diffuse, marked, lymphoplasmacytic enteritis and colitis consistent with IBD. In addition, a focal diverticulum of the proximal jejunum with partial stricture and distal duodenal distension, myocardial fibrosis, a hepatic abscess and bronchointerstitial pneumonia secondary to aspiration were noted. Bacterial cultures of antemortem blood and the hepatic abscess were positive for *Bacteroides melaninogenicus* and *alpha-hemolytic Streptococci*, *E. coli*, and *Staphylococcal* spp. Megaesophagus as sequela to the upper enteric obstruction and marked IBD contributed to this animal's anorexia and cachexia. Based on the gross lesions and histopathologic evidence, chronic immunosuppression due to steroid therapy to treat inflammatory bowel disease might have predisposed this animal to the mixed bacterial infection. Finally, the death of this animal was most likely due to a combination of aspiration of gastric contents associated with megaesophagus and septicemia due to multiple bacterial agents including *B. melaninogenicus*.

P34 Ovarian Teratoma in a Cynomolgus Macaque (*Macaca fascicularis*)

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An 8-y-old, female, cynomolgus macaque (*Macaca fascicularis*) presented for a quarantine entrance examination upon arrival to the institution. Physical examination findings and results of routine hematologic and serum biochemical analyses were unremarkable. Serial tests for tuberculosis, virus isolation for Type D retrovirus, and serology for SRV-2 and *Macacine herpesvirus I* were negative. Thoracic radiographs were considered within normal limits during exit quarantine procedures 1 mo later. On physical exam a 3-cm, firm, round, mobile mass was detected on abdominal palpation. There were no other significant findings, including results of follow-up blood work and review of daily observation sheets. Abdominal ultrasound demonstrated a round, cystic structure composed of mixed echogenic tissue. An ultrasound guided fine needle aspirate was obtained from a cystic area and submitted for cytology and culture. Cytologic examination revealed an exudate consisting of degenerate neutrophils. Aerobic culture of the aspirate was negative. An abdominal exploratory was performed and a severely enlarged right ovary measuring approximately 3 cm in diameter was identified, resected and submitted for microscopic examination. No other abnormalities were identified in the abdomen at surgery. Recovery was uneventful and to date no further clinical concerns have been reported. An ovarian teratoma was diagnosed on microscopic examination. This benign, cystic neoplasm compressed a rim of remnant ovarian tissue and contained well-differentiated tissues representing all three germ layers (ectoderm, mesoderm, and endoderm). While ovarian teratomas are not common in macaques, reports and retrospective studies in the literature suggest that teratomas should be considered a differential diagnosis for freely moveable masses in the caudal abdomen.

P35 Different Method for Fenbendazole Treatment of Pinworm

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It has been a standard practice to use fenbendazole medication to treat for pinworm infestation in rodents. The routine exercise is to purchase commercially available rodent diet with added fenbendazole. This routine results in an increase in labor and at least double the cost of regular feed. When treating large outbreaks, this increase can result in the facility running at a deficit. Our facility had an outbreak that occurred in 4 of our larger facilities over a period of 4 mo. These outbreaks affected around 5,000 rodent cages. To address this enormous cost, we started looking for an alternative treatment, using fenbendazole medication. We chose to use the medication in water. Because of the duration of the outbreak, we were able to test the water route alongside the medicated feed. The concentration in standard medicated feed is 8 mg/kg, in water we choose 15 mg/kg (stock solution of 100 mg/mL). We used hydropac pouches and added16 mL of fenbendazole medication in 9 oz of water. With a slight shaking of the pouch (performed daily, pouch is on the outside of the cage), the solution would stay in suspension for at least 2 wk, when the bag would be changed. Fenbendazole is very stable in water. We kept the animals on the medicated water for 6 wk (3 treatments). Fecal samples were taken after 4 wk (pinworm eggs can be viable up to 28 d) from completion of the treatments and tested using PCR. All animals were negative by both routes of administration. It costs US$1.75 per cage to treat by medicated feed compared with US$0.16 per cage with medicated water (this includes the labor to make the medicated water). This method greatly saves in labor and cost to the facility.

P36 Clinical Management of a Rabbit Hindlimb Ischemia Model

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Hindlimb ischemia via ligation of the femoral artery or its associated branches is the most commonly used technique for modeling human peripheral arterial disease in rodents and rabbits. Animal models vary in ischemic severity based on the location and extent of arterial occlusion. Complications with major reductions of femoral blood flow or complete excision of the femoral artery include impaired surgical wound healing, incisional dehiscence, acute and chronic pain, hindlimb paresis, self-injurious limb damage, or digit necrosis. A pilot study was performed in a rabbit hindlimb ischemia model using a variety of proximal and distal femoral ligations with the aim to optimize reduction of arterial blood flow to achieve research goals while minimizing clinical complications. Pharmacologic and nonpharmacologic modalities were employed to minimize pain and distress during the study period. Surgical wound dehiscence was commonly associated with the use of sterile skin staples and was minimized by the use of an intradermal or mattress suture technique for incision closure in combination with meticulous aseptic surgical technique. Our clinical experience indicates that incisional dehiscence or failure to appose all incision margins during surgery is difficult to repair in the postoperative period, especially greater than 12 h postsurgery. Postoperative analgesic therapy included buprenorphine and carprofen administered subcutaneously for 48 to 72 h with transition to oral NSAID therapy. Neuropathic pain management was considered but not utilized due to adequate response to NSAID therapy. Mild hindlimb paresis was observed for 10 to 14 d postoperatively and correlated with the degree of arterial blood flow restriction. Resting boards were provided in the cages and preferentially used by the rabbits. Neither self-injurious limb damage or digit necrosis was observed. Adjusting the ischemic severity while ensuring strict aseptic technique, avoiding skin staples, and using proper and timely incision apposition techniques minimized the complications of this model.

P37 Development of Lumbar Port Model in Rhesus Macaques

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Lumbar catheterization is a procedure utilized for the collection of nonhuman primate (NHP) cerebrospinal fluid (CSF), a frequent requirement in biomedical research. The procedure is percutaneous, noninvasive, and effective for single sample collection, but has proven unreliable for serial CSF collection and as a temporary procedure requires repetitive anesthesia for reinsertion. A new permanent and subcutaneous NHP lumbar port model was developed permitting reliable access to lumbar CSF in NHP with a minimally invasive procedure and eliminating repetitive anesthesia. Six adult male rhesus monkeys were utilized. A 3.5-Fr catheter and low volume port were used. The animal was positioned laterally and a skin incision made over one of the vertebral spaces between L4 and L6. An 18-gauge epidural needle was inserted and CSF flow established. A 21-gauge catheter was inserted through the needle and advanced. CSF flow was confirmed through the catheter and the needle removed. The catheter was attached to an access port, which was secured subcutaneously. Postsurgically the lumbar ports were checked to confirm CSF flow daily for 2 d, weekly for 4 wk, and then monthly. Four of the 6 animals had successful implantation with continued ability to obtain from 0.5 to 1.0 mL of CSF via aspiration or gravitational flow. The postsurgical range of ongoing duration was 4 mo to 1 y. Rapid serial CSF samples were obtained from all 4 animals. Lumbar ports were not patent in 2 animals 3 to 5 d postsurgery. Nonpatency in these animals was attributed to the position of the lumbar catheter. The NHP lumbar port model was successfully developed in rhesus macaques and is an appropriate replacement for temporary lumbar catheterization where the reliable collection of CSF is a requirement without repetitve anesthesia.

P38 Kyphosis and Muscular Dystrophy-Like Changes in a Nestin-Green Fluorescent Protein (GFP) Transgenic Mouse

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An 8-mo-old, nestin-GFP transgenic female mouse (*Mus musculus*) presented to veterinary staff for hunched posture. This animal was on an IACUC-approved breeding protocol. Physical exam revealed curvature of the spine; no other abnormalities were noted. None of the mouse pups from her litters displayed this abnormality, although another female mouse of similar age displayed hunched posture as well. Due to this undesirable trait in a breeding animal, the mouse was euthanized via CO_2 asphyxiation. At gross necropsy the mouse had marked kyphosis in the thoracic vertebrae. The skin, thoracic and abdominal viscera, and central nervous system were grossly unremarkable. Histopathology of the hindlimb skeletal muscles revealed bilateral multifocal myofiber degeneration and regeneration with variation in myofiber size. These changes in the skeletal muscle are similar to those described in mice with muscular dystrophy; however, this phenotype was unexpected in this strain. Nestin is an intermediate filament protein; with expression in adult animals limited to stem and progenitor cells. Nestin is transiently expressed in regenerating skeletal muscle, nerves, and newly formed vessels and has been used as a neural progenitor cell marker. It is possible that the GFP expression in the nestin myogenic stem cells is causing the observed pathology in skeletal muscle, possibly by disrupting their contribution to muscle homeostasis, since these mice did not sustain any injuries that would have led to widespread muscle damage and regeneration. Alternatively, the nestin-GFP transgene may have disrupted expression of another gene essential to muscle homeostasis. To the authors' knowledge, this is the first report of kyphosis and muscle degeneration and regeneration in a nestin-GFP transgenic mouse.

P39 Ketamine and Dexmedetomidine as a Maintenance Anesthetic for Nonhuman Primates Undergoing fMR and PET Imaging During Administration of Specific Nicotinic Receptor Agonist

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General anesthesia choices for nonhuman primates (NHPs) are limited, especially in studies requiring stimulation of specific neuro-receptors. We performed a NHP imaging study involving evaluation of nicotinic and dopamine receptor activity, using dexmedetomidine in combination with ketamine as a total intravenous maintenance anesthetic (TIVA) for imaging sessions lasting 4 to 8 h. Results of a literature review suggested that this regimen would have minimal impact on these receptors and provide adequate levels of anesthesia. Adult male rhesus NHPs (*Macaca mulatta*) (n = 5), were premedicated with ketamine HCl (7 mg/kg) and dexmedetomidine (0.02 mg/kg) intramuscularly, and maintained with ketamine HCl (4 to 6 mg/kg/h) + dexmedetomidine (2 to 4 µg/kg/h) intravenously for each imaging session. Respiratory rate (RR), heart rate (HR), end tidal (Et) CO_2, O_2 saturation (SPO_2), and noninvasive blood pressure (NIBP) was recorded every 5 min. Saline 0.9% was administered intravenously (5 to 10 mg/kg/h). Atipamezole HCl (0.2 mg/kg) was administered intramuscularly at the end of each session. Images were collected on an MRI scanner with a Brain PET insert. Dopamine receptor (D2) availability was assessed by measuring raclopride binding potential (BP) in the striatum (caudate and putamen). ^{11}C-raclopride was injected with 5-6 mCi activity as a bolus/infusion (50/50), followed by a nicotine challenge (0.3 to 0.5 mg/kg) 35 min later to assess changes in brain activity. This was repeated with a 2-h interval, while imaging PET and pharmacoMRI (phMRI) simultane-

ously. None of the animals in this study experienced adverse events under anesthesia or during recovery. Relevant fluctuations (during the first 30 min compared with the end of the scan session) in physiology showed only: $EtCO_2$ 41.2 ± 1.7 compared with 39.9 ± 2.6; systolic NIBP 86.5 ± 4.3 compared with 93.3 ± 4.3 ($P < 0.05$ paired t test); diastolic NIBP 53.7 ± 4.5 compared with 52.9 ± 4.7; and HR 89.4 ± 2.5 compared with 86.6 ± 2.7. Average recovery time to sternal recumbency was under 60 min after completion of imaging and atipamezole HCl injection. Average D2 BP measured using PET and ^{11}C-raclopride was compared with previous study data using either isoflurane or the awake state. Using one-way ANOVA, measurements of BP in awake rhesus monkeys (2.56 ± 0.23) were not significantly different from dexmedetomidine (3.18 ± 0.14) whereas it was significantly different from isoflurane (2.18 ± 0.18 $P < 0.01$). phMRI of nicotine stimulation showed consistent patterns of activation with nicotine across numerous brain regions as seen with prior studies in rodents and humans. Long term (> 6 h) TIVA with dexmedetomidine + ketamine allowed for the study of nicotinic and dopaminergic receptors with little or no interference. Further, this anesthetic regimen shows potential as an alternative option for long term noninvasive imaging studies in rhesus macaques. Additional evaluation is needed to establish efficacy and/or potential variability in additional animals, age groups and gender as well as other NHP species.

P40 Spontaneous Collision Tumor in a Swiss Webster Mouse

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A 3 month old Swiss Webster (Crl:CFW(SW)) male mouse with no previous experimental manipulations presented with a 1.4 cm × 1 cm swelling on the proximal left front limb that was firm on palpation. The mouse was smaller than cage mates with a body condition score of 2.5 of 5 with normal ambulation and no pain response noted during manipulations. Top differentials included neoplasia and congenital deformity. Four days later the swelling had increased in size and a gross necropsy and histopathology were performed. Atypical lymphocytes consistent with lymphoma were distributed in multiple tissues and hyperostosis in the sternum, stifle, and vertebral column were observed. Both conditions are commonly associated with advancing age and endogenous retroviruses, hence a unique finding in a young mouse. Postmortem radiographs revealed a well-demarcated boney lesion with smooth, round contours within the diaphysis of the left proximal humerus. Microscopic examination of cross-sections of the boney lesion identified a collision tumor, defined as the existence of 2 or more tumors in the same organ or site. The tumor was composed of a benign osteoma and a malignant rhadomyosarcoma. Previous reports of collision tumors include a single report in a 1-y- old ICR mouse, a 2-y-old Djungarian hamster, and a 7-y-old mixed breed rabbit with varying tumor origins and presenting locations. The Swiss Webster mouse is a general multipurpose outbred model used for safety and efficacy testing. The most common tumor in this mouse stock is pulmonary in origin with an incident range of 8% to 24%, with increased incidence correlating with age. To the authors' knowledge, this is the first report of a collision tumor in a Swiss Webster mouse.

P41 Progressive Hindlimb Paresis in an Aged Sprague–Dawley Rat

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A 3-y-old female Sprague–Dawley rat presented with a 6-wk history of bilateral progressive hindlimb paresis, with the left limb more severely affected than the right. The rat appeared otherwise healthy and in good body condition. The rat had previously been used in behavioral studies and no other experimental manipulations had been performed. The rat was weakly ambulatory in the rear limbs and tended to drag the left more than the right. Overt signs of pain were not elicited during palpation of the vertebral column. Cranial nerve exam findings and forelimb proprioceptive reactions were normal. Proprioceptive reactions were absent in the hindlimbs while the patellar and cranial tibial reflexes remained intact. These findings suggested a lesion localized to the thoracolumbar spinal cord (T3 to L3). The animal was monitored and provided supportive care; however, due to the progressive decline in condition, euthanasia was elected. On necropsy, there was brown to gray friable tissue surrounding the descending aorta and ventral surface of the vertebral column extending from T4 to T12. Based on the age, clinical presentation and gross findings, top differentials for the lesion included lymphoma and atypical arteritis. Histologic examination of the brown friable tissue showed multifocal clusters of brown adipose tissue. Though the brown fat appeared well differentiated and did not invade into the spinal canal, the distribution was expanded beyond what would be considered normal in an animal of this age and was consistent with a benign hibernoma. While spontaneous hibernomas have been reported in Sprague–Dawley rats, it is a rare neoplasm with an incidence history of less than 0.1%. Further examination of the vertebral column showed marked, extensive, bilaterally symmetric radiculoneuropathy characterized by dilated myelin sheaths, axonal atrophy and abundant cholesterol clefts. Spontaneous radiculoneuropathy has been reported in aged rats and is characterized by variably severe demyelination of the ventral spinal nerve roots presenting as progressive hindlimb paresis. Together these pathologic findings present a unique combination of lesions in a geriatric rat and demonstrate the importance of histopathology for diagnosis of the cause of clinical disease in aged animals.

P42 Withdrawn

P43 Spontaneous Cataracts in Aging Laboratory Rabbits of an Inbred Strain

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This study investigated the occurrence of spontaneous cataracts in a breeding colony of the inbred EIII/JC strain of New Zealand white rabbits (*Oryctolagus cuniculi*). Thirteen cases (8 females and 5 males) of cataract were identified in a group of 51 EIII/JC inbred rabbits (27 females and 24 males) with a morbidity of 25.5%. The mean age of the rabbits identified with unilateral or bilateral cataracts was 42.8 mo in contrast to the mean age of 27.7 mo of the entire group of 51 rabbits. Additionally, 7 cases (5 females and 2 males) of cataracts were identified in a group of 21 EIII/JC-HLA.A2.1 transgenic rabbits which were produced by continual backcrossing of rabbits from a HLA-A2.1 transgenic line to the EIII/EJ rabbits for more than 9 generations. The EIII/JC- HLA.A2.1 transgenic rabbits showed similar morbidity (33.3%) and mean age (40.9 mo) for the development of cataracts as the EIII/JC rabbits. In both groups, none of the rabbits younger than 37 mo developed cataracts while 13 of 14 (93%) EIII/JC rabbits aged 37 to 49 mo and 7 (63.6%) of 11 EIII/JC-HLA.A2.1 transgenic rabbits aged 37 to 43 mo developed cataracts. In contrast, none of 78 outbred rabbits with a mean age of 29 mo (10 to 67 mo) developed cataracts. Results of this study indicate that the occurrence of spontaneous cataracts in this inbred strain (EIII/JC) of rabbits were strictly age related and consistently transmitted through inbreeding.

P44 Nonlethal Diarrhea and Intestinal Pathology Syndrome in a Colony of NOD.Cg-*Prkdc*^{scid} *Il2rg*^{tm1Wjl}/SzJ (NSG) Mice

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Mice within a NSG breeding colony were reported for having soft feces that would stick to the sides of the cage. No other clinical signs were noted. Mice bred and grew similarly to unaffected mice in the

colony. Offspring of the affected parents also developed the same clinical signs but otherwise were bright and alert. All animals in the colony were housed within ventilated cages and provided sterile caging, irradiated rodent feed and chlorinated water via an automatic watering system. The room and cubicle housing these mice is serologically negative for rodent pathogens, including norovirus, although routine bacteriologic screening is not performed. Approximately 1% of a total of 1,875 mice over 3 generations produced in the colony were affected, both adults (G2s) and preweanlings (G3s). Five mice demonstrating soft feces were euthanized for postmortem examination. Grossly, mice had an enlarged cecum and a distended colon. The entire gastrointestinal tract was collected for histopathologic examination and was fixed in 10% buffered formalin. Samples were sent to 2 independent pathologists and each identified prominent, diffuse vacuolation of the mucosal epithelial cells, primarily in the cecum and anterior colon. Mild apoptosis and hyperplasia with minimal inflammation was also seen in affected areas. Histologic staining of sections of large intestine with Periodic Acid Schiff and Alcian Blue revealed that vacuoles did not contain polysaccharides, mucopolysaccharides or acidic mucins. PCR analysis revealed that feces were negative for *Helicobacter* spp. and *Clostridium* spp. This is the first report of cecal and large bowel vacuolation in NSG mice and represents another veterinary and husbandry challenge in the care of these immunocompromised mice.

P45 Cage Cards: The Front Line of Communication

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The Comparative Medicine (CM) group is responsible for three vivaria, serving more than 100 researchers and technical staff. Within CM there are 20 animal care (ACs) specialists between level ACI and ACIV with various research experience that provide the daily animal care and husbandry. The ACs and other facility users deal with a multitude of animal holding room and cage specific requirements on a daily basis. These range from biosafety designation to special dietary requirements, environmental enrichment, and specific husbandry requirements. The group also maintains breeding projects that require additional cage level tracking and documentation. The ACs work closely with the researchers and IACUC staff on disease models and specific clinical conditions or health monitoring requirements. Over several years CM has developed and implemented various visual identification and labeling systems to effectively and clearly communicate requirements to the ACs, researchers, technicians, managers, and veterinarians. These identifiers are reviewed and updated regularly. Through the implementation of these systems CM has reduced inconsistencies, improved communication, and gained efficiencies in animal care and disease model management in our program.

P46 Feeding Fiber to Rabbits on a High-Fat Diet Improves Appetite and Wellbeing without Impeding Blood Cholesterol Levels

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Keeping rabbits on high-fat diets for atherosclerosis models can be challenging. Naturally, rabbits are herbivores with a need for increased roughage to prevent gastrointestinal complications. High-fat rabbit feed is available for research; however, palatability is often reduced as feed does not remain in pellet form. In this study, New Zealand white rabbits underwent a bilateral iliac atherosclerosis inducing procedure. Two groups of rabbits were fed 150g of high-fat rabbit feed (1% cholesterol, 6% peanut oil) daily for 6 wk. The first group (G1, n = 9) was fed only high-fat diet, the second group (G2, n = 11) was given a handful of kale daily along with high-fat diet. The daily feed intake was measured for each rabbit and an appetite score (0 to 3) was given based on amount consumed. Feed was kept in closed bags at 0 °F then at 40°F after the bag was opened to ensure the feed maintained pellet form throughout the study. Bodyweight was measured pre-operative. Rabbits received daily injections of test drug or control for the duration of the study. Plasma cholesterol levels were measured pre-operative, mid study, and before euthanasia. Every G1 rabbit (9 of 9) did not eat high-fat feed for 2 or more days throughout the study. Animals were fed regular feed until their appetite increased. Then the regular feed was gradually replaced with high-fat feed. Less than half of the G2 rabbits (5 of 11) went off feed during the study. Data was compared between the 2 groups using 2 sample t-tests. Overall, animals that received daily kale in addition to the high-fat diet (G2) consumed more feed per day, had higher mean daily appetite scores, and higher mean body weights than animals fed only the high-fat diet (G1). G2 also had higher cholesterol levels than G1. Even though there was a difference in blood values for each group, there was no significant difference to hypercholesterolemia, remaining consistent to the study model. Additionally, G2 animals were noticeably calmer during handling for procedures. Based on our data, supplementing properly stored high-fat feed with greens, such as kale, results in increased appetites and more positive human interaction while maintaining sufficient plasma cholesterol levels in rabbits.

P47 Evaluating the Viability and Cost Effectiveness of Water-Based Gel Cups to Water Bottles for Weaned Mice (*Mus musculus*)

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Providing a secondary source of hydration for newly weaned mice (*Mus musculus*) is a common practice in facilities that use automatic watering systems. The standard operating procedure at our facility consists of adding a water bottle with a sipper tube to cages at the time of weaning and leaving it in place until animals are able to efficiently use the automatic watering system. However, water bottles are prone to leaking and can potentially flood the animal cage which can result in hypothermia and/or death. Additionally, water bottles require a large initial investment and are laborious for husbandry staff to wash, fill, empty, and autoclave. Single-use, commercially available, water-based gel cups have grown in both availability and popularity in recent years. They are both convenient and easy to use but can be expensive. The purpose of this study was to compare the clinical effectiveness and overall costs of using water bottles compared with water based gel cups for hydration support of newly weaned mice. To determine if mice would use the water-based gel cups, a cup was placed in each cage (n = 20) at the time of weaning and left for 5 to 6 d. The mice were evaluated daily by the same laboratory animal health technician for clinical signs of dehydration and overall health. Cups were weighed daily to measure rate of consumption. To determine the labor cost of using water bottles, husbandry staff members (n = 4) were timed performing the various tasks needed for processing. Those costs were then added to operational expenses such as washing and autoclaving a load of water bottles. The initial investment of the water bottles was not included in the cost assessment. Husbandry staff members were then timed processing the water-based gel cups and the calculated labor costs were added to the price of the product. The overall time savings and resultant reduced labor costs of using the water-based gel cups was significant compared with processing water bottles. However, the cost of the cups offset any financial gain by reduced labor costs, eliminating overall savings for institutions already owning water bottles.

P48 Bacteriologic Evaluation of Extended Sanitization Intervals for Mouse Cage Accessories

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Rodent cage sanitization interval extension is an attractive cost-saving measure if it does not cause environmental deterioration that negatively impacts animal welfare. Prior studies support filter top and wire-bar lid sanitization intervals of up to 180 d. However, the *Guide for the Care and Use of Laboratory Animals* states that enclosures and their accessories should be sanitized at least every 2 wk. AAALAC expects accredited institutions to use data-driven performance standards to evaluate frequencies exceeding the *Guide* recommendation. To examine their bacterial load over time, filter tops and wire-bar lids on 14 mouse cages were left in place, though cage bottoms and bedding were changed at least every other week. Subsequently, replicate organism detection and counting (contact plates) plates were pressed to these accessories at locations selected for proximity to mice, food, and bedding. This was done in two mouse housing facilities that use different individually ventilated caging (IVC). Accessories in both facilities had mean contact plates counts of 0-1 colony-forming units (CFU) when the study began, and counts up to 50 CFU, a standard cutoff value, were considered acceptable at subsequent time points. Two weeks following study initiation, only 43% (6 of 14, $P < 0.001$) of wire-bar lids in facility 1 had acceptable contact plates counts. In facility 2, 93% (13 of 14, $P = 0.5000$) were acceptable at 2 weeks, but only 39% (4 of 14, $P < 0.0001$) at 3 weeks. In both facilities, increased incidence of unacceptable contact plates counts was accompanied by visible soiling of surfaces adjacent to food, further validating these results. This suggests that a 2- or 3-week wire-bar lid sanitization interval is appropriate in these facilities. When filter tops were tested, 86% (12 of 14, $P = 0.2407$) in facility 1 and 79% (11 of 14, $P = 0.1111$) in facility 2 had acceptable contact plates counts at 4 wk. Thus, filter top sanitization intervals of 4 wk (or possibly longer) are acceptable in these facilities. This study contrasts with prior reports that supported 90-d wire-bar lid sanitization intervals possibly due to differences in contact plates plating technique or IVC equipment used. It also emphasizes the need for ongoing validation of husbandry practices in a site- and equipment-specific manner.

P49 Implementation of a Novel Delivery Method for CO_2 Euthanasia in Laboratory Rodents

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CO_2 gas is the most commonly used euthanasia agent of laboratory rodents. Despite its widespread use, there are multiple concerns around this euthanasia method. In response, the 2013 Edition of the AVMA Guidelines for the Euthanasia of Animals has addressed these issues and given refined recommendations. With the publication of new guidelines we re-evaluated our current system. Two commercially available systems were originally considered: a preset regulated flow system (PFS) and an automated fill system. Both were found to have variable functionality issues within our facility, due to our inhouse fixed CO_2 delivery pressure of 8 PSI. Many of the systems require a CO_2 source of 15 PSI or greater. After much consideration and preliminary research it was determined that a system using engineered tube flow restrictors was recognized as a well-controlled, cost-effective method to accomplish euthanasia that met all AVMA guidelines. In order to implement such a system, collaboration was implemented with a company to design, test, and certify appropriately sized gas tube flow restrictors. The system was assembled and pilot tested in our facility to ensure that it functioned properly before implementing. Staff was notified through email that they had a new didactic training course. The new systems were installed within our vivarium and staff was encouraged to email identified super users or the site veterinarian if they needed further training or had issues with the system. Overall the new system was successful in accomplishing our goal of delivering CO_2 to euthanize rodents, in compliance with AVMA guidelines. Further development and improvements occur continuously as feedback and events arise.

P50 Liquid Diet Use in Swine

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Swine are a common animal model used in gastrointestinal research where access to the upper or lower gastrointestinal tract (GIT) is necessary for device or equipment evaluation. This requires that the target area in the GIT be devoid of ingesta. Methods for emptying the digestive tract include fasting, liquid diets, enemas, and laxatives. Although some of these methods are effective, some result in incomplete clearance of the GIT, administration of enemas and laxatives can be challenging, and longer fasting periods are stressful to the animal. We wanted to evaluate the effectiveness of a liquid diet to successfully clear ingesta for endoscopic access in the stomach and colon and for longer term use in postoperative animals. The diet was made in house using store bought ingredients, was readily consumed, and cost effective. Liquid diets were given for 1 to 3 d prior to endoscopic procedures (access to the stomach, duodenum, colon, and biliary duct) to Yorkshire swine or up to 28 d postoperatively for esophogeal stenting procedures to Yucatan swine. We have found that adding a liquid diet meal prior to the 24-h fast can produce an emptied stomach and feeding a liquid diet for 3 d prior to surgery can successfully empty the colon. Liquid diet may also be used postoperatively to allow healing of the gastrointestinal tract. Preliminary data suggests swine on this diet can gain weight at an expected rate and maintain body condition scores and normal blood values for up to 4 wk. Feeding a nutritionally complete liquid diet prior to or after surgery when needed for gastrointestinal procedures has been a positive refinement for laboratory swine at our facility. Feeding a liquid diet can be effective, reduce stress, and maintain balanced nutrition. This provides an alternative or adjunct to fasting which facilitates the approach to the gastrointestinal tract, while maintaining animal health and welfare.

P51 Enhancing Environmental Enrichment without Breaking the Bank

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Although our enrichment program meets all USDA, AAALAC, and IACUC standards, we wanted to do more for the animals. Due to a limited budget, we were faced with the challenge of enhancing our program without increasing spending. We began by forming an environmental enrichment committee to develop and implement new ideas, discuss the outcomes, and make changes as needed. Enhancements made to our program include creating monthly species-specific treat menus, holiday enrichment parties for all USDA-covered species, positive reinforcement training for dogs, open-area and play cage time for rabbits and primates, and increased social housing and toy rotations. We found that using monthly species-specific treat menus enables greater variation while decreasing food waste. For holiday enrichment parties, staff members donate boxes and paper rolls and are invited to help assemble the "gifts," which are filled with various toys and treats tailored to the specific species. This provides an alternative enrichment experience from the standard daily treats and toys and occurs around every major holiday. Positive reinforcement training for the dogs was initiated to provide more human contact in addition to the time spent during daily husbandry duties, health evaluations, and exercise time. Training tasks include leash walking, sitting, and playing fetch. The training sessions result in calm, attentive dogs that enjoy the tasks and human interaction. The rabbits and primates actively make use of the play areas and have displayed decreased stereotypic behaviors. Throughout the revamping of our program, we recruited various staff members that were not previously involved with enrichment duties. The additions to our program provide enhanced enrichment for the animals, brought department-wide awareness to the importance of environmental enrichment, provide a fun team-building activity for staff apart from the normal work day, and require

little cost for equipment and supplies by using donated and repurposed materials, and regulating the amount of food treats purchased.

P52 Etruscan Shrews (*Suncus etruscus*) as a Model Organism for Biomedical Research

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Etruscan shrews are the world's smallest mammal by mass, with the average adult weighing 2 g. Their small size allows them to be readily used for multiorgan analysis, and makes them ideal for functional live imaging of the brain for neuroscience research. We have recently begun housing Etruscan shrews received from a captive colony. To our knowledge, this is the first colony of Etruscan shrews at an American research institution. Using these animals for biomedical research provides unique challenges and opportunities for innovation in animal husbandry and handling. As with any animal species, Etruscan shrews need to be housed in caging compatible with their natural environment and behavior. Etruscan shrews are insectivorous and eat a live diet consisting of crickets and mealworms. Their high metabolism results in frequent consumption of large volumes of live prey. Another consideration is their sensitivity to light cycles, such that their socialization, mating behaviors, and reproductive success are highly dependent on lighting. We have devised housing and husbandry practices to meet these unique needs. Shrews are housed in micro-isolation rat cages with bedding consisting of autoclaved peat moss and hardwood chip bedding. Enrichment includes nestlets, egg crates, PVC tubes, and mouse igloos. Shrews are housed at a temperature of 73 °F and a humidity range of 40% to 60%, with a12:12-h dark:light cycle. Breeding shrews are housed at a temperature of 75°F and a humidity range of 40% to 60%, with a10:14-h dark:light cycle. Breeding under these optimized conditions yields an average litter size of 2 to 6 cubs after a gestation period of 25 to 28 d. In addition, Etruscan shrews used in biomedical research may require individual identification, tissue collections, anesthesia, and other routine veterinary management that requiring novel solutions for their small size and unique physiology. Establishment of this colony allows for a novel translational medicine research organism.

P53 Verifying Sterilization of Materials in a Gnotobiotic Facility

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Our institution maintains a gnotobiotic research animal facility (GRAF) housing germ-free or defined flora mice within flexible film isolators. Materials are introduced into isolators using sterilization cylinders exposed to steam/heat sterilization. Confirming that a cylinder has been properly autoclaved before introducing its contents into an isolator is vital. When there are multiple people working in this type of facility it is critical to verify processes have been completed adequately. Previously, cylinder preparation and adequate processing relied on visibility of autoclave tape around the periphery of the cylinder (partially covered by opaque vinyl tape) and autoclave cycle documentation. To better verify sterilization of cylinders, existing processes were enhanced and new procedures introduced. Cylinders are sealed with a mylar cover and secured with autoclave tape and vinyl tape as before. A new modification introduced a steam sterilization indicator card secured with autoclave tape to the front of internal cylinder contents. The card documents materials, date prepared, initials, and isolator which the materials are to be introduced. The prepared cylinder is then autoclaved. Verification of the sterilization cycle occurs by documenting cycle parameters. Each cylinder can be assessed for exposure to adequate steam heat conditions by visual inspection of both peripheral autoclave tape and internal indicator card color change. The card is visible through the mylar film sealing the cylinder and to an individual introducing materials into an isolator. The card serves to verify that the cylinder is attached to the intended isolator and has been exposed to adequate steam heat sterilization as confirmed by color change of the indicator, as well as the autoclave tape securing the card. Cylinders may also contain a biologic indicator within the load. An indicator card on an isolator clipboard flags a hold on supplies pending favorable test results. Once the card is initialed and filed by staff the introduced supplies may be used. Adding the sterilization indicator card to our standard operating procedures has substantially reduced the risk of isolator compromise by inadvertently introducing materials which have not had adequate processing.

P54 From Swinging Single to Peacefully Cohabitating: A DIY Way to Managing Rabbit Socialization without Breaking Your Budget

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The *Guide for the Care and Use of Laboratory Animals* requires that all animals, without veterinary or study exemptions, be socially housed. Our current caging only allows enough floor space to house one large rabbit per cage. With the *Guide* requirement in mind, our animal resources department has been housing rabbits in individual caging and socializing them three times a week in a socialization cubicle. The three main problems with this socialization technique is having an additional room or cubicle is needed to set up the socialization area, the time required to move the rabbits and watch them to ensure they do not fight with each other, and then sanitizing the cubicle for the next group of rabbits to use. From moving the rabbits to the socialization area, observing and moving them back to their cages, to cleaning time, it takes the care staff approximately 1 h/d to socialize just a few rabbits. To fix this problem, we collaborated with a machine shop on campus to develop a tunnel system that is built into the existing rabbit cages. This tunnel system was built using inexpensive materials from a local home improvement store. This tunnel system has the ability to be capped if two rabbits do not get along with each other or it can be left open indefinitely. It is easily sanitized in the cage wash machine with the rack. After observing the rabbits on a daily basis they use the tunnel and move from cage to cage frequently. Being able to house our rabbits with this tunnel system has saved us approximately 2.5 h/wk in setup and observation, saved our department money by not having to purchase new caging, and allowed our department to save floor space for other animal use.

P55 First Aid for Rodent Breeding Colonies: A Veterinary Treatment-Kit Program for a Modern, Large-Scale Murine Production Facility

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Management and veterinary care of breeding colonies in a new, large-scale, and expanding rodent production facility is labor intensive. Efficient and innovative work practices are essential to ensure that appropriate care is provided every day. Having veterinary supplies readily available and training husbandry staff to provide standard treatments as part of their regular husbandry responsibilities facilitates timely intervention. In order to enhance veterinary treatments in our breeding colonies, a point of use veterinary treatment-kit program (Vet Kit) was developed for use in all animal holding rooms by animal care technicians (ACTs). During initial site occupancy, veterinary supplies were brought to individual animal rooms by veterinary staff as needed, which was an inefficient process that increased risks of cross-contamination. Leveraging the skills of our professionally trained and experienced husbandry staff (100% AALAS certified at ALAT or greater, average of 10 y work experience), veterinary kits containing basic supplies for veterinary

treatments and routine health surveillance sampling were placed in all holding rooms. Additional training was provided to ACTs so they could perform health treatments for common clinical conditions such as trauma wounds, dermatitis, dehydration, prolapses, or malocclusion. Over time, the program evolved based on changing needs, ACT feedback, and competency. Under the direction of the veterinary staff, ACTs were given the responsibility to develop and manage the Vet Kit program. Results of the program have been positive. Decreased response times for clinical treatments and enhanced animal care have been observed and direct ACT oversight has improved inventory management. New products were added, unnecessary products were removed or quantities were adjusted as needed, resulting in less wastage and cost savings. The program has led to broadened ACT staff development by including animal treatment responsibilities, raising overall job satisfaction at the facility.

P56 Repurposing and Recycling an Institutionally Retired Enrichment Device

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One aspect of maintaining and updating an environmental enrichment program is regular review of enrichment devices and their current uses. The review includes maintaining an accurate inventory to reflect the animal census, evaluating species-specific use, product sustainability and the replacement of older items with new and novel devices as needed. Items that are removed from the regular rotation are disposed of, sold to another institution, or repurposed whenever possible. Our institution removed from rotation an open-bottomed, red, triangular, high-temperature, polycarbonate enrichment device that was previously used for mice. This structurally sound device was removed from device rotation to accommodate a new device being implemented. Following evaluation, this retired device was determined to be usable as a repurposed device. The triangular device was able to be inexpensively repurposed in-house, into a novel customized 3-tier hanging enrichment device that can be used across multiple species compared with the initial single species use. The hanging device was created by drilling through the center crease of 3 polycarbonate triangles with a 5/32-in. drill bit and were linked together using 9 in. of 304 stainless steel ¾-in. link chain with 3 in. of chain separating each individual device. The device could then be hung from a supporting structure within an animal's cage with a stainless steel quick link. Due to center chain placement, the triangles can be manipulated as a whole or by the animal turning each individual triangle, thus increasing the forms of manipulation and novelty. This device can be further enhanced by including nutritional enrichment, such as honey or by freezing part or all of it in a water and fruit mixture. In comparison to a commonly used commercially available hanging device that serves a similar purpose, the inhouse customized device had a cost savings of 49%. By repurposing and recycling an animal enrichment device that was taken out of rotation, we were able to create a simple, cost effective, novel device that can be used by multiple species at our institution.

P57 Techniques for Improving Efficiency in a Gnotobiotic Facility

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Over the last several years interest in gnotobiotics has grown significantly. Gnotobiotic (defined bacterial species present) and axenic (microbiologically sterile) mice provide a valuable research tool to help elucidate how the microbiota impacts health. Gnotobiotic and axenic mice are typically housed in flexible film or semi-rigid isolators. To prevent contamination from environmental sources, the interior of the isolator, the air supply, and all husbandry supplies for the mice must be sterile. Maintaining gnotobiotic and axenic mice under these strict conditions is labor intensive and time consuming. Over the years, we have implemented many techniques which help us operate our gnotobiotic facility more efficiently. A sampling of these techniques include packing food and bedding in cotton drawstring bags, using a mock supply cylinder containing biologic indicators in each steam sterilizer run, and using a Class II Type A2 nonducted biosafety cabinet for short-term experiments. Packing the food and bedding into cotton drawstring bags allows for quick counting during supply inventory and provides a mechanism for securing opened but unused portions of food or bedding within the isolator. The mock supply cylinder containing biologic indicators provides quick assurance that the supplies are sterile. The use of the biosafety cabinet allows us to do short-term experiments without undertaking the time-consuming process of constructing and sterilizing additional isolators. By using these techniques and others, the time savings have allowed us to expand our colony and accommodate more projects to keep pace with the growing interest in microbiota research.

P58 Novel Ergonomic Device for Rodent Water Bottle Cap Removal

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Maintaining an ergonomic and safe work place is a key component to reducing chronic injuries amongst laboratory animal care staff. Many of these injuries are the result of cage-changing activities. Often, one of the most ergonomically challenging tasks is removing slip-type rodent water bottle caps. To address this issue we developed a novel and ergonomic method for the removal of these caps. This method involves the use of a device constructed from polyvinyl chloride tubes that have been assembled to form a T-shaped handle. This device allows the operator to remove water bottle caps quickly and efficiently with minimal ergonomic risk compared with removing each cap without this device. The use of this device has greatly reduced the number of chronic hand injuries in experienced staff members and has prevented these injuries from occurring in newer staff members as well. We believe that the use of this device is an easy and inexpensive solution to a common occupational health problem that can be readily applied at other institutions using rodent water bottles.

P59 Laboratory Husbandry and Management of a Challenging and Unique Species: The Spotted Hyena (*Crocuta crocuta*)

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Our animal care program houses a variety of unique species, each requiring their own specific husbandry protocols, as well as additional enrichment and safety protocols. One such species, the spotted hyena (*Crocuta crocuta*) necessitates an especially concerted effort in terms of personnel safety. Given their intelligence and social structure, husbandry and enrichment present unique challenges for the animal care program. Routine husbandry tasks become potentially more hazardous and thus, additional safeguards must be put in place. For example, personnel must work in pairs, direct physical contact with the animals is prohibited, and all entrances and exits to animal enclosures are color coded to ensure security. In addition, boredom and weight gain, due to a combination of reduced activity and restricted enclosure size, are common concerns among these captive animals. Possessing some of the most powerful jaws on earth, hyenas are capable of laying waste to many standard enrichment devices. There are few objects that a spotted hyena cannot destroy and devour within minutes, which can potentially lead to lacerations, gastrointestinal obstructions or perforations, and fractured teeth.

Some examples of effective enrichment include providing pumpkins to prompt rolling behavior, water troughs and feed bags to induce play behavior, eucalyptus branches to promote scent marking, and leaving felled trees in enclosures to encourage den making. Here we discuss several aspects of our hyena husbandry and enrichment program, a novel program designed to facilitate natural behaviors and increase mental and physical health in our hyena colony, while upholding appropriate safety standards. Though our facility has the only known captive hyena research colony, the lessons learned in managing this colony are widely applicable to other research programs housing socially intelligent or potentially hazardous species. Many institutions maintain colonies of large carnivores, scavengers, and herbivores with well-developed social intelligence, including nonhuman primates. The general approach to safety and enrichment for hyenas is undoubtedly applicable in these situations, regardless of the species in question.

P60 Developing an Enrichment Plan for Water-Regulated Nonhuman Primates

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Environmental enrichment for nonhuman primates is an essential requirement in laboratory animal research. The Animal Welfare Regulations and the *Guide for the Care and Use of Laboratory Animals* state that the facility's enrichment program must enhance the psychologic wellbeing of nonhuman primates, as well as promote species-specific behaviors. Oftentimes, environmental enrichment plans include the provision of various food items including produce. However, many nonhuman primates involved in neuroscience research require strict regulation of daily water intake, thus prohibiting enrichment items with high water content is scientifically justified. As a result, alternatives to simply offering fruit or vegetables are critical to supporting a stimulating and enhanced indoor housing atmosphere. Daily enrichment is primarily offered in the form of manipulanda placed inside or on the outside of the cage which are rotated out daily to provide variety while avoiding monotony and habituation. Some commonly available commercial enrichment devices may successfully consume much of the nonhuman primates' time, but do not allow for a variety of healthy offerings to be successfully placed inside the device. Furthermore, these devices have a history of breaking easily. The nonhuman primate care staff in collaboration with the veterinary staff developed a plan to include new devices of varying difficulty built inhouse that would offer added possibilities in terms of dry food items to place inside while still maintaining the desired time consuming quality. These devices were constructed of readily available materials such as polyvinyl chloride pipes, metal chain links, metal clips, and epoxy. In addition, this plan included specifications for visual and auditory enrichment, caretaker interaction, and increased cage space via play cages and cage extensions. This enrichment plan has proved to be successful in enhancing the environment of our water-regulated nonhuman primates and has decreased the incidence of stereotypic behaviors as well.

P61 An Easy Solution for Social Housing Larger Guinea Pigs in Research

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The standard microisolation caging for guinea pigs, *Cavia porcellus*, allows for group housing up to a combined weight of 350 g. However, as mandated by the USDA, research facilities must socially house guinea pigs with compatible conspecifics regardless of size. Lack of appropriate sized caging is not an acceptable reason for single housing a social animal. We investigated purchasing species-specific caging that allows for social housing larger guinea pigs, but its use is limited to one species. We have standard cages that allow for housing ferrets or rabbits, but are not suitable for social housing guinea pigs. Other facilities have made attempts at social housing guinea pigs. Unfortunately, the modifications were not user friendly. To solve these issues, we have modified rabbit caging for social housing larger guinea pigs. This easy modification allows for social housing guinea pigs as they increase in size over time and can be modified back to rabbit housing when needed. Sanitization is essentially the same as cleaning a rabbit cage using an automatic cage wash system. In addition, the cage design works well for daily husbandry and clinical observations because animals are easily accessible and the cage provides a less obstructive view. This is a simple inexpensive modification to standard rabbit caging and provides group housing for guinea pigs as they grow.

P62 Take Your Child to Work Day in an Animal Research Facility? Improving Perceptions Using 3Es: Education, Engagement, and Enrichment

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Children are exposed to many views about animals in research regularly, whether at school, through peers, or media sources. These views impact their perceptions of animals in research, usually negatively. Participation in Take Your Child to Work Day allows animal research facilities to provide children positive experiences involving animal research. Many people working with animals in research are proud of their work and would love the opportunity to share that work with children. However, a common problem faced is animal facility rules/regulations which restrict or inhibit those opportunities. We have established a program that allows our animal research facility to participate in Take Your Child to Work Day while upholding facility rules/regulations. Age eligibility, number of participants per workshop/session, and application procedures are clearly defined. Letters are sent to participants explaining the program, rules, and expectations for Take Your Child to Work Day. The program consists of several hour-long workshops, including interactive education sessions focusing on animal use in research, equipment demonstrations, and vivarium tours. Employees volunteer to manage, facilitate, and/or participate in each workshop. All participants must follow facility standard operating procedures. The IACUC reviewed the program. We use Take Your Child to Work Day as a form of youth outreach focusing on improving perceptions of animals in research using three Es: Education (presentations, demonstrations, workshops, tours), Engagement (participants, employees), and Enrichment (human, nonhuman). Participants share their experience in this program with teachers and school peers, thus increasing our reach. Success of this program is illustrated by active participation for 8 consecutive years with continued growth in number of participants, from under 100 to more than 200 per year. We continue to receive positive feedback and hope to inspire other animal research facilities to consider participation in a similar program.

P63 Using Positive Reinforcement Training to Refine Daily Medical Treatment of a Cynomolgus Macaque

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Medical care of nonhuman primates in a laboratory setting can be challenging due to the safety concerns inherent in handling conscious animals. A mature, adult male cynomolgus macaque presented with a chronic foot condition that required daily treatment by the veterinarian and husbandry staff. Traditional care was time consuming and somewhat stressful, requiring the use of a pole-and-collar system to place the animal in a chair, perform the medical treatment, and return the animal to the home cage. Since handling a mature

male cynomolgus requires 2 technicians to ensure the safety of the animal and the staff, this daily treatment was also a significant resource drain. Our objective was to use positive reinforcement training (PRT) to teach the animal to voluntarily participate in the treatment. We present the PRT plan used to teach the animal to present his foot for treatment while in his home cage. The successful implementation of this plan decreased stress for the animal reduced the time needed to care for the animal and increased safety for both the animal and the operations staff.

P64 The Future of Mouse Room Monitoring: iMouse, an Integrated System

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iMouse is a pilot project that aims to automate procedures and activities that occur in a mouse room, eliminating the need for signs and paperwork including daily logs. Furthermore, this project will act as a channel or live stream between the animal facility in Qatar and the main animal facilities in New York City allowing researchers and animal care staff from both campuses to communicate more effectively and efficiently. Researchers and animal care staff will also use a suite of integrated applications developed by the Center of Comparative Medicine and Pathology. This customized web-based application facilitates and streamlines many interactions and transactions with both the Research Animal Resource Center and the IACUC. Since this project requires simple and available resources, it will promote economy of space and easy setup. The system is composed of the following two components. The first component consists of three tablet units, one inside the mouse room used for accessing the suite of applications, as well as a replacement for the printed signs that are currently mounted in the mouse room that display SOPs and policies. The second tablet will be placed outside of the room to view room schedules, procedures, requests, technicians, and tasks assignments. The third tablet is a mini tablet that will be available for use cageside by both researchers and animal care staff. The second component consists of two docks in which two of the tablet units are mounted into the walls to ensure secure access/login to the tablets. iMouse is considered the first in this area and a unique tool that will bring the future of animal facility monitoring to our hands digitally and globally.

P65 Going Green: It's Not What You Think

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Producing a consistent and abundant supply of wheat grass for our locust colony is important to both their health and reproductive capability. Growing this grass has posed a number of challenges, including reduced germination and mold growth, after planting. We examined our own handling and storage methods to insure seed quality at time of planting. A number of questions were formulated to focus on our internal processes. These included: (1) why was mold growing on seeds planted on top of the soil? (2) Were seeds being stored at correct temperatures in our facilities? (3) Were new saucer containers providing adequate drainage and air circulation? We found that storage conditions, including temperature and humidity, can have an effect on seed performance. Therefore, seeds must be stored in a cool and dry environment to maximize performance. While we could not confirm the storage conditions of our supplier, we could obviously document our own. Originally, seeds were stored in a cooler at our receiving dock when delivered. They were kept there until being moved to our locust room for final storage prior to use. Our locust room temperature averaged 85 °F. Seed stored above 60 °F can affect its ability to germinate. Our old saucer containers had half inch holes cut in the center for drainage and air circulation. New saucer containers were purchased that had no holes. We placed 5 pen size holes on the bottom of these saucers. We learned that temperature, moisture, and air circulation are critical for the growth of these seeds. The seeds were sitting in moisture which contributed to mold growth. Through research, we found that baking soda could assist in diminishing or completely eliminating mold growth. We began a process of soaking our seeds in water and baking soda. The final combination of seed storage in a controlled environment with adequate drainage and moisture control in growth containers and the elimination of baking soda resulted in substantially increased wheat grass yields. Not only are we providing adequate amounts of grass to keep our locusts healthy and reproductive, we are also supplying grass to our rabbits and nonhuman primates for enrichment.

P66 Ammonia Production and Nasal Histopathology in Mice Housed in 4 IVC Systems for 14, 21, or 28 Days

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We compared ammonia levels and nasal histopathology in mice housed in 4 individually ventilated caging (IVC) systems with 14-, 21-, and 28-d cage change intervals. One IVC system used disposable cages; the remaining 3 used nondisposable cages. Air flow was set according to the manufacturers' recommendations. All cages were supplied with irradiated 1/8-in. corncob bedding, paper nesting material, 18% protein chow, and reverse osmosis water. The IVC systems were placed in the same room at 71.4 to 76.8 °F temperature, 28.7% to 38.5% relative humidity, and a 12:12-h light:dark cycle. Nine cages each with a Crl:CD1 (ICR) (CD1) female with a litter of 9 1-d-old pups ("pup cages") and 9 cages each with 5 CD1 males weighing 18 to 23 g ("male cages") were placed on each rack. Ammonia levels were monitored using a portable gas monitor, measurements being taken in the middle of the cage at mouse level. At 14, 21, and 28 d, mice in 3 pup and 3 male cages from each system were euthanized and examined for anatomic lesions and histologic lesions of the respiratory and olfactory mucosa. Repeated measures polynomial regression showed a rapid rise in ammonia, followed by a controlled plateau period, followed by an uncontrolled rise in ammonia ($P < 0.0001$ for pup cages; $P = 0.0034$ for male cages). Because ammonia levels were similar at 14 and 21 d, it was unclear whether the daily or cumulative ammonia exposure is most important. Histopathologic evaluation suggested that cumulative exposure was more important: lesions were observed from 2 of 16 (13%), 7 of 16 (44%), and 10 of 16 (63%) cages at the 14-, 21- and 28-d time points, respectively. Nevertheless, detailed analysis showed that, regardless of the actual length of exposure, both the cumulative daily exposure ($P < 0.0001$) and the acute level of exposure on the final day of measurement ($P < 0.0001$) were highly predictive of nasal mucosa lesion severity in a linear fashion. These data illustrate the importance of intracage ammonia levels as a performance standard for mouse husbandry and welfare.

P67 The Longevity of a Nonhuman Primate Social Housing Strategy: A Review of Group Housing for Rhesus Male Macaques Maintained on Active Protocols in Biomedical Research

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Regulatory mandates promoting the psychologic wellbeing and addressing the social needs of nonhuman primates have been well described. In response to these directives, in 1992, we initially reported a successful group housing strategy for instrumented rhesus macaques accessed routinely for biomedical research. Small groups of macaques were successfully established and maintained. These groups consisted predominately of males with subcutaneous CNS

catheters and intravenous ports, which were removed from their groups for 0.5 to 20 d with successful return. This social housing strategy did not impede any research requirements or results, and has been effectively and continuously used for 22 y within our facility. A review of the social housing history from 2003 to 2014 was done for 28 male macaques ranging in age from 2 to 20 y. The type of instrumentation implanted; psychologic status; and the number, success, duration, removal and returns to groups for each animal were recorded and analyzed. Eighty-two percent of the macaques were instrumented with subcutaneous CNS catheters, cannulas, or lumbar and intravenous ports. Each macaque, on average, was involved in 2.4 groupings; 30% were involved in 3 to 6 groupings. On average the grouping success rate was 65% and 5.4 m in duration. The longest group duration was 61 m. There were 113 removals from groups with a 92.8% successful return rate. All grouped macaques were accessed, on average, at a frequency of once/animal/month with successfully removal/returns for 92.9% after 1 to 4 h, 35.7% for 2 to 7 and 17.9% for 14 to 35 d of absence from group. Grouping and return failures were attributed to aggression. The longevity and effective use of this social housing strategy has been successfully demonstrated. Instrumented male rhesus macaques can be socially housed and maintained on active research protocols.

P68 Ground Squirrel Round-Up: The Development of a Quarantine and Health Monitoring Program

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Thirteen-lined ground squirrels (TLGS), *Ictidomys tridecemlineatus*, possess several anatomic and physiologic features unique to this mammalian system. Historically, TLGS have been used as a laboratory animal model for studies of hypometabolism as they undergo a natural, seasonal deep torpor. Additionally, their unusual retinal neuroanatomy, which encodes a color visual spectrum, serves as a unique mammalian model for investigating retinal physiology and visual processing. Specifically, TLGS retinas are cone photoreceptor dominant, comparable to the central human retina. We work with a principal investigator whose ophthalmic research uses TLGS to map the neuroanatomy of the inner retina. As the total number of TLGS in research is low, the availability of purpose-bred animals from vendors is limited. Typically, we obtain unconditioned, wild-caught animals from a commercial vendor. Wild-caught animals are susceptible to a range of diseases and conditions which can introduce unknown study variables. To ensure the biosecurity of our permanent colonies and to safeguard the health of the animals for welfare and study purposes, we have developed a comprehensive quarantine and health monitoring program for incoming and permanent colony animals. This program has evolved over the years based on the prevalence of pathogens identified in our wild-caught colonies. New components of the program include sentinel monitoring and bi-therapy deworming with follow-up fecal analysis. As working with purpose-bred animals would minimize the need for most components of this program and resolve the welfare impact on wild-caught populations, we have also initiated an inhouse breeding program. Small numbers of litters have been produced over the last few years and with time, we anticipate replacing wild-caught animals with an inhouse, purpose bred colony.

P69 Effect of Adult Male Canine Tooth Modification on Group Welfare in Rhesus Macaques (*Macaca mulatta*)

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Social housing is one of the most important strategies to ensure good welfare in rhesus macaques. Unfortunately, this also comes with the risk of conspecific trauma, which when severe or recurring often leads to removal of injured animals and/or aggressors from the group. Adult male rhesus macaques have large canine teeth that serve no significant dietary role, but are adapted for social aggression and capable of inflicting serious injury. Historically, canine crown reduction (canine cutting or vital pulpotomy) was performed to reduce the risk of trauma from adult males. This process can result in infections and chronic oral pain, leading the USDA to consider the management practice unacceptable. Canine tooth blunting, without penetrating the pulp cavity, is considered an acceptable alternative in some situations. However, the efficacy of any method of canine tooth modification in reducing trauma has not previously been demonstrated. To obtain a more complete view of the welfare effects of canine tooth modifications, 9 groups of rhesus macaques (1072 total) were studied. The groups consisted of multimale/multifemale family lines in half-acre corrals. Adult males (n = 38) from three corrals were assigned to each condition: maxillary canine crown reduction (n = 12), canine tooth blunting (n = 12), or no manipulation (n = 14) of the canine teeth. Trauma in all occupants of these cages was scored and aggressive interactions were sampled twice weekly during the following breeding season. Mixed-model ordinal regression analyses show that the severity of laceration and puncture trauma increased with the rate of contact aggression by adult males, but was lower for animals in the blunt (treatment × male aggression: $\beta = -3.33$, se = 1.51; $P = 0.03$) and cut conditions (treatment × male aggression: $\beta = -1.71$, se = 1.20, $P = .16$). These preliminary results indicate that both canine crown reduction and blunting reduced the severity of trauma, contributing to improved welfare for the group. Further analysis is planned to evaluate the number of animals removed from social housing due to such trauma. Longitudinal monitoring of dental pathology is ongoing for adult males in all groups to directly compare the rate of complications for canine crown reduction and blunting.

P70 Successful Social Housing of Mature Male Cynomolgus Macaques in Mixed Sex Rooms

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We are committed to ensuring all our animals have the highest level of care and welfare. To this end, our social housing program includes placing all animals in pairs or groups. We have a rate of almost 100% success social housing juvenile and sub-adult animals, as well as adult females. Social housing of sexually mature males can be a challenge. Some publications suggest that it is not advisable to attempt adult male introductions in mixed sex rooms of rhesus macaques (*Macaca mulatta*). However, separating animals into single sex rooms introduces a potential scientific confound as well as operational inefficiencies, and to our knowledge this data has not been published for cynomolgus macaques (*Macaca fascicularis*). Data was collected from 2 sites that are actively social housing mature males. For the purposes of data collection, sexually mature males were defined as 5 y of age and 5 kg or greater. As is common in toxicology research, all animals had a narrowly defined weight range. A pair/group was considered successful if they had maintained compatibility for a minimum of 2 wk. All social housing attempts were made in rooms with females present and visible to the males. A total of 21 rooms were analyzed, and 81% of the rooms surveyed had success in pairing the majority of males in the room. Fifteen rooms had a success rate of 75% to 100% (median 92%) with more than 280 male animals being socially housed. This data shows that mature males can be successfully socially housed in rooms with females. The ability to compatibly socially house mature males in mix sex rooms does not compromise welfare and allows for greater flexibility of vivarium space usage.

P71 Assessment of Inhouse Refurbishment of Automated Water Valves on Flood Incidence

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Automatic watering systems provide many advantages over other methods. They are less labor intensive and provide unlimited water on demand. However, water valve failures are known to occur causing significant morbidity and mortality to rodents within closed caging. Eight years after facility commissioning, cage flood frequencies in a vivarium with an average daily census of approximately 9,000 mouse cages began to noticeably increase. Due to the increased incidence of flood events, an investigation into current valve function concluded the need for diffuse valve refurbishment. However, the cost of facility-wide valve refurbishment by the manufacturer was high. A pilot study was performed to assess the viability of inhouse refurbishment of water valves using manufacturer provided instructions, tools, and parts as compared with manufacturer refurbished valves. In a single housing room containing 11 racks, water values of 2 randomly selected, 70 cage racks were either provided inhouse refurbished valves or valves refurbished by the manufacturer. The racks were then monitored for floods. The valves on the remaining racks remained un-refurbished as controls during the study. Our results demonstrated an equal number of cage floods on racks with inhouse and manufacturer refurbished valves equal to 0.89 floods/rack/30 d (n = 1 rack/treatment, 269 d). In comparison, racks with nonrefurbished valves had a flood incidence of 1.94 floods/rack/30 d (n = 9, 222.9 ± 57 d) over an equivalent period of time. This was a greater than 2-fold reduction in floods with valve refurbishment independent of refurbishment source. A cost analysis concluded that inhouse refurbishment resulted in a savings of $5.35 per valve, including an average of 2 min in staff time for service and materials. Based on the results of this pilot study with limited sample size, we concluded that inhouse refurbishment was less expensive and equally effective as manufacturer based refurbishment. Thus, the decision was made to continue with inhouse refurbishment of water values using manufacturer provided materials. Additional analysis will continue as valve refurbishment occurs to ensure that inhouse replacement continues to be as effective both in costs and cage flooding incidences.

P72 A Global IACUC Council: Harmony through Synthesis

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Humans have benefitted immensely from scientific research involving animals, with virtually every medical achievement in the past century reliant on the use of animals in the developmental process. Research using animals is highly regulated, but remains an emotive issue with the public. The Corporate Policy on Animal Care and Use recognizes the value of animals in research while balancing public concerns demanding the highest standards of animal care and welfare. Our policy has underlying principles that transcend site and national boundaries by emphasizing that the company should behave in a globally consistent manner. But when you have a gamut of variable research operations to consider, how can you create a uniform mindset that is conducive to maintaining flawless animal care, welfare, and ethics while respecting the autonomy of 10 different IACUCs/ethical review boards in 3 different countries? You devise a Global IACUC Council, of course! The Global IACUC Council, comprised of major stakeholders involved in the oversight and assessment of the animal care and use program at each site, engages members to enhance internal communication and cooperation on animal welfare and ethical issues and ensure compliance with applicable standards, guidelines, policies, laws, and regulations. Colleagues may express interest in joining the Council, may be nominated by site or global heads, and are then appointed by an internal multi-divisional governance body. Representation on the Council by animal users, regulatory, veterinary, quality, or other key colleagues who sit on site IACUCs allows for a well-rounded perspective on issues. Site IACUCs still retain integral autonomy to direct activities at a local level, but are expected to follow and enforce all guidelines adopted by the Council. Transparency, information sharing, and candid dialogue are key contributors to the success of this unique network. The Council adds value at many levels: globally, by increasing consistency and maximizing effectiveness through development of global IACUC guidelines; regionally, by identifying key opportunities for resource sharing and standardizing processes to facilitate movement of research activities or personnel between sites; and locally, by providing common resources to enhance compliance, minimize redundancy of effort, and establish a diverse network for discussion of complex issues. The Council helps to encourage efficiency through harmonization, standardization, synthesis, and implementation of best practices by adopting principles and standards that assist in managing regulatory risk.

P73 Artificial Insemination in a Colony of Duchenne Muscular Dystrophy Canines Using Affected Males

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When working with canines that have a recessive × chromosome-linked genetic disease such as Duchenne Muscular Dystrophy, successful breeding does not always produce a DMD-affected offspring. Since only male offspring are affected, litters born into our colony with a high ratio of female puppies have not always produced affected male offspring. The pairing of a wildtype male to a female DMD carrier results in only a 25 percent chance of producing an affected offspring. The resultant lack of affected DMD offspring stretches resources and, in many cases, creates canines unable to be used in the study. To create a larger colony of affected DMD offspring, affected DMD adult males can be bred to female DMD carriers; whereby, with two affected × chromosomes it is possible to have female DMD-affected offspring which results in a 50 percent chance of producing an affected DMD offspring. Unfortunately, affected DMD adult males are unable to breed naturally because of severe deterioration of skeletal and cardiac muscle. Without natural breeding as an option, artificial insemination (AI) of DMD carrier females with fresh semen from DMD-affected males was the chosen course of action. The DMD carrier female needs to be monitored throughout her heat cycles. When signs of proestrus (vaginal swelling and/or blood-tinged discharge) are noted, vaginal cytologies are collected and examined daily to monitor the estrous cycle. Once the female is in estrous AI starts and continues until the vaginal cytologies show signs of diestrous. So far we have three successful AI litters with a 60% incidence of DMD in the resultant pups. The DMD-affected colony has greatly increased since the start of the AI breeding and is helping in controlling the amount of unusable canines breed in the colony, as well as the resource used to care for them.

P74 Methods of Pregnancy Confirmation for Timed Matings

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The study of embryo development necessitates the isolation of embryos for analysis at specific embryonic dates. When using timed mating breeding, a copulatory plug does not always indicate pregnancy, so alternative methods of pregnancy confirmation should be used. Not all facilities have the ability to check for pregnancy via ultrasound, so other methods can be used to confirm pregnancy. Female mice were placed into male mice cages in the afternoon and

were allowed to remain in the cage overnight. Copulatory plugs were checked the following morning. For mice that had a copulatory plug, the initial weight was recorded. Those that did not have a plug were also weighed as controls. Mice were weighed daily as an indication of pregnancy. The duration of the copulatory plug was also monitored hourly to see if this had an effect on whether or not the female would become pregnant. Dams were euthanized at ED 10.5 to confirm or deny pregnancy. Noticeable weight gain was evident by embryonic day 7.5. External physical differences, such as enlargement of mammary glands (around ED 7.5 to 8.5), were noted in some mice. The duration of the copulatory plug was associated with pregnancy in most cases. These methods can be useful to prevent the unnecessary euthanasia of mice that are not pregnant, thus allowing further use of the mouse and reduction in animal numbers, technician time, and cost to the researcher.

P75 Sampling for Pathogens: Live Sentinel Mice vs. PCR Swabs from Exhaust Plenum on Individually Ventilated Cage (IVC) Racks

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Most laboratory animal facilities monitor mouse colonies for pathogens that can negatively impact research. Our current health monitoring program consists of exposing 3 sentinel mice to soiled bedding from 70 colony cages during biweekly cage changes. Samples are sent to a commercial laboratory quarterly alternating between PCR (quarters 1 and 3) and serology testing (quarters 2 and 4). Hoping to improve detection methods and reduce the number of mice necessary for sentinel testing, we decided to evaluate whether PCR testing using swabs collected from a single location on the exhaust plenum of each IVC rack would be equivalent to results obtained from testing sentinel animals. Our racks are equipped with HEPA-filtered supply air, and the exhaust is ducted directly out of the room through the building exhaust system. Eight separate mouse rooms were selected. Each room contained 5 to 10 ventilated racks. Samples were collected by swabbing the inside of each rack plenum at the terminal exhaust connection. Samples from racks in each room were pooled up to 10 samples per submission which represented a total of 55 racks. The results from the exhaust plenum samples were compared with the past year of results from sentinel mice. All pathogens detected during the previous year by using direct sentinel sampling and testing by serology and PCR were detected by the PCR-tested IVC rack exhaust plenum samples proving this to be a reliable method of pathogen detection. The use of IVC rack exhaust plenum swab samples may result in savings for labor costs, diagnostic costs, sentinel exposure time, and more importantly reduce numbers of live mice used as sentinels. Comparison of samples from sentinel mice compared with IVC rack exhaust plenums will continue to be performed in an effort to gain confidence and acceptance of relying on this alternative method of pathogen detection.

P76 Verification of Sanitation Standards for Micro Isolator Filtered Cage Lids

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According to The *Guide for the Care and Use of Laboratory Animals*, enclosures and accessories, such as [cage] tops, should be sanitized every 2 wk. Extended change out times have been proposed for cage lids on individually ventilated cages (IVCs) that do not directly contact the animal. IVC lids were tested for their ability to act as a barrier to compounds within cages and as a fomite for transmission to animals between cages. Sanitation levels of mouse IVC lids were tested every 2 wk for 24 wk using contact plates and ATP monitoring system. Contact plates pass level was defined as 0 to 15 colonies and set the standard for ATP pass level of ≤ 17 RLUs. FVB mice were housed in cages treated with glow powder. Black light was used to determine the spread of powder from the cage floor to the lid and IVC rack. Lids positive for fluorescence were placed on clean cages containing untreated FVB mice and black light was used to determine the spread of powder from the lid to the cage bottom. At 2 wk, approximately 30% of cage lids passed according to contact plates testing and this level remained consistent through 18 wk. In glow powder-treated IVCs, fluorescence was not detectable within adjacent cages or within cages from treated lids. Currently, approximately 25% of cage lids are sanitized every 2 wk. Sanitizing all lids every 2 wk would increase labor cost > 75%. The significance of the 30% contact plates pass rate remains unclear given the arbitrary nature of the guideline; however, extending sanitation intervals for nonanimal contact accessories remains a significant source for cost savings. The use of glow powder indicates that filter tops are effective at containing material within IVCs and suggest that cage lids could be changed every 18 wk.

P77 Refinement of Receiving and Packing Procedures to Ensure Health and Welfare in Rodent Shipping

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Availability of unique genetically modified mice has helped researchers all over the world find the perfect model for their studies. The National Institutes of Health (NIH) expects mouse resources generated with the aid of NIH funding to be shared within the scientific community. It is imperative that shipping of valuable rodents between institutions is performed correctly. The primary goal was to develop a strategy to improve the shipping and receiving process by providing better communication between the shipping coordinator, quarantine facility, husbandry staff, investigator, and outside shipping and receiving institutions. Digital photography was introduced into the rodent import process, and simple step-by-step instruction sheets were created for the rodent export process. Quarantine staff was asked to photograph all mice and cage cards upon receipt. Photographs were forwarded to investigator staff to alleviate and/or communicate concerns regarding shipment. Photographs were taken of shipping containers if problems were identified such as broken dividers and open, ripped, or re-taped containers. Pictures of inappropriate packaging were sent to the shipping coordinator, investigator, and originating institution to open dialogue about concerns, ensure appropriate reporting, and guard against future problems. Easy-to-follow steps for packing mice were written and sent to husbandry staff responsible for packing shipments for export. Packing instructions included a list of materials needed, correct identification of the mice to be shipped, and how to handle paperwork and cage cards. Information on how containers were packed was also sent to the receiving facility for each shipment going out. Feedback from husbandry personnel preparing shipments for export, as well as receiving institutions was used to refine packing instructions. These simple and inexpensive procedures have proven valuable to ensure health and welfare of valuable rodents during shipping.

P78 Maintaining Germ-Free Animals on an Opti Mouse Rack

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Experimental studies on germ-free animals in most institutions are typically performed in sterile isolators. These studies are often short term (2 to 12 wk) and usually only use a small number of cages (2 to 6). For the facilities this means breaking down and recycling several

isolators a month resulting in increased labor for the facility staff and down time for investigators. The process of breaking down, recycling, and testing is time consuming and can take up to 3 wk. Not only does this process affect the investigator's ability to start the next experiment, it requires a good deal of a technician's time, which could be spent in other ways. With so many negatives associated with this process, we sought to maintain germ-free animals on a conventional mouse rack. Cages with their own dedicated sterile supplies were maintained on a ventilated rack. All cages were housed on an Opti rack. All materials were either autoclaved or double irradiated and sprayed into the hood with 1-3-1 with a chlorine dioxide-based sterilant. Each group of cages had their own dedicated cage that contained irradiated feed and bedding. Autoclaved water bottles were sprayed in as needed. Cages were changed within a horizontal flow clean bench. Sterile gloves were used and hands sprayed prior to cage changing. Gloves were changed for each group. Fecal samples were collected weekly and tested inhouse and monthly sent out to a diagnostic lab. Investigators were trained to work in a sterile manner in a laminar flow hood prior to taking the responsibility of working with the germ free mice and changing cages. Cages were maintained germ free for 16 wk before the study was ended. We are now continuing this process with other investigators and studies.

P79 Autoclave Compared with Double Irradiated Food for Germ-Free Animals

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We maintain a germ-free facility in excess of 30 isolators. When working with germ-free animals, autoclaving feed is the traditional method used to maintain sterility. Our concern was twofold. In terms of consistency, was the feed for every cage receiving the correct exposure time during the autoclave cycle to ensure sterility? In addition, autoclaving typically causes the feed to become so hard that it is difficult for the animals to bite down on. Could the hardness of the feed after autoclaving affect animal health or our breeding results? Double autoclaving was out of the question because of it not only affecting the nutritional value of the feed, but the concern that the degree of hardness would only worsen. We decided to try irradiated diet instead. A bag of irradiated feed from various lots was sent to a diagnostic lab to test for sterility. *Bacillus* was detected in some of the samples. Working with scientists we discovered that single irradiation doesn't always eliminate all organisms, especially *Bacillus*. However, double irradiating the feed might solve this problem. We reached out to all the feed vendors to see if they carried double irradiated diets but had no success, nor were they willing to offer this as a service. Instead, we worked with a source that if we sent the irradiated feed directly to them would irradiate the feed once again. This program has been in place for over 2 y. Each lot of feed is tested upon arrival and all have been determined to be sterile. This method of double irradiation has proven to be beneficial for the animals as well. Both the health and breeding of our colony is equivalent to what we find in our SPF colony.

P80 Successful Communication of the Response Plan During a Mouse Pathogen Outbreak

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Coordinating the response to a mouse pathogen outbreak can be challenging in a large academic institution. Communication of test results and the resulting isolation and treatment measures must simultaneously be provided to investigators, animal care and use program staff, and IACUC members. If this is not accomplished in a quick, coordinated manner, the result can be confusion about isolation practices, further spread of the pathogen, and distrust between investigators and the animal care and use program. During an outbreak of mouse rotavirus, a process was developed by our veterinary, operations, and administrative leadership team to optimize communication between the animal care and use program and the research community. This process involved a carefully timed series of emails that provided descriptions of the pathogen, immediate responses, and invitations to meetings to discuss the pathogen response plan. Meetings were arranged with the entire research community to explain the response and the impact on the researchers in the rooms where the pathogen was detected, as well as on the mouse research community as a whole. Veterinarians and operations managers from the animal care and use program then met with each individual lab affected by the pathogen to complete a survey about their research needs and provide a detailed explanation of the pathogen response plan. Extensive follow-up communication continued throughout the course of all testing and treatment by email and additional meetings, as well as thorough weekly updates on the animal care and use program's website. This process has proven to be successful in ensuring transparency to the research community. It was very well-received by our investigators and resulted in excellent compliance with our pathogen response plans. Due to this positive response from the research community, the process has been tailored and utilized for subsequent pathogen outbreaks at our institution with equally successful results.

P81 Is Weekly Sanitation of the Entire Mouse Cage Really Necessary?

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The *Guide for Care and Use of Laboratory Animals* recommends that solid-bottom cages be cleaned weekly. There are many reports relating to frequency of bedding change; however, there is no published information supporting the biologic rationale for weekly change of the solid cage itself. Decreasing the frequency of cage-washing could have considerable impact on labor, energy, and water-use efficiency in a rodent facility. In this study, organic contamination of cages that had bedding changed but no additional sanitation for 6 wk was compared with contamination of cages that were completely sanitized every 7 d. Adult female ICR mice were housed in polysulfone static microisolation cages with autoclaved bedding (corncob and crinkle paper). Two control cages containing 4 or 2 mice were completely changed (including cage bottom and lid, feed hopper, and bedding) every 7 d. Four experimental cages housing 2 to 4 mice were not changed or washed, but soiled bedding was dumped and autoclaved bedding added at d 7, 14, 21, 28, and 36. The ammonia concentration was measured in each group prior to dumping the bedding. Contact plates and ATP samples were obtained from inside the cages after the bedding was dumped in both groups. Body weights were taken at every time-point for all cages. There were no significant differences in body weights, ammonia levels, ATP counts, or colony forming units as measured by contact plates between control cages that were replaced with freshly sanitize cages every week, and experimental cages that had only soiled bedding changed. This study provided preliminary data relevant to establishing a validated frequency for sanitization of rodent caging. Future studies are planned to validate these findings, including behavioral testing on mice to evaluate effects on wellbeing.

P82 The Design and Use of an Automated Euthanasia Station

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Exposure to CO_2 continues to be a common method of euthanasia for rodents. However, there can be issues associated with using CO_2, such as leaving the CO_2 chamber on and draining the CO_2 supply

tanks, controlling the flow rate to meet AVMA guidelines, or assuring that the duration of exposure to the CO_2 is sufficient to assure euthanasia. In addition, the methods need to be straightforward and uncomplicated. To address these concerns, we developed a simple and inexpensive method of automating our existing euthanasia stations. The euthanasia station consists of an adjustable regulator, a flow meter, and a programmable timer to control the amount and duration of gas flow into a standard euthanasia chamber. These stations were developed from supplies readily available in parts and supply catalogs, and they can be used in a central animal facility or easily adapted for use in individual investigator laboratories. The regulator and timer are preset by facility personnel to prevent accidental adjustment by the users that could result in either insufficient or excessive flow of CO_2 into the chamber. Activation requires the user to push a single button to initiate the flow of CO_2 into the chamber. Once activated, CO_2 flows into the chamber until the present timer counts down to zero (5 min for our system). The use of the automated euthanasia station has decreased the incidence of drained CO_2 tanks in our facilities, thus decreasing costs for CO_2. This system has also made the mechanical process of using the CO_2 chamber easier for the users, which has facilitated training and decreased problems with insufficient CO_2 exposure.

P83 Sand Pools as a Novel Enrichment for Rabbits

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For anyone who has housed rabbits in an artificial plastic environment, what to provide as a nonfood enrichment can be an issue. Nonfood enrichment, or environmental enrichment, can be any toy, engaging sights or sounds, or procedures that productively stimulate the animal, but does not necessarily increase the daily caloric intake. Environmental enrichment allows animals to engage in species typical behavior that may reduce the stress of the laboratory environment. While a wide number of behaviors can cross species, a typical behavior found in rabbits is digging. Our staff used 43-in. wide round plastic pools as a digging ground. The pools were filled with 3 inches of play sand. Play sand was chosen because it can be bleached, sanitized, or autoclaved and is easily commercially available. The entire pool was enclosed with a plastic playpen to contain the rabbit while still providing it with visibility of other rabbits in neighboring playpens. The rabbits were given between 45 min to 1 h to use the pool. Afterwards, any feces is scoop removed and properly disposed of. Initially the rabbits were uncomfortable in the sand pools, but acclimated overtime and began to use the sand pools as intended. We have noticed less stress-based aggression or hiding with the rabbits that used the pool regularly. In addition, our staff is able to more fully observe and evaluate the movement of the rabbits in our care. This novel enrichment also allows the rabbits to safely engage in a species-typical behavior, as well as giving them a larger space to exercise their muscles.

P84 Sterilization of Isolator Equipment with Vaporized Hydrogen Peroxide

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Sterility is of paramount importance for successful work with germ-free animals. Isolators are generally sterilized with peracetic acid introduced via compressed air in an open system. Due to the corrosive properties of this chemical, it is legally required to wear gas masks or respirators. The aim of this study was to develop and validate a safe procedure for efficient sterilization of isolator equipment by means of vaporized hydrogen peroxide (VHP) in a closed system. This method was tested for rigid isolators, semirigid transfer-isolators, and the working area of a special clean bench. The fumigation was performed at a pressure of 200 Pascal controlled by the H_2O_2-generator. A special program was developed for each type of equipment and validated by means of chemical and biologic indicators as well as microbiological swab testing. Twenty resp. 15 chemical and biologic indicators were placed at different locations in the isolator and the transfer isolator. All indicators showed a complete color change and a kill of all spores of the test organism *Geobacillus stearothermophilus* in a concentration of 10^5 and 10^6. A successful sterilization was ascertained also in locations hard to reach like sleeves and gloves. All 10 microbiological swab tests showed no growth. Prior to working with germ-free animals, the working area of the clean bench was decontaminated with a 5 log reduction, demonstrated by 15 indicators. Sterilization of all mentioned equipment was successfully reproduced 3 times and hereby validated. This method is effective for the sterilization of isolators and the transfer of different supply materials into the isolator. This has been proved by more than 6 mo of successful practical work. In conclusion, this VHP fumigation is a good alternative to the sterilization of germ-free isolator equipment with peracetic acid. This method shows a high efficacy and good reproducibility. It is easy to use and validate and offers significant advantages in terms of environmental and occupational safety.

P85 Influence of Nutritional Supplements on Growth of Weanling Mice

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Genetic engineering of mice presents husbandry challenges, including rate of mouse weanling weight gain. One local colony commonly has pups whose weaning weight at 21 d is 8 g or less, which was detrimental to both their survivability and overall health. In an effort to determine which dietary supplement(s) would best help pups gain weight, we evaluated various forms of murine supplemental nutrition and its contribution to weight gain in mouse pups. Using rodent chow on the cage floor as a control, we evaluated the efficacy of supplying moist food made inhouse and two bacon-flavored commercially available supplements (one pellet form and one a thick mash). The results were compared across colonies involving 10 strains of mice. Strains included gene knockout, transgenic and mutant mouse models, and their various crosses. Supplemental nutrition was offered to the adolescent mice ad libitum starting at 18 d of age. The pups were weighed at 18 d of age, at weaning (20 to 21 d), and at 3 and 7 d postweaning. Fresh supplemental nutrition was supplied as needed. All forms of supplemental nutrition increased weanling weights. However, the degree of weight gain varied greatly, and differences in pup weight gain may have been attributable to other factors (for example, litter size, age of dam, and pup starting weight). Only 3 strains showed a distinct preference for one nutritional supplement. One strain, OT1 P50 cRel, gained more weight when offered regular chow supplied on the cage floor, a 9% increase among males and a 2% increase among females, compared with the most effective supplement. Two strains preferred the bacon-flavored mash, however. Male and female PKC OT1 weanlings given bacon-flavored mash gained 12% and 21% more, respectively, than those of this genotype given chow. In addition, for the strain B7 ½ DKO, male mice gain 34% and females 8% more on the bacon-flavored mash. In conclusion, no one supplement ensures that all genetically engineered mice flourish and gain weight. Different strains prefer different dietary supplements. The results of this experiment indicate that more research is necessary, specifically to control for other variables that can affect weight gain in pups.

P86 Remodeling of the Animal Facility for Common Marmoset (*Callithrix jacchus*)

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As biopharmaceutical researches are advanced, nonhuman primates (NHPs) have been used increasingly in safety and efficacy evaluation for drug development. The common marmoset (*Callithrix jacchus*) is useful and valuable for those areas especially. In view of its small size in comparison with other NHPs, such as rhesus monkey or cynomolgus monkey, the common marmoset is of great value for researches. At first, to import 20 common marmosets from CLEA, Japan and to take approval as the quarantine facility by Animal and Plant Quarantine Agency of Korea, we remodeled our animal facility. And, according to law and regulation related to animal quarantine, we set up preparing/procedure room, animal holding room, and animal isolating room. As the animal holding room was originally used for rabbits, the room environments, including temperature and humidity, were adjusted from 20 to 24 ℃, 40% to 60% to 22 to 26 ℃, 35% to 50%, respectively, for the common marmoset. After cleaning and disinfection, cages for common marmosets with automated water supply system were installed. During the legal quarantine period, we recorded animal health status (feeding, body weight, evacuation, activity) and room condition daily and found that there was no evidence that the room condition remodeled was not acceptable to the common marmoset. Therefore, we provide knowhow about the procedure for import of common marmosets, including preparation for import quarantine based on the actual operation for other facility and suggest common marmosets are a suitable laboratory animal to keep in an animal facility without additional construction and equipment in Korea.

P87 A Novel Method for Sanitizing Rack-Mounted Automatic Watering System Valves on the Rack

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Rack-mounted automated watering systems used to provide water to laboratory rodents were developed to reliably deliver water to research animals and to decrease labor and costs. It could be said that one aspect of reliability is to reduce human errors, for example, personnel forgetting to refill water bottles and staff forgetting to replace water bottles after removing the bottles from the cage. In solving one problem, others were created. Primarily, sanitation of lixits between cages containing different groups of rodents and valve failure due to inadequate cleaning and maintenance of the automatic watering system. In many institutions the racks and attached watering systems may be washed or sanitized as infrequently as twice per year. These methods of cleaning/sanitation do not effectively sanitize the automatic watering system valve itself. Chemical disinfectants may be used to wipe the outside of the automatic watering system between cages. However, these chemicals are not able to affect organisms inside the automatic watering system and can leave a residue that may affect the animals' health and the study. Additionally, debris such as saliva, feed particles, bedding, and fur are deposited on and in the automatic watering system as the animals drink. Current methods of sanitizing these valves include removal of the valves from the rack and steam sterilization in an autoclave. Many institutions may not have adequate personnel or equipment to clean/sanitize the valves as frequently as needed to reduce pathogens and remove debris inside each valve. A portable steam cleaning system is being investigated as an alternative to conventionally cleaning procedures. This poster will demonstrate the effectiveness of a portable steam unit in reducing/eliminating organic material and debris from automatic watering system valves used in rack mounted automated watering systems. Additionally, the data will show that this new method is rapid enough to be used as frequently as needed to prevent contamination, due to organic contaminants in automatic watering system valves, without the need for increased labor or removal from racks.

P88 Does a Novel Diet Supplement to Rodents Cater to the "Love Match" Needed for Successful Reproduction?

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Genetically engineered rodents can potentially have reduced fecundity. A new supplemental diet (Love diet) for both male and female rodents that is rich in omega-3 fatty acids has been shown to improve reproductive performance in various animal species. Compared with standard rodent breeding diet's 7.5% fat content, Love diet has 14.3% fat content because of its high composition of fish oil. Here, we report the efficacy of Love diet by investigating various reproductive performance indexes (litter size, weaning rate, and average pup weight at days 7, 14, and 21 of age) of newly setup breeding trios of several transgenic strains historically reported as poor breeders in our institutional breeding program. Ad libitum standard rodent breeding diet was provided to mice with (treated group) or without (control group) continuous 6-mo supplementation of Love diet starting at 2 wk prior to breeding setup. Placed at the cage level to also provide an opportunity for foraging, the Love diet pellets were consumed like treats by the mice. All cages were supplied with similar bedding, nesting, and enrichment items. Although, the average numbers of pups born and weaned were not different ($P < 0.05$) between groups, pups born to groups given Love diet had increased average body weight. The ability to maintain breeding colonies of various rodent models are vital to continued innovations in biomedical research and, novel products like Love diet can be used to potentially improve fecundity in these animals.

P89 Factors Influencing Mus m1 Allergen Levels in a Mouse facility

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Animal allergies are a major occupational hazard for laboratory animal workers. Minimizing their occurrence requires information about the levels of the allergens to which the workers are exposed and the factors influencing those levels. We measured Mus m1 levels in the animal rooms and in the corridors of a vivarium predominantly populated by mice a) on days with no cage change, b) during cage change, and c) 3 h after cage change. We also measured the allergen levels in the cage wash (dirty and clean sides) and in an office, and evaluated the effects of the room's cage load, the mouse strain, and of using hoods during cage change. The mice were housed in individually ventilated cages. The number of room air changes per hour (ACH) was 10 and the air pressure in the rooms relative to that in the corridors was either –0.05 in. or +0.05 in. depending on the mice strain housed in the room. At each sampling location, we collected 2.4 m^3 air samples using rotary pumps connected to filter-equipped allergen sampling cassettes. The air samples were analyzed for Mus m1 using an ELISA method with a detection limit (DL) of 0.04 ng/m^3. The allergen levels were below the DL in almost every room not undergoing change. In rooms being changed, the allergen levels ranged from 0.05 to 0.38 ng/m^3 but they, in most cases, returned to below DL 3 h after the change. The Mus m1 levels in the corridors on no-cage-change days were below the DL; during cage change, however, measurable allergen levels (0.05 to 0.09 ng/m^3) were observed in half of the cases. The highest allergen levels (0.26 and 0.38 ng/m^3, 55 cages) occurred with CD1 mice and the lowest with NOD (0 and 0.05 ng/m3 with 154 and 167 cages); because the NOD rooms used hoods and the CD1 room did not, the effects of strain and hood may be confounded. There was a trend for a relationship between room cage load and allergen levels in Nu/Nu mice (R^2 = 0.73, P = 0.14); more data is needed to verify that this is the case.

P90 Optimization of a Radio Frequency Identification Scanner for Animal Census Data Collection

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Animal research facilities need accurate and timely information about their animal population. Radio frequency identification (RFID) scanners can be used to facilitate this process reducing the time to collect the census. Unlike bar codes, RFID tags read from a distance without needing a direct line of sight to the reader. This results in much faster reads for the RFID cards significantly reducing data collection time, but present challenges because the reader may pick up cards from adjacent rooms. When we switched to using the RFID scanner, our initial studies using the out of the box RFID scanner resulted in reading cage card information not only from the target room but also from adjacent rooms. We set up a study to optimize the antenna gain settings at which the reading of the cage cards in the target room is maximized while minimizing or eliminating the reading of cage cards in other rooms. To achieve this, we placed a known number of RFID enabled cage cards on a plastic cage rack in the target room and in rooms surrounding the target room. We then scanned the cage cards in the target room from several angles and tallied the number of in-target-room matches and misses, as well as the number of cage cards from adjacent rooms. We used gain settings of 15, 18, 20, 21, 22, 24, 27, and 30 decibels. In-room misses decreased as the gain increased. At settings higher than 22 dB, the cards from adjacent rooms started to increase. Our study results indicated that a setting of 21 was considered optimal for these rooms and cages.

P91 Radio Frequency Identification and Code Scanning Comparison Study

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Animal research facilities need accurate and timely information about their animal population. Frequently the information is obtained through manual counts or using some technology such as a bar code scanner. We investigated the use of cage cards with embedded radio frequency identification (RFID) tags as a replacement for a system based on bar code scanning. Unlike bar codes, RFID tags can be read from a distance without needing a direct line of sight to the reader. This enables them to be read at faster speeds and, we theorized, would lead to shorter data collection times. We also measured error rates (misses, extras, rescans, communication errors) with both systems. The data presented here are from 5 full animal census collection periods conducted using both technologies in parallel. The number of cages counted per census ranged from 1678 to 1904. The times reported were the times actually spent counting the cages. The results indicated that the RFID system scanned the cage cards faster ($P < 0.01$) than the bar code scanners (0.55 s compared with 1.65 s per card), with no differences ($P > 0.05$) in error rates. The error rate with the bar code system (cage cards missed + cage cards re-scanned) was 0.18% while the error rate with the RFID system (cage cards missed + cage cards read from an outside location) averaged 0.19%. The error rates for the bar code system did not include communication errors or software pop ups. The results of this study indicated that using the RFID technology the animal census data collection time can be reduced by as much 60% to 70% with no changes in accuracy, freeing up time for more value-added tasks.

P92 Establishing a Zebra Finch Breeding Flock for Research

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Zebra finches (*Taeniopygia guttata*) are not a common research subject; consequently, little data is available regarding the establishment and maintenance of a breeding flock as it pertains to specific research demands. Initial purchase of multiple founder birds (of unknown age) was made from a number of local vendors to ensure genetic variation. Five pairs of birds (male:female ratio 5:5) were placed in 36 × 18 × 18-in. flight cages. The birds were kept at 14:10-h light:dark cycle, at a temperature of 61 to 81 °F, and humidity levels between 30 to 70%. The installation of true full spectrum lights, including UVA and UVB (7100 K) wavelengths, mimic natural lighting thus promoting species-specific behaviors. To keep dominant birds from monopolizing preferred foods, multiple perches and several feeding stations were placed in the cage. To provide adequate nesting sites and eliminate competition and/or aggression, 7 bamboo breeding nests were placed at various locations within the cage. Nesting material consisted of cotton string, shredded paper, and hay (observed to be the most commonly utilized material for nest construction). Food and water were available *ad libitum*. A standard fortified seed mix was provided daily, which consisted of millet, cereal seeds, and canary grass. During egg production, dietary requirements for birds increased; egg-based food was provided 3 times a week as a supplemental form of protein, along with green leafy vegetables 2 to 3 times per week. Additionally, cuttlebones and sprays of *Seteria* millet were offered to increase foraging and species-specific behavior. After an initial acclimation period, males started building nests, singing, courtship displaying, and allopreening. Human interaction was decreased to afford the birds a feeling of security and to reduce stress. After monogamous pairs formed, nest building increased; eggs were noted approximately 6 to 10 d later. Good standards of welfare and wellbeing, along with an understanding of the diversity regarding bird behavior and physiology, are essential for breeding success.

P93 Freebies for Felines

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Institutions are in a never-ending budget battle and items like environmental enrichment tend to get overshadowed. The challenge for laboratory animal technicians is to put on their thinking caps and find novel, low-cost enrichment items for animals in their care. Keys to implementing proper and appropriate enrichment are to not only have veterinary oversight, but also to understand the natural behaviors of your animals. For anyone who has worked with felines, they know just how, finicky, and playful they can be. Years ago we began identifying low-cost enrichment items that would normally be discarded, like plastic banding from pallets, empty cardboard boxes, and wadded up paper. These new items are easily hand sanitized every 2 wk or discarded when worn, thus saving time and money by not having to use a cage washer. At the same time, we implemented an enrichment trial process to determine if a proposed item was safe and enticing for our frisky felines. Subjectively, we discovered that the cats tend to use the low-cost items more frequently than the expensive, store-bought items. Additionally having a formal system to evaluate each item prior to widespread distribution provided the opportunity for formal feedback, approval by the veterinary staff and transparency to investigators. In conclusion, an approach to environmental enrichment, as described, ensures that everyone wins. Staff and animals are enriched and exercised, and environmental enrichment costs are more manageable. The most important enrichment tools available are, of course, the technicians. Without their dedication, passion, and compassion, none of this would be possible.

P94 Increasing Compliance in Surgical Operations

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Assuring compliance within surgical operations in a cost recovery animal care program with high surgical volume can be a challenging

task. In the face of continuing financial constraints, we considered modification to our existing resources and training program in the evaluation of means by which we could improve compliance. We determined that most compliance issues were related to the following: 1) a delay between provision of training and implementation of training; 2) a gap between our curriculum and the specific surgery undertaken; 3) a gap between the methodology used in our curriculum and that used by investigative staff; and 4) a gap between our curriculum and lab specific training. To address these issues, we elected to divide the existing surgical training program into two components: a theoretical and a practical, hands-on session. The theoretical material was presented on a DVD that investigative staff completed on their own time. Their proficiency in this material is tested as a condition to enrollment in the hands-on session. This modification allowed for increased efficiency in the allocation of our existing training staff and shifted the emphasis onto the more practical components of the module. We also redesigned the curriculum to be a best fit to the methodology most commonly used in our facilities. To address the remaining causal factors contributing to compliance issues, we elected to establish post-training follow-up sessions tailored to the specific surgery undertaken by the lab. This allowed for establishment of proficiency in basic surgical technique as implemented through a specific surgical methodology. Through this comprehensive approach we were able to achieve a significant reduction in compliance issues related to our surgical operations.

P95 Using Project Management Principles to Evaluate and Implement New Cagewash Chemicals

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Using strategic planning and project management principles, we evaluated and implemented newly marketed acid and detergent cagewash chemicals, across 9 cagewash facilities. We used SWOT analysis during project planning, identifying the new products' potential strengths (reduced cost and improved safety profile) and weaknesses (unknown efficacy); opportunities to implement the change (new facility could be used as independent test site); and potential threats (stakeholder resistance) to the use of new products. To obtain buy-in from departmental leadership, we provided a comprehensive project plan including our experimental design to measure sanitization efficacy, and a timeline to complete the project that included routine progress reports. We then tested both the old and new detergent and acid products in the rack washer, assessing sanitization of rabbit cages and pans, and soiled feeders and toys from large animal pens. We also tested old and new acid products in the tunnel washer, assessing sanitization of mouse cage bottoms and lids, water bottles, and stoppers. For each wash, we swabbed designated areas on the equipment, and measured bacterial reduction between pre- and post-washing using contact plates plating, and reduction in adenosine triphosphate using bioluminescence monitors. We repeated each test for each type of equipment 3 times. We observed no significant difference in bacterial reduction ($t(11) = 1.963$; $P = 0.08$) or adenosine triphosphate reduction ($t(11) = 0.992$; $P = 0.33$) between the old and new products, in the rack washer trials. In the tunnel washer, we observed no significant difference in bacterial reduction ($t(11) = 0.894$; $P = 0.47$), or adenosine triphosphate reduction ($t(11) = 1.142$; $P = 0.31$) between the old and new acid products. In accordance, we transitioned to the new chemicals at most facilities, and our reserves of the old products will be used within our largest facility. Over the next year, we will monitor changes to equipment longevity in facilities using the old and new products. When fully implemented, we anticipate a 37% reduction in annual chemical costs.

P96 Detection of Infectious Agents by Exhaust Air Dust PCR on an IVC Rack with Cage-Level Low-Efficiency Air Filtration

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The successful use of exhaust air dust (EAD) PCR testing of IVC racks has been previously reported for fur mites and for small panels of agents. Our laboratory and others have published findings that many commonly reported agents do not transfer efficiently to bedding sentinels. Using large panels of assays, we have obtained feasibility data supporting that EAD testing could be used as an alternative or addition to sentinel-based health monitoring for IVC racks with unobstructed cage-level air filtration. To gather more data on EAD testing and to investigate the impact of air flow obstruction, we performed a controlled study on an IVC rack with disposable cages containing an incorporated low-efficiency cage-level air filter. Per each independent side of the IVC, 5 cages each with 2 pet shop mice were used to provide soiled bedding to 4 sentinel cages. Screening was performed monthly for 3 mo. Of the 16 infectious agents detected in the pet shop mice, at 3 mo, 5 were detected or moderately detected in sentinel cages, whereas 11 were detected by PCR testing on a filter inserted prior to the manufacturer prefilter assembly. PCR testing of swab samples representing vertical plenum/gasket area or manufacturer pre-filters yielded poor results. PCR testing of sentinel cage air filters detected 13 agents over the combined 3-mo period, and testing filters covering the entire aperture of the vertical plenums over a 5-d period post study detected 12 agents. Contrary to feasibility data collected previously for IVC racks without obstructed airflow at the cage level, most protozoa were detected on cage filters but not other EAD sampling locations on the current rack. Despite this deficiency, some of the EAD sampling locations yielded detection of the majority of agents of which most were not detected in bedding sentinels. Findings of this study suggest that PCR testing of concentrated EAD on this IVC rack can improve the detection of agents over traditional soiled bedding sentinel use.

P97 Investigation of Real-Time PCR-Based Pathogen Screening for Monitoring Mice in Flexible Film Isolators

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PCR panel-based pathogen screening has become more economically feasible because sample types can be pooled to some degree and there is a potential to reduce or eliminate sentinels and the associated husbandry, shipping cost, and multiple traditional screening methods. To address pathogen screening for thousands of flexible film isolators within our facilities, we proposed considering the use of real-time (rt)PCR-based pathogen screening for replacement or partial replacement of sentinel use. Based on a pilot study using flexible film isolators containing either pet shop mice or rats, we identified optimal sample types for the detection of a large array of rodent pathogens. To further investigate alternative isolator monitoring, we designed a larger study to compare rtPCR-based screening of direct sampling and environmental samples with contact sentinels screened by traditional methods in 13 isolators containing genetically modified mice. Direct PCR sampling included pooled feces and body swabs and pooled environmental sampling, including an exhaust port and floor swab. In this investigation, contact sentinel use and rtPCR equally detected MNV and *S. aureus*; however, there were minor to substantial false-negative findings by sentinels use for all other agents detected. Both direct sampling and environmental sampling detected most agents by rtPCR, except *Pneumocystis* and *Cryptosporidium* which was found only in the environmental samples. Average estimated target copy numbers was highest by direct sampling for *Helicobacter*, *Campylobacter*, *S. aureus*, *K. pneumoniae*, *P. mirabilis*, *P. pneumotropica*, *Murine norovirus*, *Spironucleus*, and *Entamoeba*, but highest by environmental samples for *K. oxytoca*, *P. aeruginosa*, and fur mites. We conclude that PCR testing of noninvasive samples in combination with environmental samples improves the detection of infectious agents of mice maintained in flexible film

isolator over sentinel use by traditional screening methods.

P98 Gaining Efficiency through Implementation of Computer-Based Training

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We are committed to the ethical treatment and high-quality care of all animals used in research. Two important strategic imperatives are to never waver from our pursuit of providing flawless animal care and welfare and to maintain compliance with external, internal, regulatory, and AAALAC standards and to leverage our scale and expertise to harmonize and create efficiencies and flexibility globally. To achieve these goals, the Comparative Medicine (CM) Department implemented a Global Training Program that has required standardized training, measureable competency criteria, trainer, and subject matter expert (SME) qualifications, and electronic documentation. To further create efficiencies in training while standardizing the information transferred and retained, CM has implemented several computer-based training (CBT) modules for disseminating information that does not require hands-on instruction and evaluation. This has increased our efficiency by allowing trainers to focus on applied technical training and permitting trainees to complete the CBT training at their own pace. Also included in CBT courses are knowledge checks at the end of each section which ensures that each trainee has retained the required information.

P99 Development of Safe Handling and Husbandry Practices in the Reservoir for Lassa Fever Virus, *Mastomys natalensis*, in the Animal Biosafety Level 4 Laboratory

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We recently acquired a breeding colony of *Mastomys natalensis* from Mali, Africa, to study the reservoir of Lassa fever virus (LASV). *Mastomys* have proven to be much quicker and more escape-prone than most laboratory mouse models, therefore, have the higher potential for escape and bite incidences. LASV is a select agent and must be worked within an Animal Biosafety Level-4 (ABSL-4) laboratory. Several parameters to obtain safe and practical methods for Mastomys husbandry and handling had to be re-evaluated. These include: 1) a revised caging system; 2) sedation of the *Mastomys* within the microisolation cage before removal of animals for any procedure, including routine husbandry practices and/or experimental manipulations; and 3) restricting all work to small, confined procedure rooms for ease of recovery in the event of an escape. In addition to changes in basic husbandry and handling practices, baseline hematological, blood chemistries, and histologic parameters for this novel animal model needed to be established. The implemented handling precautions have reduced the risk of escape and/or bites for individuals handling the *Mastomys* research colony. In addition, the established hematological, blood chemistry and histologic parameters have proven invaluable to the ongoing research with this animal model.

P100 Statistical Modeling and Computer Simulation Approach for the Assessment of a Health Monitoring Program for Immunocompromised Mice within a Flexible Film Isolator

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Health monitoring is a critical component of good biosecurity practices in a laboratory animal facility. Best practices for health monitoring of animals are essential to prevent disease introduction, as well as transmission to other naïve animals in the colony. Timely and early identification of infection is crucial in a commercial breeding facility, especially in immunocompromised rodents. To the authors' knowledge, there have been no published studies using data-driven formulation or evaluation of sentinel health monitoring programs in flexible film isolator settings using computer simulation. A discrete-event computer model, created for this purpose, was based on different assumptions and parameters and was used to assess various proposed sentinel programs for breeding colonies of mice maintained within 162-cage flexible film isolators. The following assumptions and parameters were included in the model: number and type of sentinel animals, frequency of testing, number and frequency of dirty bedding transfer to sentinel cages from colony residents, rate of spread of infection, type of infection (bacterial or viral), length of incubation time (using typical values for both bacteria and viruses) and sensitivity/specificity of the diagnostic test. For each set of parameters under consideration, the model was utilized to simulate 10,000 replications of a random infection. Results included median time (and number of testing intervals) needed to detect infection and also percentage of infection undetected during each testing interval. With this process, we were able to theoretically predict the effectiveness of potential improvements in our sentinel rodent health monitoring program in flexible film isolators. Computer modeling is an innovative approach that could also be utilized in academic, commercial, or other laboratory rodent operations in determining the most appropriate health monitoring program under various circumstances.

P101 Searching the Literature for Animal Housing and Handling Information

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Animal caretakers, veterinarians, and researchers often look to the literature for ways to refine animal housing and handling procedures at their facilities. Locating this type of information can be tedious since authors and indexers infrequently and inconsistently use terms like "environmental enrichment," "animal use refinement" or even "animal use alternatives." This discussion walks information seekers through the process of conducting a search to find housing and handling ideas in the literature. This includes suggestions for selecting appropriate databases, websites, and primary sources such as journals, books, and conference proceedings; identifying useful housing and handling terminology; developing search strategies that link keywords correctly with Boolean operators; and discovering and saving relevant results. The ability to create robust searches is essential when looking for information on any topic and knowledge of appropriate databases and terminology can lead to ease of retrieval.

P102 Useful Rodent Enrichment: Party in a Bag

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Based on standard practice, all laboratory animals should be given enrichment for physical and mental wellbeing. However, depending on the type of enrichment, it can become costly or impede with accurate health observations. We assessed different types of enrichment to determine if we could identify one that would be the least labor intensive and preferred by our mice. We compared singly housed male and female mice under 3 enriched conditions, a cotton bag stuffed with paper strips, a circular disk of bound paper strips, and our standard facility enrichment (a toilet paper roll, paper strips, and a cotton fiber square). Two cages, containing either a single male or single female mouse (weanling aged) were setup for each type of enrichment, and observed for a 3-wk period. A score chart was developed and photographs were analyzed to determine the use of the enrichment and nest quality by each mouse. The enrichment was

replaced weekly. The males were indiscriminate towards the type of enrichment and had better quality nests earlier on than the females. By the second week all mice of both sexes formed nests with the standard enrichment and the cotton bag enrichment. The female mice in the same time period separated the circular disk enrichment, however did not create a nest with it. In summary, the least labor intensive and most preferred enrichment amongst our mice would be the cotton bag stuffed with paper strips. Not only can it be used as enrichment for mice but it allows for easy health observations by animal care staff. It can also serve as a visual indicator of mouse health status by latency to build and the quality of their nest.

P103 The Use of a Mock Animal Room for Training and Validation of Animal Technicians

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Business process improvement (BPI) involves a series of steps to identify, analyze, and improve processes within an organization to meet new goals and objectives. We used a mock animal room to implement BPI with regard to performing animal observations. Animal care technicians are responsible for both observations and husbandry of the animals. All animals are observed twice daily during the standard work week and once daily on weekends and holidays. To verify that husbandry and animal health issues were being identified in a timely manner, a mock animal room was created. Fifteen cages staged with common rodent husbandry and health issues (for example, no food, low water bottle, dead mouse, etc.) were planted in the mock animal room. Technicians were asked to do their regular daily observations in the room. They were evaluated on identification of the planted cages and the amount of time required to complete the observations. Only 2 out of 8 technicians located all of the anomalies (average = 12.8 of 15). Time required to complete the observations ranged from 7 to 25 min. Based on these results, a standard operating procedure for conducting observations was developed and all technicians were re-trained to follow this procedure. Follow-up testing was conducted using the mock animal room with new scenarios. The mock animal room provided a tool by which discrepancies in animal observations could be identified, new processes could be developed and analyzed, and improved performance could be validated.

P104 Comprehensive Documentation of Training for Researchers and IACUC Members

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In the laboratory animal field, training comes in many forms and from many sources, making proper and accurate documentation of training difficult. Hands-on training for animal procedures and instructor-led training are generally easily documented since this training is done in the facility by a trainer or subject matter expert (SME). Training that is acquired by attending external meetings is often difficult to document. The NIH *Guide for the Care and Use of Laboratory Animals* states that "institutions should provide appropriate education and training to members of research teams…to ensure that they have the necessary knowledge and expertise for the specific animal procedures proposed and the species used." Part of this necessary training includes the researchers keeping current in their field of expertise. Since most of this type of training is acquired off site, documentation of attendance at these meetings or seminars is typically inconsistent. Most of our training is documented in a Learning Management System (LMS). Our global training team enters completions into the LMS as competency criteria are met for skill based techniques. Computer-based training is entered electronically into the LMS as courses are completed. Prior to implementing our comprehensive documentation system any training acquired through external meetings or seminars was not documented in the LMS because these meetings were so diverse and specific to each researcher. This training attendance was typically stored in a folder by the trainee but not added to the LMS. Therefore, when training transcripts were pulled from the LMS, these meetings and seminars were not listed. To rectify this problem, Comparative Medicine devised a simple but effective method of collecting the necessary information on attendance at external meetings and seminars for all colleagues involved in animal based research. This information can now be easily uploaded into our LMS and comprehensive transcripts can now be pulled that contain all training acquired by each colleague.

P105 Evaluation of 4-Week Intervals When Changing Grids on Small Rodent Cages

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According to the *Guide for the Care and Use of Laboratory Animals* it is recommended to change the wire bar lids/grids that hold the water bottle and pelleted feed on small rodent cages every other week. The process to change the wire bar lids is time consuming and provides staff with a large workload in changing and washing them. By changing the wire bar lids every 4 wk, instead of every second, we can reduce the workload, costs, and animal stress. In association with an independent company we have made measurements using an ATP meter that measures the amount of ATP. This provides a measure of cleanliness and hygiene on the wire bar lids. ATP shows live activity and is found in all living cells such as bacteria, yeasts, and fungi. Therefore these measurements do not show residue of food and dust. Our acceptable values are 0 to 50 fmole. The tests were performed as one sample on day 0, one sample after 2 wk and one sample after 4 wk. These measurements were made in a variety of cages with males or females, group housed or single housed. We have measured the time consumption for cleaning and washing the wire bar lids. In addition, the cost regarding the washing of wire bar lids was calculated. The results of this study show no significant difference in cleanliness at week 4 compared with week 2. At the same time costs are reduced when washing every 4 wk instead of every 2 wk. We therefore conclude that changing the grids can advantageously be carried out every 4 wk.

P106 Evaluation of Individually Ventilated Cages and Microisolation Cages on Hydration Status in Neonatal Mice

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Transepidermal water loss (TEWL) represents a major component of water balance, and TEWL measurement is a new technique for quantification of water turnover in mice. The effect of ventilated caging on the hydration of neonatal mice was studied as an indication of health status in 4 common laboratory mouse strains: SKH, nude, C57BL/6, and FVB. Ten cages for each strain were set up as monogamous breeding pairs and observed daily for 6 wk. Mice were housed under static conditions on a free-standing rack, or an individually ventilated rack (IVC). Date of birth was recorded as day 0. Starting day 1, cages were changed, and pups were counted and tattooed. Weight and TEWL measurements were recorded daily as an indication of hydration status through day 10. Intracage humidity and temperature were measured daily. Mortality was determined from the number of pups remaining at day 10 compared with day 1. One litter per cage was followed for data collection and pups were sexed and weaned at 21 d. There was no significant difference in the number of pups born per litter per strain when comparing static to IVC housing. The ratio of males to females approximated 50% for all strains and total numbers of litters per strain was not different for static compared with IVC housing. Pups from all strains demonstrated linear weight gain and TEWL level; however, static housed pups

had higher TEWL for SCID, SKH, and FVB strains by d 10. Temperatures within IVC ranged from 75.6 °F to 78.4 °F compared with 70 ± 0.1 °F in the room. Humidity within IVC ranged from 39.5% to 60% compared with 24% ± 2% within the room. These data indicate that pups housed in standard IVC do not have increased water loss through nonfurred skin and do not display signs of dehydration as indicated by TEWL and weight gain.

P107 Implementing a Visual System for Rodent Cage Identification during Disease Outbreak

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At a large land grant institution with an annual census of over 160,000 mice, communication between diagnostic laboratory personnel and husbandry and investigator staff can be a challenge when excluded agents are identified on routine sentinel testing. The primary concern is communicating the importance of keeping mouse colony cages with their assigned sentinel cages throughout the entire testing process. Maintaining dirty bedding sentinels with the correct colony group ensures the effectiveness of disease transmission and detection. A visual identification system using novelty stickers was implemented when excluded agents were detected in 8 rooms used by multiple investigators in a single vivarium. This system allowed personnel and lab staff to keep colony groups separated and in contact with the correct sentinel cage throughout the testing and eradication process. Small stickers, approximately 0.25-in. diameter, were used to preserve cage card visual clarity but still easy to identify and locate. Sticker packs containing 5,100 stickers were purchased at a cost of US$13.49 each. Rooms involved had a minimum of 5 ventilated mouse racks and averaged 120 cages per rack. Each rack was divided into 4 quarters with an allocated sentinel cage. Each rack quarter was assigned a unique sticker which was affixed in the upper right hand side of the cage card and with a corresponding sticker and written rack location on the sentinel cage. A total of 160 sentinel cages and 2 packs of stickers (cost less than US$30) were used during the eradication process. Minimal technical time was needed to apply the stickers and maintain an adequate stock of additional cards for PI use. Sentinels were tested monthly until 6 consecutive negatives were achieved. This simple and inexpensive system allows for rapid identification, easy tracking, and notification of personnel when positive animals are identified in large complex mouse colonies.

P108 Successful Population of Athymic Nude Mouse Breeding Colonies in Flexible-Film Isolators Using Intensive Microbial Screening

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Our project to populate breeding colonies in 23 new flexible film isolators was driven by the need to rapidly expand our Hsd:Athymic nude-*Foxn1*^{nu} (Athymic Nude) mouse colonies that met our microbial health standards. In developing this plan, 2 methods were considered: embryo transfer rederivation or intensive screening of current isolators for populating other vacant isolators. Due to time constraints, we chose the second method, and developed an enhanced plan of reviewing the microbial health status and genetic history of animals within the isolators to determine appropriate donor colonies. Once these donor isolators were identified, more advanced microbial health screening was carried out, and genetic testing was performed to confirm concordance with the athymic nude genetic background. Upon approval of donor isolators, a small number of animals were transferred into new, sterile isolators via carefully planned and well-controlled processes. These newly established colonies were then subjected to intense sentinel animal exposure and were monitored to ensure that the transfer was not compromised and the transferred colonies met our microbial health standard. Our process produced 23 newly populated isolators over several months' time. This resulted in athymic nude mice being available to our clients that had been sourced from already existing colonies. These isolators, maintained at three North American sites, still remain free of microbial pathogenic or secondary opportunist bacteria and fully meet our microbial health and genetic standards since initial population in late 2012. In conclusion, while our flexible-film isolator colony expansion procedures may not be appropriate in all situations, in instances where only a relatively short period of start-up time is available and embryo transfer rederivation is not a feasible option, we believe our procedures are a relatively fast and effective approach to consider.

P109 Vaccinating for Compliance

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Monitoring of animal use protocols for compliance is a requirement of the Animal Welfare Act, the *Guide for the Care and Use of Laboratory Animals*, the Public Health Service Policy on Humane Care and Use of Laboratory Animals, and the United States Government Principles for the Utilization and Care of Vertebrate Animals Used in Testing, Research, and Training. Although the regulations are the same for all institutions, there are differences in how each institution applies the regulations to their program. These differences can lead to noncompliance when investigators move from one institution to another. An ideal method of addressing these types of issues is to provide some immunity to new PIs. The compliance team at this institution employs the use of a series of "vaccines" to ensure compliance for investigators new to the institution as well as existing investigators. The components of the "vaccine" entails visits, advising, conducting protocol discussion, congruency checks, inoculations of regulatory updates, noncompliance support, and electronic mechanisms for tracking and follow-up. The compliance "vaccine" program has shown to be an effective means of preventing noncompliance and for providing ongoing compliance oversight.

P110 Elimination of Mouse Hepatitis Virus Infection in a Large Mouse Colony through Biocontainment in IVCs

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Hepatitis virus (MHV) is among the most prevalent mouse viruses interfering with research results in contemporary animal facilities. MHV is highly contagious and transmitted by direct contact, feces, and air. Fecal shedding of MHV in immunocompetent mice is short (1 to 2 wk) and ends after seroconversion. Infection of susceptible young mice maintains the infection chain. Biocontainment in individually ventilated cages (IVCs) might stop the infection chain. An MHV positive colony of about 8,000 mice was moved from open cages to IVCs without hygienic rederivation. The cage system in a large barrier was changed sequentially room by room over a period of 4 mo, and mice housed in IVCs were changed in cage changing stations thereafter. MHV seroprevalence of retired breeders or experimental mice in the 13 rooms of the barrier at the time of IVC introduction was between 21% and 94%. The colony was composed of various strains of immunocompetent genetically modified mouse lines mostly on a C57BL/6 background used for both breeding and experiments. Breeding was not discontinued when the cage system was changed. Health monitoring in in open cages and later in IVCs was performed with used bedding (UB) sentinels. All UB sentinels had been positive for MHV at all quarterly tests of the previous 2 y. To closely monitor changes in MHV prevalence after introduction of IVCs, once a month, all mice grown 12 to 16 wk old in one of the rooms were tested for MHV antibodies by ELISA (positive results confirmed by an IFA). Hygienic monitoring of the whole barrier was continued by UB sentinel monitoring. MHV seroconversion of young susceptible mice in the one room tested dropped from 40% to 0%

within 4 mo after introduction of IVCs. UB sentinels monitoring the whole colony became negative for MHV within 1 y and stayed negative for the following 2 y. We conclude that use of IVCs interrupted the infection chain. Introduction of IVCs might be a strategy for MHV elimination even in big mouse colonies.

P111 Custom-Designed, Self-Contained, Mobile Rodent Anesthetic and Surgical Station

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The provision of a dedicated work space with appropriate anesthetic and surgical equipment during the conduct of rodent survival surgery is emphasized in regulatory guidance and meets current standards of veterinary care. Acquiring space and equipment can be challenging in busy scientific laboratories with space limitations and recent funding scarcity. In order to provide a resource that would address this problem for investigators, a self-contained, mobile rodent anesthetic and surgical station was designed and developed. The unit is a custom mobile tower with multiple drawers, cabinets, and drop-down shelves that contains all equipment and work surfaces necessary for rodent survival surgery, including an isoflurane vaporizer with oxygen tank and anesthetic waste gas scavenging, external heat source, clippers, expendable injection and surgical preparation supplies, and personal protective equipment. The unit is constructed from materials that are all easily sanitized. Investigators can schedule use of the unit as they would a rodent operating room and be confident that they are using equipment compliant with regulations and best practices. The custom-designed, self-contained, mobile rodent anesthetic and surgical station is a creative and practical addition to our animal research program and received a commendation from AAALAC site visitors during a recent triennial accreditation site visit.

P112 Improved Workflow and Ergonomic Safety Practices in Soiled Cagewash Operation

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The Animal Resources Department (ARD) manages a mouse barrier facility housing approximately 14,000 IVCs on a 7- to 10-d cage changing cycle. Cagewash processes approximately 3,000 cage bottoms daily. Over the past 2 y, ARD has had the highest injury incidence rate with most injuries occurring in soiled cagewash. In collaboration with our EH&S department, we identified a poorly distributed workload with improper ergonomic practices. These resulted in unnecessary repetitive strain injuries, which directly impacted employee time on the floor and productivity. Originally, technicians were dedicated to either scraping/dumping or loading the tunnel washer. During these tasks, technicians were rushing and displayed bad habits of twisting, banging cages, and using excessive force, which caused musculoskeletal injuries. Even though these tasks were being performed very fast, output was below expectations and produced several bottlenecks. To address these issues, we restructured workflow by dividing dumping, scraping, and loading procedures into 3 separate stations with rotations every 2 h. A neutral 2-hand technique was developed to improve biomechanics. Dumping removed all bedding in 15% of cages and the remaining cages were manually scraped using novel ergonomic scrapers. Despite this restructured workflow, we still experienced morning delays and an uneven flow of soiled cages from husbandry resulting in frequent equipment downtime and backlog of soiled cages at the end of the day that impacted workload on the following morning. By modifying the operations work schedule to a staggered shift 2 h apart, we eliminated this backlog and gained maximum efficiency and throughput from the equipment. Technicians were no longer overwhelmed and under pressure to catch up from a backlog of previous work as they began their day. As a result of these efforts, we observed an increase in productivity, a decrease in repetitive strain injuries, and an increase in employee morale, all of which led to a better work environment.

P113 A Simplified Method to Identify and Reduce Flooded Rodent Caging

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Rodent cage flooding can present a significant animal health concern, since prolonged animal exposure to water can cause animal hypothermia. However, rodents must have access to water as it is essential to their health and welfare. The means of water delivery can impact flooding frequency, whether it is because of training or equipment malfunctions. Although there are a variety of water delivery methods, water bottles and water pouches are the focuses of this discussion. The number of flooded cages was tracked and it was identified that the majority of leaks and flooding occurred shortly after cage changing was complete. Based on these findings, an evaluation was conducted of the entire cage changing process. Cage assembly was identified as a potential point of improvement. To reduce the occurrence of flooded caging, our program developed a prescreening process. This allows us to identify leaking water bottles and pouches prior to housing animals. The additional advantages are reduced labor and reduced risk of water and cage contamination. Water bottles and pouches are prepared in the clean room by the technicians and are not handled again, not even at the cage changing process. The amount of labor dedicated for rodent cage assembly and cage changing was analyzed using a time and motion study. It was discovered that preassembling entire clean cages, to include bedding, hoppers, water bottles or pouches, and filter top presented only minor labor increases. Cage changing with fully preassembled cages presented significant time savings, due to the reduced number of cage handling steps, which we believe also reduces the risk of potential contamination. In summary, by restructuring existing cage assembly tasks, a pre-screening method was established which allowed us to identify and reduce flooded caging, decreasing animal health occurrences related to hypothermia, reducing overall labor required to cage change, and minimizing the potential of water and cage contamination.

P114 Electronic Animal Health Reporting Made Simple

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In many laboratory animal facilities the task of health reporting is the responsibility of the animal care staff (ACS). When commercially available electronic systems are not in place, manual means to create and exchange animal health reports (AHRs) can cause unnecessary delays for animal treatment. Having an efficient process in place is crucial for the welfare and wellbeing of the animals and a successful program. Our program has streamlined the process by using an electronic AHR form which can be implemented easily in other animal facilities to enhance the service provided to the veterinarian, principal investigator (PI), and supporting research staff. We eliminated the process of hand writing AHR's and scanning them into PDF format for email notification. Instead, an electronic AHR form in PDF format was created, which can be edited accordingly and emailed directly from the animal holding room. Along with the electronic AHR form created to fit the needs of the program, our program uses standardized health report emails, distribution email lists, and portable laptops (Wi-Fi needed), in order to expedite the animal health reporting process. By developing this system and creating the electronic AHR form, the reporting process flows with

greater ease for the vivarium, veterinarian, and research staff. The results are: (1) ease in reporting, (2) consistency in delivery, (3) an electronic library (4) faster turnaround time, (5) and most importantly, happier animals. Upon implementation, the laboratory animal research program experienced a decrease in additional follow ups needed and a faster response for treatment approvals. The administrative requirements are minimal in order to implement these steps for faster reporting and response. The electronic reporting is also convenient for veterinarians or researchers who are remote or not readily available at all times.

P115 Colony Management of Genetically Engineered Mouse Models

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Genetically engineered mouse models (GEMM) have been proven to be a powerful tool in drug discovery and biomedical research. As models become more complex and the demand increases, it is vital that new tools and strategies are developed and implemented to improve efficiency, meet the increasing demand, and mitigate for risk. We have adopted traditional methods and developed new innovative tools for high throughput colony management. We developed a colony management system (CMS) that uses barcoding and real-time data entry, allowing tracking of all relevant information down to the individual mouse level and brings all the information about a colony together in one place. CMS enables electronic tracking of health issues, directed cage change (DCC), inhouse genotyping of samples, and efficient colony management. In addition, implementation of new strategies such as cryopreservation and ultrasound has helped to address the 3Rs. The ability to generate reports to identify trends in colonies has significantly streamlined the production of experimental cohorts. Cryopreservation and termination of retired strains prevents the production of 115,000 animals per year. High-throughput ultrasound confirmation of pregnancy in plugged females allows us to reduce the number of animals required for timed-pregnant experiments by 40%. Taken together, these approaches have allowed us to streamline the production of mutant mice while conserving resources.

P116 Ultrasound Confirmation of Pregnancy in Genetically Modified Mice Reduces Resources While Enhancing Reliability

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The mouse is widely used as a model to study embryonic development, which requires tracking conception dates through identification of a copulatory plug. Due to variability in male fertility, approximately 40% of plugged females are found not to be pregnant at the time of embryo collection. Lack of reliability of this method creates a major impact to our collaborators' experimental timelines and wastes the complex mutant mice. The traditional way for assessment of pregnancy in mice is direct visual observation or abdominal palpation. The reliability of these methods prior to E12 depends on the skill of the technician and is dependent on litter size. To address this problem, ultrasound was implemented at our facility to confirm pregnancy in timed-pregnant females, and to count embryos when required. Use of ultrasound is a noninvasive, early, and reliable means to confirm pregnancy. The ultrasound process involves anesthetizing animals with isoflurane, chemical removal of the abdominal fur, imaging the animals on a heated stage, and monitoring for recovery from anesthesia. An imaging system with a RMV 704 scan head is used for imaging and a scavenging unit is used for personnel safety. We have determined that our capacity is approximately 15 to 20 scans per hour. In summary, the use of ultrasound reduces the number of animals required for experiment and also assures an adequate number of animals for the timely completion of the experimental objectives. Nonpregnant animals can be recycled for other purposes, in accordance with the philosophy of the 3Rs.

P117 Communication Framework to Maintain Containment within an ABSL2 Large Animal Facility During an Emergency Heating Ventilation and Air Conditioning Shutdown

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Containment is of paramount importance when working with infectious pathogens to protect the health and welfare of animals and personnel. An emergency steam interruption, with an outdoor temperature of 2 °C, triggered an 8-h heating, ventilation, and air conditioning system shutdown. Normally to minimize the risk of pathogen escape, entry into primary or secondary enclosures are arrested once air pressure differential between airlocks can no longer be maintained. Additionally, most research protocols in the ABSL2 facility are time sensitive and require additional animal health monitoring and supportive care. The ABSL2 facility of interest was operating a clean and dirty corridor system with 28 rooms equipped with anterooms. It was housing 350 animals infected with one of nine different pathogens. The QA technician immediately set up an email communication network between facility staff, research teams, physical resource repair staff, and veterinary services. The facility technicians and facility veterinarian devised and implemented a plan to meet the needs of the research teams and adequately monitor and treat infected animals while maintaining containment within the facility. All rooms had exterior observation windows that were used for clinical observations of illness and heat stress. Animal rooms requiring entry were accessed from the dirty corridor with disposable personal protective equipment donned at the room threshold. The facility staff and research team members were required to remain in the dirty corridor for the duration of the procedures, thereby preventing contamination of the clean corridor and communication between the clean and dirty corridors was accomplished by telephone. The rapid activation of a communication network facilitated the prompt and ongoing transfer of information between all stakeholders to prevent unintentional breaches of containment, ensure project-specific needs were met, and maintain the highest level of animal welfare.

P118 Creating an Online Work Order and Maintenance Tracking System

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In a large, multi-site research institution, tracking, reporting, and resolving facility deficiencies can be difficult and inefficient. A need was found to create an online work order submission and tracking system to increase efficiency and decrease resolution times. A system that minimizes a technician's time to report deficiencies and alerts maintenance personnel soon after the deficiency is reported decreases the amount of time it takes to address the issue. By decreasing the amount of time it takes to report and correct a deficiency, technicians and maintenance team members are more efficient. The system is designed such that a user can login and quickly report many issues to the maintenance team. The maintenance supervisor can then assign a work order to a team member who has the responsibility for that system or area. As this is done an email is generated and sent to the team member assigned to the task with all the details of the task. Once the issue is worked on, the maintenance team member will update the status of the work order which then generates an email to update the person who reported the issue. Once the work is complete, the technician that originated the work order and the immediate supervisor is alerted through an email that the work is completed. The work can then be reviewed and approved by the supervisor. Once approved, the work order is closed. Time and

materials are tracked by the maintenance team member and recorded in the work order record. Work orders can be reviewed by anyone to help reduce possible duplications and allow individuals to track the progress of the work orders they have generated. Our staff designed and built this system based on Oracle Apex development platform. Oracle database software is available in several versions and this design is supported in the no-cost version and the process can be duplicated to run in multiple environments.

P119 Application of a Reusable Chemical Gel Heat Pack to Provide Rodent Cages with Supplemental Heat

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A vital role in animal husbandry is providing care to rodents found to be hypothermic after an automatic watering system failure. One method of applying heat is to use an external warming device to warm the home cage. Circulating water blankets provide constant heat but are bulky and expensive, limiting their availability. In comparison, reusable chemical gel heat packs are inexpensive and can be distributed to multiple areas to limit concerns of cross-contamination. The goal of this study was to determine if gel packs are a viable option for providing supplemental heat by determining the amount of heat generated, the duration of heat, arrangement of gel pack and cage for efficient heat transfer, and the product lifespan. Three gel packs were activated and reset 100 times, and their temperatures were measured using an infrared thermometer to determine the lifespan of the product. A second set of gel packs were used to determine the optimal placement in contact with the cage to increase intracage temperature. A variety of orientations were attempted, including placing the gel pack under the cage or under the cage card holder, as compared with a circulating water blanket which went under the cage. It was determined that new gel packs reached a maximum temperature of 51.9 °C, which decreased to 43.3 °C after 100 resets. A color change from aqua to pink and gradual weight increase of 8.7 ± 0.2 g correlated with the overall number of gel pack resets. Placement of the gel pack under the cage provided the most efficient and rapid increase in intracage temperature of all positions tested. The gel pack achieved a maximum intracage temperature of 26.5 ± 1.2 °C after 26.3 ± 4.2 min and returned to room temperature (21.7 ± 0.1 °C) after 185.3 ± 1.2 min. The maximum intracage temperature achieved by the water blanket was 30.8 ± 0.6 °C after 91 ± 19 min which was maintained indefinitely. We conclude that the gel packs were effective at increasing intracage temperature until 50 to 60 resets and the color change to magenta allowed for a standard disposal without keeping track of resets. Gel packs effectively provide supplemental heat for two hours post activation and can be used in multiple locations independent of health status with limited fear of cross-contamination.

P120 Supplemental Diet Aids Early Weaning of Crl:CD1(ICR) Mouse Pups

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Mouse pups are routinely weaned at postnatal day 21; however, it is not unusual for pups to be orphaned earlier due to a death or severe morbidity of the dam requiring euthanasia. If a foster dam is not available, euthanasia of the pups is often recommended because of the limited knowledge regarding the age at which mouse pups can thrive. We hypothesized that with supplemental diet, orphaned CD1 mouse pups would thrive at postnatal day 14 (P14) without a dam. At P7, the litters of 4 Crl:CD1(ICR) (CD1) timed pregnant females were pooled and pups equally distributed by number and sex among the four dams. At P14, each pup was weighed and randomly assigned to either a control group (dam and pups) or one of three supplemental diet groups. Supplemental diet was introduced to mouse pups by placing it on the paws and muzzle. Thereafter, supplemental diet was provided daily in a small shallow petri dish on the cage floor. Additional daily care included observation, stimulation of the genitourinary tract (supplemental diet pups), and weighing. Although there was an initial loss of weight gain seen in pups being fed supplemental diet, these pups subsequently gained weight equivalent to the control pups and there was no statistically significant difference in final weight at P21. To ensure that the early weaning or supplemental diets did not have an adverse epigenetic effect on future reproductive performance, 2 breeding pairs from each of the 3 experimental diet and control groups were maintained and bred. All breeding pairs were fertile and produced litters that were not significantly different in litter size. We conclude that early weaning of CD1 mouse pups can be successfully performed at P14 with supplemental diet and that the early weaning had no adverse effect on future reproductive performance of the affected pups.

P121 Refinement and Efficiency through Serial Microsampling: Cost:Benefit Analysis

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Advances in bioanalytic methods allow for accurate plasma drug level measurement in small sample volumes. Decreased sample volumes enable serial blood sample collection from one rodent, rather than terminal or sparse sampling from multiple rodents. Decreased variability in data from serial microsampling would therefore be expected with a discrete pharmacokinetic profile from individual animals rather than the composite profile generated from multiple animals. While the resulting reduction in animal use was a clear benefit, the technical challenges associated with the shift from terminal or sparse sampling remained an obstacle preventing broad adoption of the refinement. We therefore conducted a cost/benefit analysis to assess internal and external costs, animal use, and potential data output from terminal sampling, sparse sampling, and serial microsampling. While sparse sampling results in decreased external costs, internal animal care costs, and animal use, technician costs are increased and data output is decreased by half. Serial sampling, however, results in decreased external costs, internal animal care costs, animal use, and technician costs with a minimal decrease in data output. In addition to annual reduction of total costs by approximately US$200,000 and reduction of mouse use by 90%, corresponding reduction of compound requirement and other unquantifiable savings, such as compound resynthesis as well as increased efficiencies were considered. Based on these findings, senior management supported broad adoption of serial microsampling as a standard for all mouse pharmacokinetic studies and strategies to overcoming obstacles in adopting this refinement for realized benefit were identified.

P122 The Use of Lean Management Principles to Enhance a Nonhuman Primate Environmental Enrichment Program

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Sewage back-up and overflows in the rooms of our primate facility initiated an analysis of the factors contributing to the clogging of the drains. An investigation reviewing the materials responsible for the clogging of the drains revealed that the use of paper trays, paper bags, or other nondissolvable materials was the main cause of this problem. These nondissolvable materials were being used as the main delivery devices for novelty food items as part of the nonhuman primate (NHP) environmental enrichment program. Rather than eliminate these essential enrichment items from the program, we

sought to improve delivery of food enrichment by implementing the use of an expanded array of delivery devices made from materials that would not clog the drains. We developed a system composed of 6 dissolvable delivery items with 7 fillers, which could be used in different combinations resulting in more than 40 distinct novel food items. As part of the process, we created a visual standard operating procedure, outlining the process for making each individual item, in order to improve the efficiency of training all of our nonhuman primate staff. An additional benefit of this process was that we were able to standardize the nutritional component of our NHP environmental enrichment program while providing novelty to the animals on a consistent basis. In conclusion, the quest to solve a simple drain clogging problem resulted in a transformation and significant positive long term impact on our NHP enrichment program.

P123 Light Exposure in Different Positions in Ventilated Mouse Racks

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Light exposure is a potential experimental variable for research rodents. Even when rodents are housed in identical cages, position on the rack and in the room can result in greatly different light exposure inside the cages. Using a light meter placed inside the cage, interior light levels were mapped for each cage position on our double-sided ventilated mouse racks. Light levels outside the cages varied with position in the room, but were typically twice as high at the level of the top row of cages as at the level of the bottom row of cages. Interior light levels were much lower than exterior levels and typically varied 4-fold between the top and bottom row of cages. Objects less than 5 ft from the rack (for example, other racks, walls, personnel) could cast shadows that decreased interior light levels by more than 2-fold. At maximum room light levels, exterior levels ranged from 450 lx at the level of the bottom row of cages to 800 at the top row, but interior levels were approximately 10 lx in the bottom cages and approximately 45 lx in the top cages, a 4-fold difference. Cages on the edges had significantly higher light levels than cages that were surrounded by other cages; the top edge cages had levels of 65 to 85 lx, and in general light levels were approximately 2-fold higher in edge cages than in the adjacent cages at the same height. Empty spots behind or below the cage being measured had a negligible effect on light levels. Empty spots above or to the side increased light levels 50% to 100%, depending on location. This detailed knowledge of the light levels in different cage spots was used to design studies to assess whether specific tumor cell lines exhibit light-sensitive tumor growth curves, and can be used to assign study animals to cage locations with equivalent light levels.

P124 Innovative Solutions through the Use of a Suite of Integrated Applications

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As the availability and power of software development increases, the opportunity to create innovative solutions to existing demands grows. RARC has seized an opportunity, with the development of an animal research management system, which is a unique application combining modules that streamlines IACUC administration, protocol development/review, cage census, animal ordering, animal health, and billing. Managing an animal care program requires precise data entry/record keeping, which is easily affected by human error and is time consuming when processing information manually. This software package was designed in response to these issues and serves to replace multiple methods that communicated independently of one another. Rather than purchase an existing electronic management system, which would need to be customized for our needs and which would offer less flexibility, RARC elected to develop a system unique to our needs. This system is designed to handle all administrative operations, including tracking census, billing investigators, and monitoring protocols. This automatic record keeping increases data integrity by reducing human error. All records and reports are offered in one location, in a manner convenient and accessible to researchers, veterinary and husbandry staff. By enabling each function to communicate with one another, efficiency, accuracy, and detail have increased. An accurate census and cage tracking system has allowed for investigators to be properly billed. With a more detailed view of total workload, scheduling has become more even. Animal health information is sent directly to veterinarians or investigators, and emergencies are handled more efficiently. This innovative response has allowed for a safer and more enriching environment for all.

P125 Evaluation of a Novel Disinfectant with Residual Action

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A novel organo-functional silane technology (OFST) disinfectant that binds treated surfaces acts by disrupting an organism's cell membrane on contact. The manufacturer indicates that organisms do not metabolize the active compound and do not become resistant. This colorless, odorless, and nonleaching compound was found to be safe and effective against a broad spectrum of viruses, fungi, bacteria, algae, and yeast. We compared OFST to a standard disinfectant using an established disinfection protocol, except that each disinfectant was applied once, by applying each compound to standard vivarium surfaces (for example, floor, wall, ceiling) and evaluating sanitation over a four week period using replicate organism direct agar contact plates to evaluate sanitation. Each compound was applied according to manufacturer's directions. Following a one-time application of the standard disinfectant and OFST compounds provided comparable results in weeks 1 and 2 of the study for the 3 surfaces studied. Bacterial counts continued to rise on the standard disinfection method during weeks 2 and 3. During weeks three and four the standard disinfection method failed contact plates plate evaluation while the OFST passed. The potential exists to reduce the labor expenditure for sanitation while providing equal or superior sanitation effectiveness.

P126 Streamlining Protocols with the Use of an Online Procedure Library

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The process of writing and reviewing Animal Study Protocols (ASP) can be time consuming for both the principal investigator (PI) and for the IACUC to review, especially when the protocols are on paper. Since we are a large institution with more than 1,000 protocols and 250 investigators, the time spent on both sides of the protocol submission process was both lengthy and consumed a lot of paper. Because the majority of reviews are handled as designated member review, and all of the protocols are housed in filing cabinets, the same procedure on one approved protocol could have undergone a completely different set of comments and revisions in the review process as the same procedure on a different protocol, even for the same PI. This can lead to inconsistent reviews and frustrated PIs, as well as the potential for noncompliance issues or incomplete protocol reviews. With the help of the PIs and a vendor, we were able to find a product allowing us to create a procedure library that the PIs could use to save laboratory procedures and substances across different protocols and reviewers could use to see which procedures have been previously approved. While the protocol is still reviewed by the reviewers in context to research aims, having the library has helped eliminate 1 reviewer on one protocol contradicting another reviewer from a previous version of the same protocol; this leads to less contradicting reviews. Also, if a new standard is available for that

procedure/substance the lab can easily see all the protocols in which it is deployed and generate multiple amendments to deploy the change to the related protocol with one click. Since deployment we have had great success and acceptance from the PIs as well as the IACUC.

P127 Determining Best Practices for Cage Bedding Volumes for Individually Ventilated Cages in Vivaria

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Bedding is a key variable in research using mice. Despite the fact that bedding is an integral component of the mouse's environment from birth to death, there is much that remains unknown about its impact on welfare and research. The gap in published data regarding ideal volumes of bedding recently came to light at our facility with the use of a newly purchased automatic bedding dispenser. Bedding the cages at a volume of 800 mL of corncob bedding for individually ventilated cages, as had been historically performed at our institution, was problematic using the new equipment and also not consistent with reported volumes of 300 to 400 mL. At stake were significant cost savings. However, providing evidence to stakeholders that the animal's health would not be compromised in the face of declining appearance of the cage was of key concern. This study compared daily ammonia levels, gross bedding appearance, temperature, and humidity across 3 bedding volumes (300, 400, and 800 mL), with bedding appearance evaluation for 3 additional volumes: 500, 600, and 700 mL. Environmental parameters were recorded over the duration of a cage change interval (14 d) and a 3-stage observation scoring system was adapted to provide a daily combined score for subjective determination of the cleanliness of the cage. With consideration of the 3Rs we used 4 cages of male and 2 cages of female mice, housing 5 mice per cage at different ages and strains from another study. During the course of the study ammonia levels remained undetectable (<200 ppm) for all bedding levels and there were no significant differences in temperature or humidity. Visual appearance scores were highest (best visual appearance) for the 500- and 600-mL volumes over the duration of the study. We have decided to use 550 mL of bedding for our cages. The process of systematically evaluating cage appearance scores and environmental parameters has resulted in conservation of institutional resources, appropriate function of equipment and buy-in from key stakeholders.

P128 The One-Minute Plan for Improving Communication

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Communication that is clear, direct, and brief is critical in engaging the research community and keeping them updated with regulatory requirements, resources changes, and the provision of training resources. Our department supports approximately 70 principle investigators, about 400 researchers, more than 200 animal use protocols, and an average daily census of more than 20,000 animals. Direct feedback from faculty and researchers pointed anecdotally toward shortcomings with communications between investigators and Comparative Medicine (CM) personnel regarding regulatory changes and policy evolution. A large-scale survey conducted by CM in 2013 formally demonstrated that a lack of communication between investigators and CM personnel results in investigator frustration, regulatory compliance issues, and reduced efficiency in the research process. A "1-min a month" email initiative was developed based on feedback from the survey. We present a mode of communication that asks researchers to commit 1 min each month, only 12 min/y, to keeping up-to-date with information about CM that directly affects their research. These clear, direct, and brief monthly emails are sent to more than 150 researchers within the organization. The emails contain succinct summaries of relevant information, including regulatory requirements, resource availability, internal policy changes, and training opportunities with hyperlinks to detailed news stories on the CM website. These emails take less than 60 s for researchers to read, while providing them with essential information for the conduct of their work. The email initiative has been well received by researchers within the organization and has resulted in more traffic for CM website resources, increased dialogue between investigators and CM personnel, and overall satisfaction from investigators and their staff about the value and convenience of this novel method of communication.

P129 Do You Know Where Your Animals Are?

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As stated in the Code of Federal Regulations Title 42 Public Health, Part 73 Select Agent and Toxin, 73.17 Records sub part 2, an accurate, current accounting of any animals or plants intentionally or accidentally exposed to or infected with a select agent must be maintained. The system that was in place at our BSL3 facility failed when one of many logs was not correctly filled out and then found during an inspection. To correct the deficiency the logs were refined so that the system became more streamlined. We start by initiating the census sheet, which are color coded by shipment when animals arrive and used daily. During their stay an Animal Use Log is filled out when animals are manipulated or euthanized. At euthanasia a Carcass Disposal Tag (with same color code as shipment) is completed and placed on the bag. The carcass bags are then refrigerated. When the animals are ready to be autoclaved out, an appropriate color coded, Carcass Disposal Log is then initiated. This log is completed when the autoclave cycle is done, showing the removal of animals from the biosafety level 3 laboratory. This system reduces the principal investigator's paperwork burden, as they fill out the Animal Use Log and Carcass Disposal Tag now compared with all of the logs. It is the responsibility of the animal care technician to complete the Census Sheet, Animal Use Log, and Carcass Disposal Log now. This also provides data entry consistency from start to finish. This system created a paper trail that tracks all animals from receiving to autoclaving with ease for all to follow. Since implementation, data entry errors have been eliminated.

P130 The Lone Surgeon: Maintaining Aseptic Technique When Performing Unassisted Survival Surgery Procedures

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The "lone surgeon" is an individual who performs rodent surgery procedures without the help of an assistant. Aseptic technique, while important, can feel like a burden when working unassisted, with equipment that cannot be sterilized, and when coordinating multiple animal procedures to maintain a specific experimental deadline. In turn, proper aseptic technique may not be followed, especially by less experienced surgeons. We have gathered guidance for maintaining aseptic technique while performing surgical procedures without assistance. This guidance will assist with facilitating research goals while maintaining compliance with research regulatory requirements and standard veterinary care. Specific topics include performing multiple surgeries in a day, presurgical planning, specialized equipment use, recordkeeping, and emergency preparedness. This guidance, coupled with video resources and training available at research institutions, will help disseminate the importance of aseptic technique when performing surgical procedures in rodent animal models. Even the lone surgeon can appreciate the need for proper asepsis and easily integrate these tips and techniques into their standard operating procedures.

P131 Neonatal Evaluation of Nursing Baboons

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Proactive management of breeding colonies requires being able to evaluate the health status of every member with minimal disruption of the animal groups. Most of the time this is accomplished by observing the animals as they move themselves around the enclosures, watching for behavioral clues that someone is not feeling well, acting differently, or has visible injuries. Trying to assess the health status of an infant "in arms" is difficult as so little of the baby may be seen if the mother is competent or tries to keep her baby hidden. This Infant Assessment System was developed to evaluate the health of mother raised infants. The first challenge was to identify traits noted in infants that were vigorous and survived the crucial first days of life. These four traits, skin color, clinging, nursing, and vocalization were the building blocks for the newborn assessments we developed. Newborn infants should have very distinctive red skin coloration. This coloring seems to fade to pink within 24 h after birth but may persist for 48 h. Any color other than this bright red and or pink could indicate that the infant is compromised. Clinging behavior can be difficult to gauge if the mother is supporting the infant well but it should be grasping the mother with all 4 ft. We like to see infants "high and tight," actually clinging to the mother's chest with their face on the nipple and no light showing between them. Nursing is the next critical step in evaluating health and vigor. Just being in the right place is not a reliable indicator the evaluator must see the mouth on the nipple. Vocalization is a big health indicator. Healthy secure infants are quiet infants, cold or hungry babies are not. We have had a few instances where an infant was too weak to vocalize but they were identified by the previous indicators. We also look for the following signs, alertness and activity level, general appearance (posture, size, clean, dry, etc.), and whether their eyes look bright, dull, or sunken. A new indicator we are looking at is tail carriage. Once the appraisal is done you are equipped to make decisions about whether any intervention is needed on the infants' behalf.

P132 Toys and Technicians: 2 Keys to a Successful Enrichment Program for Singly Housed Felines

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Social or group housing is well known as the ideal environment for maintaining cats in a research setting as it allows specific behaviors such as play, grooming, and climbing. When social housing is not possible, enrichment items and personnel interactions are essential to providing a stimulating environment. We wanted to know which enrichment items consistently allowed our cats to demonstrate species-specific play behaviors and whether personnel being present impact their level of play. A feline infectious disease project necessitating singly housed cats was conducted at our institution. We wanted to maximize the opportunities for interaction with enrichment items and identify which items they spent the most time playing with. Twenty one 14-wk-old female SPF cats were housed singly for an 11-wk study. Eight different enrichment items were rotated through their environments. Scoring was done both while the technician was present and remotely recorded video. Items were scored using a number ranking-system of low-1, medium-2, and high-3 activity levels. Three toys showed a higher level of interaction both with and without the technician present; a basic cellophane crinkle ball (2.5), a heavy duty zip tie attached to the cage bar (1.9), and a crumpled piece of paper (2.0). As the clinical conditions of the cats were affected by the virus, their overall level of play with no personnel present dropped significantly while their level of interaction with the technician maintained high. Based on this data we determined that species-specific behavior depends critically on interaction with animal caretakers and/or laboratory personnel as cats become ill in this study. We conclude that when personnel are not present, enrichment items that are most likely to encourage play and therefore species specific behaviors should be provided to singly housed cats.

P133 Environmental Enrichment for *Xenopus laevis*

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Environmental enrichment has become an important aspect of animal husbandry within research facilities. Such enrichment has been shown to improve the quality of life for research animals and provide a more natural habitat. Different enrichment tools are used to benefit several species of research animals. Our aquatic species *Xenopus laevis* lacked enrichment and this issue was largely unaddressed. We often observed physical signs of stress such as red leg, sores on the nose, and excessive skin sloughing. They also startled easily and remained in the back of the tank. In order to reduce stress and improve enrichment in the aquatic environment, we brainstormed different types of enrichment we could experiment with. In a colony of 16 frogs we began by introducing various enrichment tools into the frog's environment, such as plastic nonleaching aquarium plants, colored rocks, and tunnels. The frogs were also moved to larger tanks and socially housed. During this process, the frogs were monitored for reduction in signs of stress and for any behavioral changes. We rotated the different types of enrichment through the cages and observed to see which were more effective. Later we introduced weekly food enrichment using frozen bloodworms as a secondary enrichment. We considered the introduction of plant enrichment the most successful. Since introducing plant enrichment we have not had a single case of red leg, sore nose, or excessive skin sloughing. Frogs with plants also startled less from any room movement. Hand feeding and using food enrichment resulted in frogs presenting themselves at the front of cages frequently, resulting in the frogs being more interactive with the technicians at feeding time. It was also noted that when a frog went from social housing to single housing it showed signs of a depressed-like state, with a notable loss of appetite. When returned to a socially housed tank its normal behavior and appetite returned. Overall, by providing our frogs with an enriched habitat they showed no physical signs of stress, adapted to our technicians, and became easier to handle. By interacting with the frogs on a regular basis and learning each frog's normal behavior, it became easier to identify problems. This has helped our technicians to alert researchers of possible environmental or stress-related issues before any physical symptoms become evident.

P134 An Evaluation of Commercially Available Nesting Materials on the Complexity of Deer Mice (*Peromyscus maniculatus*) Nests

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Nesting is a natural behavior of the deer mouse (*Peromyscus maniculatus*). Provision of nesting material promotes species-typical behaviors such as burrowing, nest building, and foraging. Wild deer mice use a variety of substrates to build complex nests including plant material and synthetic fibers. The purpose of this study was to determine which type of commercially available nesting material promoted laboratory housed deer mice to produce similar intricate nest building behavior typically observed in their wild counterparts. Cages were housed on a ventilated rack with ¼-in. corncob bedding. An existing breeding colony was evaluated (37 cage average) which contained a cage combination of all males, all females, or breeding pairs. At each cage change, all animals received a new type of nesting material. Five commercially available substrates were tested including square compressed cotton, cylindrical compressed cotton, small pieces of rolled paper scattered throughout the bedding, brown crinkled paper, and white crinkled paper. Nests were evaluated 24 h after cage change and scored for complexity on a scale of 0 (no manipulation) to 5 (complete dome). Nest complexity was compared between breeding pairs and single sex cages and also between males

and females. Cages housing only females had the highest average complexity score. Approximately 37% of cages using either type of crinkled paper had material strewn throughout the cage with no or very small nests built. Despite that observation, brown crinkled paper had the highest complexity with an average score above all other types of substrate. The rolled paper material scattered throughout the cage bedding scored the lowest, despite this group being reevaluated at 48 h to provide additional time for foraging. This study has demonstrated that deer mice build more complex nests with brown crinkled paper compared with other commercially available substrates and that increased nesting complexity is impacted by sex. As wild deer mice often use a variety of nesting materials, more research is needed to evaluate if provision of multiple substrates increases nest complexity.

P135 Selection of Appropriate Food for Germ-Free Mice: Sterility and Nutrition Analysis

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Selecting a diet for germ-free animals can be challenging due to sterility requirements and maintenance of adequate nutritional value. Penetration of adequate heat into the dense food pellets is difficult during the autoclave process and can lead to contamination of the mice. Autoclaving food also reduces the level of many nutrients, making nutritional analysis essential for germ-free mice. To determine the optimal diet for our new germ-free facility, we tested 5 autoclavable diets (5010, 2018SX, 2018, 2919, and 7012). Diets were autoclaved at 250 °F for 45 minutes, which is a standard autoclave cycle in for germ-free facilities to ensure adequate kill of bacteria is achieved. Multiple quality control methods were used to test the sterility of the pellets. We used bacterial spore strips and multiple aerobic and anaerobic culture testing procedures for quality control purposes. Food was packaged in a metal tray and then placed in sterilizing cylinders to mimic the standard set up in germ-free facilities. After testing, 2018SX proved to maintain sterility the best out of all the foods tested so it was selected to be tested prior to and after autoclaving for thiamine (Vit B1), Vitamin A, Vitamin C, lysine, and methionine content. Based on the combined sterility testing and the food analysis, 2018SX was determined to be the optimal feed for our germ-free facility. To date, there has been no contamination or nutritional health issues linked to the use of 2018SX at our facility.

P136 An Innovative Restraint Device for the Placement of Intravenous Tail Vein Catheters in Anesthetized Mice

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Consistently accurate intravenous tail vein administration of radiopharmaceuticals in anesthetized mice is a critical component of successful PET/CT imaging in the preclinical setting. Injectable anesthetics are often not suitable for PET imaging and commercially available mouse restraint devices do not accommodate mice under inhalant anesthesia. There is a need for a restraint device that allows for the administration of radioactive material via tail vein catheter while mice are under general inhalation anesthetic. The device must be of a durable, nonporous, sturdy material that will allow for thorough disinfection practices to prevent cross-contamination and to suit a facility accommodating immunocompromised mice. It must provide a method for attachment and adjustment of the anesthesia nose cone, easily support placement and positioning of mice of different sizes, and enable gentle extension of the tail for injection or catheter placement. The device described here satisfies all of these criteria. Solid surface countertop proved to be the ideal material for the base platform. It is nonporous, easily cleaned, impervious to bacteria, and is often used in hospitals and for laboratory countertops. The platform's dimensions are 10 × 2 × 2 in. It is sturdy but light enough to be easily moved to the bench or laboratory hood. A beveled trough allows the mouse to rest on its side. A removable piece of slotted plastic enables the technologist to extend the tail such that the lateral tail vein is positioned for easy access. A vinyl plastic strip held in place by magnets secures the anesthesia nosecone to the base. All components of the device meet IACUC standards for cleaning and sanitation. This device has been used for the administration of radiopharmaceuticals by multiple staff members in all PET studies since its development. It has also proved useful in nonimaging studies for the intravenous administration of pharmaceuticals or contrast agents when the animals have pump implants or their size prohibits the use of standard conscious restraint. This device has facilitated the successful administration of radiopharmaceuticals via tail vein catheter for all studies in our facility since its inception and is now an established part of our standard operating procedure.

P137 Teaching Laboratory Rodent Research Techniques under the Tenets of Situated Learning Improves Student Confidence and Promotes Collaboration

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A targeted needs assessment performed at our institution revealed that the online system used to train researchers on performing techniques with animals did not provide opportunities to practice skills, introduce learners to animal care staff, nor satisfactorily support researchers' needs to become comfortable with laboratory animal species. To correct these deficiencies, a series of hands-on training sessions, framed theoretically in situated learning, was developed. This theoretical framework asserts that learning for everyday living (in this case, performing laboratory animal techniques) happens when people interact within the community while using the tools at hand (that is, the instruments and jargon of the field). From this perspective, the students work alongside the instructor as apprentices. The instructor creates increasingly challenging learning opportunities as students work toward independently performing techniques, which is an educational method called scaffolding. This method has been recommended for use in training in laboratory animal medicine because its goal is a student who can independently and proficiently perform the given technique. To test our hypothesis that teaching from this perspective improves comfort levels with laboratory animals and promotes collaborative relationships between animal care and research personnel, a mixed-method design involving online surveys (first survey, $n = 45$; second survey, $n = 35$) and semistructured interviews ($n = 10$) was used. Quantitative results revealed that students became more comfortable with laboratory animals and were more likely to contact animal care personnel due to participating in the training program. The qualitative arm of the study identified specific features of the training program that improved comfort levels for students (seeing then doing, working in small groups, learning within a comfortable environment, and building collegial relationships). These results support teaching rodent research techniques from the practical and theoretical approach of situated learning.

P138 Management of the Risks Associated with the Care, Welfare, and Treatment of Animals

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An element of reputational risk is inherent when working with laboratory animals. Therefore, managing this risk and others associated with the care, welfare, and treatment of animals is an important factor for the success of an animal research facility. Using a 5-Step Risk Management Process which includes risk identification, risk assessment and prioritization, risk owner appointment and risk analysis, risk treatment, and upward reporting and monitoring, we

are able to manage risk at all levels of the organization. Employees are also expected to identify and escalate any encountered risks so that they can be appropriately managed. Appointment of an accountable risk owner for the care, welfare, and treatment of animals, as well as a risk coordinator and an established governance structure in combination with a robust internal control framework ensures that risks such as transportation of animals, externalized animal work, and infiltration are proactively managed. We will present our governance structure, provide more detail on the 5-Step Risk Management Process, provide a high level overview of identified risks, and discuss potential mitigation steps.

P139 Evaluating the Necessity of Automatic Watering System Charging for Automated Watering Systems at Cage Change

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Optimizing animal husbandry includes minimizing ergonomic risk and maximizing efficiency while maintaining animal wellbeing. At our facility, an automated watering system is used with cage integrated water valves. Facility standard operating procedures require staff to charge the automatic watering system on mouse cages before use by placing the cage in a charging station and maneuvering the automatic watering system until water is visible. Inquiries with the vendor and colleagues regarding why cage charging is considered necessary resulted in numerous, inconsistent justifications for this step. Our theory suggests omitting this step will reduce ergonomic risk to caretakers due to less cage maneuvering and save time resulting in increased efficiency without compromising animal welfare. To test our hypothesis, 14 cages of mice were used. Half of the cages were charged in the charging station (control group) while the other half were not charged (experimental group). The mice were then weighed, numbered and a clinical assessment was performed by a registered veterinary technician (RVT) blinded to the experiment. For 5 consecutive days the same RVT weighed, assessed skin turgor, and visually evaluated each mouse for signs of dehydration. After a brief washout period, the control and experimental groups were switched and the study repeated. Weights, skin turgor, and visual health evaluations were all within normal limits for both groups at all time points, demonstrating no animal welfare concerns. To evaluate efficiency, 10 caretakers were timed 3 times setting up 10 cages in a biosafety cabinet with and without charging the automatic watering system. Removing automatic watering system charging reduced cage preparation time by 63%, translating to approximately US$25,000 a year in cost savings for the animal care program based on 10,000 weekly cage changes. Based on clinical assessment of these mice, charging cages appears unnecessary for automatic watering system functionality and removing this procedure from preparation results in increased efficiency during cage change.

P140 Program Development, Implementation, and Quality Control for Use of Alkaline Hydrolysis Tissue Digestion for Pathologic Waste

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Whether it is natural, experimental, or clinical, it is inevitable that research animals will reach an endpoint, resulting in the need to safely dispose of pathologic waste. There are many criteria to consider when choosing the best method of disposal with the variety of resources available, while also complying with federal, state, and local regulations. While incineration is a traditional method of disposal, there is not a licensed commercial medical waste incinerator in the state of Georgia. Pathologic waste is transported hundreds of miles to out-of-state medical waste incinerators and out of the direct control of the generator, creating a potential public health risk. Alkaline hydrolysis is a nontraditional method of disposal using a chemical reaction to sterilize and liquefy pathologic waste giving the generator full control of the process and the ability to dispose of waste on-site via sanitary sewer. A unique alkaline hydrolysis tissue digester was designed and installed in the wall between the animal biosafety level 2 and 3 facility of a building. Managing this waste disposal onsite creates additional risks to the health and safety of personnel that are involved with disposal. The goal of this project was to reduce the amount of waste transported over public roads while also protecting personnel responsible for loading and operating the tissue digester. To promote ownership of the new disposal process, stakeholders were involved throughout the project. Occupational health and safety risks were addressed by the creation of process maps, a personal protective equipment risk assessment, standard operating procedures, the identification of critical indicators for quality control, and updated personnel training. The main goal of reducing the amount of pathologic waste transported over public highways while protecting personnel safety was accomplished. Additional aspects of the program that still need to be addressed include a more durable pathologic waste bag.

P141 Isoflurane Overdose Is Inadequate as a Means of Euthanasia for Neonatal Mice

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Euthanasia of neonates is an essential component of laboratory mouse management that continues to necessitate refinement of technique. With the increasing prominence of transgenic mice used in biomedical research, specific genotypes are desired, leaving numerous unwanted neonates as candidates for euthanasia. Carbon dioxide overdose, the most common euthanasia method in laboratory mice, can take up to an hour to sufficiently euthanize mouse neonates aged less than 6 d postpartum (P6). Although the AVMA Guidelines for the Euthanasia of Animals lists inhalant anesthetics as an acceptable method of euthanasia for laboratory rodents, a previous report recommended against the use of halothane or isoflurane overdose as a means of euthanasia for mice of any age. However, these studies delivered the anesthetic through a vaporizer restricting the dose to a maximum of 5% volume of atmosphere. We hypothesized that if used at saturated vapor pressure, euthanasia of neonates by isoflurane overdose may be more efficacious than CO_2. Seventy-six neonatal mice (P0 through P2) representing 8 litters of CD1 or C57BL/6J genetic backgrounds were exposed to isoflurane at saturated vapor pressure at room temperature ° C) for 30 min. This was accomplished by placing the pups in a quart-size resealable plastic bag with a 2-in.^{2} of absorbent paper towel to which was added 0.5 mL of isoflurane immediately after addition of the mice. Sufficient space was available to prevent pups' physical contact with littermates or the paper towel. Pups lost all righting reflex and pain response by 2 min of exposure. After 30 min, pups were removed from isoflurane exposure and monitored approximately every 5 min for up to 120 min. All pups were cyanotic with no detectable signs of life. Surprisingly, after a period of time ranging from 30 to 120 min, 24% of the mice initiated gasping then normal respiration and the cyanotic skin regained a normal pink coloration. Recovered pups were euthanized by decapitation 2 to 3 min following normal breathing and coloration. We conclude that P0 to P2 mouse pups exposed for 30 min to isoflurane at saturated vapor pressure must be followed by a secondary method of euthanasia to ensure humane death.

P142 Financial Impact of Self-imposed Regulatory Burden on Animal Care and Use Program Components

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Like many animal research care and use programs (ACUP), the last decade has resulted in enhanced regulatory oversight from external

agencies such as the USDA and OLAW. In response, many academic institutions have responded by imposing internal oversight programs to preemptively address problem areas before they are noted by external agencies. During this same period, the economic downturn resulted in decreased funding to many of these same institutions with land grant universities being especially vulnerable to the economic challenges. With this background, it was theorized that overall funding for the animal care program decreased during this period, and that the resources directed to self-imposed oversight of the animal program, such as dedicated compliance staff correspond to a decrease in support of direct animal care. For this study, financial data was obtained for the period 2005 to 2013 and included funds for the veterinary care and training program, the core biomedical animal facilities, and the IACUC/post approval monitoring (PAM) staff. These 3 components of the ACUP receive direct funding from the institution, while other ancillary components do not, and therefore were not included in this study. Over the 8-y period, the number of animals used in biomedical research increased by 92%. However, funding for the overall program actually decreased by 8% when corrected for inflation. Inflation was estimated based on the mandated salary increases for personnel during this time period, recognizing that ACUP costs are primarily related to personnel and benefits. The veterinary care and training program funding was not significantly changed during this period (3% increase), while the IACUC/PAM component funding increased by 106%. This increase in the IACUC/PAM component is primarily related to the addition of dedicated PAM personnel. Concomitantly, there was a 43% drop in funding to the core biomedical animal facilities in spite of a doubling in the workload based on the number of animals used and the average daily census during this same period. Based on the results of this financial study at our institution, the increased emphasis on internal regulatory oversight by the institution was accomplished by redirecting resources away from the direct animal care component of the program. The results of this study are important for IACUCs to consider as self-imposed requirements are added to the ACUP and the financial impact of these choices on the direct animal care component.

P143 The Combination Rack as a Housing Solution

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Our institution uses commercially available individually ventilated cage (IVC) mouse racks outfitted with automatic watering. The racks were only available consisting of entirely small or entirely large breeder cages. IVC racks with small cages are the most commonly used housing type at our institution, while IVC racks with large cages used for housing trio breeding groups are less commonly used. Our mouse rooms typically have 1 to 10 IVC racks, each consisting of 140 small mouse cages each. In some of these rooms there was a need to also have 4 to 40 large mouse breeding cages. We typically use an entire IVC rack of 80 large mouse breeding cages to house only a few breeder trio cages or have housed them in static micro isolator cages. In an effort to maximize housing space and use our existing IVC units we created a combination IVC rack with interchangeable shelves and automatic watering lines to accommodate both large mouse breeder cages and small mouse cages. The original combination IVC rack was constructed by using parts and equipment which were already onsite and involved retrofitting an 8-shelf mouse breeder rack to accommodate 6 shelves of small mouse cages and 2 shelves of the large mouse breeder cages. After construction, the rack was functionally tested for performance and durability to withstand routine cleaning and sterilization procedures. After the successful implementation of our functional prototype combination rack, we have worked with the IVC rack manufacturer to construct a factory-produced rack with interchangeable shelving and water lines to accommodate both large mouse breeder cages and small mouse cages. The combination IVC rack will allow our department to expand mouse housing options using IVC racks without losing valuable floor space.

P144 Fecal Progesterone as an Indicator of Early Pregnancy in Mice

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Pregnancy detection during early stages of gestation, before outward signs are apparent, remains a challenge for investigators requiring pregnant mice or mouse embryos for developmental biology research. Current noninvasive methods of early pregnancy detection are unreliable, and invasive methods may be stressful to the pregnant mouse and/or require sophisticated imaging equipment. Our previous work suggests that pregnancy-specific urinary proteins in mice, if present, are not produced in sufficient concentration to be detected by standard electrophoresis methods. The current study focused on fecal progesterone as a potential indicator of pregnancy in mice during early gestation. Fecal pellets (1 to 2 per mouse) were obtained from female mice that were not pregnant (n = 34), or time-pregnant (plug day = E0.5) at embryonic days (E) representing preimplantation (E0.5 to E5.5, n = 12), organogenesis (E6.5 to E10.5, n = 11), early fetal (E11.5 to E15.5, n = 12), or late fetal (E16.5 to E18.5, n = 9) stages and later verified to be pregnant. A progesterone enzyme immunoassay (EIA) was optimized with nonpregnant female mouse feces to validate parallelism and accuracy, and test fecal samples were assayed using EIA by a scientist blinded to the sample identities. Numerical values were analyzed for statistical significance by ANOVA, Tukey HSD, and t test. Optimization of the EIA assay demonstrated good recognition of antibodies to murine fecal progesterone metabolites. Fecal progesterone concentrations (mean ng/g feces ± SEM) in test samples were 233.2 ± 17.1 in nonpregnant mice, 604.5 ± 123.8 at E0.5 to E5.5, 724.7 ± 139.6 at E6.5 to E10.5, 351.0 ± 40.8 at E11.5 to E15.5, and 308.3 ± 17.8 at E16.5 to E18.5. Fecal progesterone levels were significantly ($P < 0.01$) elevated at stages < E11.5 compared with nonpregnant mice. Our results suggest that a fecal progesterone assay may provide a valid noninvasive method of pregnancy detection during early gestation, before outward signs of pregnancy are apparent. Supported by an ACLAM Foundation grant to IMW.

P145 Chronic *Helicobacter pylori* Infection Causes Serum Iron Storage Depletion and Alters Local Iron Gene Expression in INS-GAS Mice (*Mus musculus*)

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Iron deficiency anemia (IDA) affects over 500 million people worldwide, and is linked to impaired cognitive development and function in children. *Helicobacter pylori* infects approximately half of the world's population, thus creating a high likelihood of overlapping risk. Previous studies suggest that a correlation exists between *H. pylori* pathogenicity and systemic iron deficiency. However, the causal effect between these two diseases remains to be defined. This study sought to determine the effect of *H. pylori* infection on systemic iron deficiency and brain iron homeostasis based on parameters including red blood cell indices, iron metabolism, and synaptic plasticity related gene expression, and behavioral outcomes in a mouse model. Two replicates of hypergastrinemic INS-GAS/FVB male mice (n = 10 to 12 per group) were dosed with *H. pylori* (*Hp*) strain SS1 or sham dosed at 7 to 9 wk of age. Mice were necropsied at 25 to 27 wk postinfection. Serum ferritin was lower in *Hp* SS1 infected mice than uninfected mice ($P < 0.0001$). The ratio of myeloid to erythroid cell precursors in bone marrow was lower in infected mice than uninfected ($P < 0.001$). Infected mice had a lower red blood cell count ($P = 0.0001$), as well as lower hematocrit ($P < 0.001$) and hemoglobin concentration ($P < 0.001$). Mean cellular volume was increased in infected mice ($P < 0.0001$), as was reticulocyte % ($P <$

0.01), and erythropoietin concentration ($P = 0.001$). Gastric expression of the iron regulator hepcidin was downregulated in *Hp* SS1 infected mice ($P < 0.05$). Expression of brain divalent metal ion transporter ($P = 0.01$) and brain dopamine receptor 1 ($P = 0.04$) was upregulated in *Hp* SS1 infected mice, consistent with brain iron deficiency, while expression of and brain derived necrosis neurotrophic factor 4 (a marker of synaptic plasticity) was downregulated in infected mice. Our data indicate that long-term infection with *Hp* SS1 caused depletion of total serum iron stores and deregulated gastric and brain gene expression related to both iron metabolism and synaptic plasticity. Red blood cell indices were consistent with anemia caused by blood loss. Ongoing studies seek to further define the relationship between *H. pylori,* iron deficiency, anemia, and cognitive function in this model.

P146 Collection of Rodent Bone Marrow for Pathology Evaluation: No Decalcification Required

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Rodent bone marrow is often requested for histologic and immunohistochemical (IHC) analysis to identify hematotoxic effects of new compounds or confirm known target biology. Standard "whole bone" procedure for microscopic bone marrow evaluation consists of femoral or sternal bone harvesting followed by fixation and tissue decalcification prior to trimming, processing, embedding and staining. Decalcification is typically performed in acid or chelating agents; however, these techniques often interfere with or are not compatible with some IHC staining protocols and if not carefully monitored could lead to distorted cellular morphology. To circumvent the decalcification step, our lab developed a bone marrow collection procedure where bone femurs are harvested, distal ends are cut, placed in an tube and centrifuged, forming a robust bone marrow "pellet" that is then fixed with 10% neutral buffered formalin for 24 h. The pellet is processed in a tissue processor, embedded in paraffin and hematoxylin and eosin stained for pathologic evaluation. To determine the benefit of removing the decalcification step from bone marrow processing "standard" whole bone and "bone marrow pellet" procedures were compared in mice and rats, using hematoxylin and eosin, special stains and decal sensitive IHC markers. We found bone marrow pellets provide greater cellular density, excellent morphologic preservation and were suitable to a wider range of IHC assays. The pellets created are free of bone fragments, eliminating the decalcification process, which shortens slide preparation time and facilitates microtome sectioning. The "bone marrow pellet" procedure provides faster turnaround time and is suitable to a wider range of staining procedures in both rats and mice, and is satisfactory for pathologic evaluation when bone marrow cellularity and composition are of importance and the tissue architecture and microenvironment are not required.

P147 Evaluation of a Novel Perfusion Apparatus in Rodents

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The brain is a tissue commonly used to assess a variety of drug therapy and disease progression models. In order to ensure results are not skewed by introduced artifacts from the uncontrolled delivery of perfusate, we developed a novel system that closely controls and maintains perfusate flow rates. Hand-driven syringe methods that can rupture vessels and cause artifacts that confound results, and syringe pumps can apply inconsistent pressure and require multiple syringe changes when dealing with larger volumes. While manufactured perfusion pump systems are available for purchase, they are not cost effective for most studies. We evaluated tissues perfused via 3 conditions, hand-driven syringe, syringe pump, and our novel design in rats. The rat serves as a good representation of a small animal model whose vessels are easily compromised. Our novel device uses a 1-L, graduated flask with a rubber stopper to seal the opening. Within the stopper, 2 nozzles are placed—one for connection to air supply for pressurizing the system and the other to carry the pressurized solution out of the flask. The solution proceeds through a hose connected to a macrodrip intravenous set where flow rate can be controlled and monitored. Delivery proceeds quickly and without intermission or fluctuations in pressure. Based on histologic comparison of brains collected using these methods, our novel device appears to successfully achieve brain perfusion with minimal tissue disruption, thus allowing both refinement of commonly accepted techniques and reduction in animal use.

P148 Comparison of 2 Variations of Blood Collection for Sentinel Surveillance Testing in Mice

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The rodent sentinel program is an essential component in laboratory animal research. It continues to be the primary means of detecting specific infectious pathogens within facilities. In this study, our goal was to compare 2 variations of sentinel testing to see if we could reduce animal numbers, costs, and time. Female CD1 sentinels were set up in two groups on 28 racks (14 racks per group). Group 1 was setup in our standard housing arrangement of 3 mice per IVC cage, one cage per rack. At the time of collection one sentinel was removed from each cage for terminal sample collection. The sentinel was euthanized and blood collected via cardiac puncture. Evaluation for ectoparasites by fur pluck testing and pelt examination, gross necropsy for lesioned organs, and examination for endoparasites by anal tape test of the perineum and visualization of cecal contents were performed. Serum samples were submitted to a commercial laboratory for testing. Group 2 was setup with the housing arrangement of 2 sentinels per IVC cage, one cage per rack. At the time of collection, one sentinel was selected from each cage and placed back in the home cage after sample collection. Blood was collected via saphenous vein and placed on a blood spot serologic assay provided by a commercial diagnostic laboratory. Evaluation for ectoparasites by fur pluck testing and endoparasites by anal tape test of the perineum from each sentinel were also performed. We found significant differences between the 2 blood collection techniques with reductions in both cost and number of sentinels required. The total supply cost for group 1 was US$366 while the cost for group 2 was US$202. There were also significant time differences (191 min for group 1 compared with 108 min for group 2). Predominantly, we found group 2 to be an effective means of sentinel testing that reduces cost, time and most importantly reduces the number of sentinel animals required.

P149 Testing the Effectiveness of Nonopioid Analgesics in Rainbow Trout (*Oncorhynchus mykiss*) Subjected to Anesthesia and Surgery Using Behavioral, Clinical Pathology, and Histopathology Changes

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Techniques to manage pain in fish are still in their infancy. Opioid analgesics have been shown to ameliorate aversive behaviors and physiologic changes when fish are exposed to noxious stimuli. However, the opioid agents are controlled substances requiring special licensing, reporting requirements, and security measures. If noncontrolled agents such as NSAIDs can be proven safe and effective, this may encourage wider use of analgesics in fish. To determine the effectiveness of 3 different NSAIDs in managing pain, 48

rainbow trout underwent an exploratory celiotomy using MS222 anesthesia. Fish were randomly assigned to one of 4 treatment groups with 12 fish per group: flunixin (0.5 mg/kg), ketorolac (0.5 mg/kg), ketoprofen (2 mg/kg), or saline. At specific time points before (baseline) and after surgery the behavior of the fish was monitored for vertical position in the water, respiratory rate, and response to food (presentation of 3 pellets). Clinical pathology was assessed 1 wk before surgery and 48 h after surgery. Fish were euthanized at 14 d after surgery using MS222 and a necropsy was performed. Tissues were collected for histopathology to evaluate the healing of the incision, tissue reaction at the injection site, and potential organ toxicity due to use of an NSAID. No significant differences existed between the treatment groups for behavioral observations and histopathology of the incision, injection site, or internal organs. Postoperative phosphorus levels were shown to be significantly higher in the control saline treatment group than the flunixin treatment group. This may have been due to a Type I false-positive error. Although significant differences between the saline treatment group and the NSAID treatment groups were not proven, it should be noted these drugs also had no adverse effects on the health of the fish. In summary, as the use of fish in research is rapidly increasing, there is an urgent need to validate effective methods to alleviate any suffering for both fish welfare and scientific validity.

P150 Loss of Folliculin Interacting Protein-1 Leads to Cardiac Hypertrophy in Mice

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Cardiac hypertrophy-induced heart failure is a leading cause of morbidity and mortality in the United States. The major risk factors associated with the development of cardiac disorders include the presence of metabolic diseases such as diabetes and obesity. Hence, understanding how metabolic homeostasis normally maintains cardiomyocyte health is essential for elucidating how to prevent the development of cardiac disorders, and to identify novel therapeutic targets. Several laboratories have demonstrated that dysregulation of AMP Kinase (AMPK), a central cellular energy sensor, and the anabolic mTOR pathway, are directly involved in the development of cardiac hypertrophy. In this study, we tested the hypothesis that Folliculin Interacting Protein-1 (Fnip1), a newly identified AMPK interacting partner, helps maintain metabolic balance and inhibits cardiac hypertrophy in mice. Using *Fnip1*-null mice we previously generated, we found that loss of Fnip1 results in increased heart-to-brain weight ratio relative to wildtype (WT) mice, which correlated in increased left ventricle wall diameter on echocardiography. Using real-time PCR analysis, we found that mRNA transcript abundance of cardiac hypertrophy factors such as atrial natriuretic peptide, brain natriuretic peptide and α-smooth muscle actin were significantly elevated in *Fnip1* $^{-/-}$ cardiomyocytes compared with WT controls. Biochemical studies indicated that AMPK, PGC1α, and mTORC1 pathways were concurrently activated in *Fnip1* null cardiac tissue, suggesting that AMPK-mediated suppression of mTORC1 is impaired in *Fnip1* null mice. However, a cardiac-specific dominant-negative AMPK transgene and long-term inhibition of mTOR signaling by rapamycin both failed to rescue the cardiac hypertrophy in *Fnip1* null mice. Thus, our findings collectively suggest that Fnip1 normally regulates cardiac morphology and function independent of AMPK and mTOR signaling. Funded in part by NIH grants K26RR024462, MMPC09MCG96, R56AI092093, P30-DK035816 to BMI.

P151 Addressing the 3Rs in Pharmacokinetic Screening

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One of the key tenets to responsible research involving animals is the adherence to the principles of "reduce, replace, and refine" (the 3Rs). As such, researchers and institutions have an obligation to find alternative methods to animal models. This can be difficult in early screening when in silico, in vitro, and in vivo data are needed to drive structure activity relationship (SAR). Traditional pharmacokinetic (PK) studies in rat typically use 6 animals (n = 3 per leg, drug dosed intravenously and orally) bled serially to estimate PK parameters. A traditional mouse design involves euthanizing mice (n = 2 per timepoint, 7 timepoints per intravenous or oral leg) resulting in approximately 28 mice being used. Early in screening, compounds are binned based on their PK parameters, and our group took the approach of less is more by evaluating what is truly needed to enable decision making on compound being tested. Instead of serially bleeding 2 groups of rats (n = 3 per leg intravenously and orally) simultaneously after dosing, we have adopted a crossover design where 2 rats dosed intravenously on day 1 are serially bled and the same animals are dosed orally on day 2 and then serially sampled again. This reduced rats used for PK study from 6 to 2. In the case of mouse PK, a serial bleeding paradigm (n = 3 per leg intravenously and orally) reduced number of mice from 28 to 6 per PK study. Since the inception of the group, the number of PK studies performed has grown 20%, in contrast the number of animals used has been reduced approximately 33%. This has been attributed to the crossover design in rat and serial sampling in mice. Additionally, PK parameters generated for compounds using the new paradigm were comparable to the old paradigm. It is also worth noting that in addition to a reduction in the number of animals used, the amount of compound utilized to perform a study is also reduced. Therefore, this approach can offer long terms resource savings.

P152 Effects of Thymol on Urine Preservation during 16- to 24-Hour Urine Collections in Rats

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Crystal thymol is a preservative that has been used to prevent bacterial growth during overnight urine collections in rodent toxicology studies. A small amount of thymol (approximately 1 crystal) is added to urine containers. Since thymol is affected by light, the collection containers should be opaque or wrapped with an opaque material like aluminum foil (AL). The purpose of this study was to evaluate the effectiveness of crystal thymol for urine preservation during routine 16- to 24-h urine collections and to determine if the procedure could be eliminated without compromising urine sample quality. Sprague–Dawley rats (n = 6 to 20 group per sex) were singly housed in metabolism cages for a 16- to 24-h urine collection procedure. Food was withheld at this time, but water was available ad libitum. Groups were as follows: thymol (+) AL wrapped (+), thymol (–) AL wrapped (+), thymol (+) and AL wrapped (–), thymol (–) AL wrapped (-). Collection tubes with 0.25, 0.50, 0.75, or 1.0 mg/mL thymol solutions (1 mL) were also assessed. Urine sediments were examined using standard urinalysis techniques for formed elements. Bacteria were not observed in 25% of the thymol (+) groups compared with 4% of the thymol (–) groups. In the 1.0 mg/mL thymol concentration, bacteria were observed in 45% of the female rat samples. While in the 0.25, 0.50, and 0.75 mg/mL thymol concentrations, bacteria was observed in 80%, 100%, and 80% of the female rat samples, respectively. All of the thymol concentrations in the male groups resulted in bacteria being observed in all samples. AL wrapped (+) and (–) did not affect the presence of bacteria in either sex. The presence or absence of thymol also did not affect the ability to assess other formed elements being observed in the samples. Although the addition of thymol as a preservative to urine collection tubes during a routine 16- to 24-h collection did tend to reduce the observation of bacteria in sediment, the ability to evaluate formed elements (for example, casts, WBC, RBC, epithelial cells, and

others) in sediment was not affected by the presence or absence of thymol. Thus, the addition of thymol to urine collection containers could be eliminated without compromising urine sample quality.

P153 Validation of Dried Blood Spot Test Collection Method for Routine Serosurveillance of Cynomolgus and Rhesus Macaque Colonies

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The dried blood spot test (DBST) utilizes dried whole blood spot sample collection technique instead of serum for routine serology. Immune and nonimmune DBST-serum sample pairs from macaques were tested by using routine simian multiplex fluorescent immunoassay (MFIA) bead panel. The immune (known positive) samples were prepared from naturally or experimentally infected cynomolgus or rhesus macaques with one or more pathogens including simian retrovirus (SRV), simian immunodeficiency virus (SIV), simian T-lymphotropic virus (STLV), and herpes B virus. The nonimmune (known negative) samples were derived from historically known negative SPF cynomolgus or rhesus macaque colonies for the above mentioned agents. Eight positive and 8 negative macaque whole blood samples were spotted on DBST cards and equivalent serum samples from the same animals were prepared. Elution of serum IgG from the cards was performed on 3 separate occasions and 3 different MFIA runs were performed on DBST-serum paired samples. DBST MFIA data from triplicate runs was compared with the serum data to evaluate diagnostic sensitivity and specificity, reproducibility, and ruggedness. A total of 624 assays were performed and analytical performance of the DBST MFIA assay including selectivity and limit of detection was found to be comparable to those obtained by serum MFIA. The diagnostic specificity of both DBST and serum simian assays was found to be 100%. The diagnostic sensitivity of individual infectious agents was 98% in all MFIA runs. The validation study shows good correlation between DBST and corresponding serum samples data. It also proves that DBST MFIA results are analytically and diagnostically equivalent to those with serum indicating that DBST is a suitable alternative to serum for routine serologic testing of macaque colonies.

P154 Enhanced Intra- and Postoperative Care Drastically Reduces Mortality and Complications after Major Cardiothoracic Surgery in Rodents

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Survival rodent models of cardiovascular diseases have become very popular in recent years due to advances in genetic engineering and molecular biology. Recent innovations in rat cardiac surgery have included the development of different heart failure models and the creation of different myocardial infarction models. However reported mortality remains as high as 40% to 60%. The aim of this study was to analyze the factors leading to mortality after major cardiothoracic surgery and design a strategy to improve outcomes. One-hundred and sixty-eight male Sprague–Dawley rats (386 ± 7 g) received left thoracotomies, 156 of which also received proximal ligation of the left anterior descending coronary artery. The first 49 animals received care consistent with that typically described in the literature. The subsequent 119 animals received enhanced care including the following components of the 3 operative phases: preoperative = administration of analgesics, monitoring of vitals including continuous pulse oximetry, electrocardiography, echocardiography as needed, core body temperature monitoring and management to prevent hypothermia and overheating; intraoperative = electrocardiography, continuous pulse oximetry, minimized blood loss, gentle tissue handling including minimal manipulation of the heart, suturing by layers, attention to atelectasis, careful postop lung function management through the use of chest tubes for air evacuation, administration of local anesthetics and nerve block; postoperative = fluid therapy to prevent dehydration, prolonged use of oxygen, further pain management, preventative treatment of arrhythmias. With the additional care and monitoring, survival increased from the first group to the second group at 48 h after infarct (51% to 93%) and at 10 wk after infarct (47% to 90%). Total complications decreased from 26 (of 49 cases) to 12 (of 119 cases). The most notable complications that decreased from group 1 to group 2 were ventricular arrhythmia leading to cardiac arrest (10 to 2), and hypoxia (4 to 1). Echocardiography and histology at 10 wk postinfarct confirmed large infarct sizes with no differences between groups. The modification of intraoperative and postoperative monitoring and management of the cardiopulmonary system allows for drastically improved survival and incidence of complications.

P155 Efficacy and Safety Evaluation of Multivesicular Liposomal Bupivicaine for Local Analgesia in the Mouse

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Postoperative analgesia to alleviate pain and distress is an important component of laboratory animal refinement. A safe, long-acting, local anesthetic, with minimal adverse effects would be greatly beneficial, improving the postoperative care of rodents by providing the option of multimodal analgesia, when in use with other analgesics such as meloxicam or buprenorphine. Recently, a new multivesicular liposomal extended-release bupivacaine has been approved by the Food and Drug Administration (FDA) as a postoperative local analgesia for human use in a number of surgical procedures. This drug is designed to provide a slow release of bupivacaine, lasting up to 72 to 96 h following perioperative administration, reducing human opioid consumption, lowering hospital costs, and reducing hospital length of stay. We assessed the safety and efficacy of this liposomal encapsulated bupivacaine as a local analgesic agent in the mouse model. Fifty-four adult female Crl:CD1 mice were dosed with either 1 or 2 mg/kg (n = 24 per dose) of liposomal bupivacaine and 6 control mice received an equal volume of injectable saline subcutaneously. Pain was assessed in groups of 3 mice at each dose level, by vocalization in response to electrostimulation at multiple time points through 48 h. Body weights were monitored daily and injection sites were assessed grossly and histologically for lesions. Subcutaneous administration of liposomal bupivacaine resulted in transient weight loss and erythematous lesions at injection sites within 7 d, with both resolving at 28 d. Vocalization was absent, suggesting local anesthesia was adequate, within 2 h at 1 mg/kg and in 2 of 3 mice at 2 mg/kg. Anesthesia was not adequate at 12 h (2 of 3 mice at 1 mg/kg and 1 of 3 mice at 2 mg/kg). After 24 h, anesthesia was present in only 1 of 3 mice in the 2-mg/kg group, and anesthesia was not present in the 1 mg/kg group. Histopathology revealed mild local inflammation (over 7 d) in both dosage groups, with mild long-term (28 d) epithelial hyperplasia and fibrosis present only in the 2-mg/kg group. This study shows that subcutaneous administration of this liposomal bupivacaine results in mild adverse effects and inadequate long-term duration of local analgesia, suggesting it may not be a useful drug for laboratory mice.

P156 Efficient Methods of Collection and Cryopreservation for Rat Blastocysts

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Genetic modification of rats is often carried out using rat embryonic stem cells (ES cells). However, large numbers of blastocysts are required to produce chimeric rats. In addition, a great deal of effort is required to tune the ES cells and blastocysts with the time schedule of the recipients. Therefore, we studied the collection of blastocysts and cryopreservation using BrlHan:WIST@Jcl (GALAS) rats. Blastocysts were collected using 2 methods. Eight-cell embryos were

collected using the oviduct and uterus flushing method from a female rat after mating, followed by in vitro culture until blastocysts (8C group). Blastocysts were also collected with the uterus flushing method (BL group). A vitrification method recently developed was used for cryopreservation, and morphologically normal embryos were examined for fetal development. An average of seven 8-cell stage embryos were collected for each female rat obtained in 8C group. However, the blastocysts obtained after in vitro culture were reduced to an average of 5, and the results were the same as the average number (5) of blastocysts collected for BL group. The percentage of morphologically normal embryos after cryopreservation for 8C group was 64%, significantly lower than 93% for BL group. Thirty-six percent of the blastocysts shrunk after cryopreservation. This caused a reduction in percentage of morphologically normal embryos for 8C group. There were no significant differences in fetal development rate after embryo transfer between blastocysts that were not cryopreserved after collection in 8C group (46%) and the cryopreserved blastocysts of 8C group (51%) or BL group (48%). The experimental results indicated that BL group is efficient for making cryopreserved blastocysts. In future, we will inject ES cells into the cryopreserved blastocysts to make chimeric rats.

P157 Efficacy and Safety of Pirfenidone in the Treatment of Canine Corneal Fibrosis

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Corneal scarring (fibrosis) stemming from an initial corneal insult is a significant cause of visual impairment across species. Currently, antifibrotic treatments aimed at mitigating the loss of vision caused by corneal fibrosis are infrequently utilized in veterinary ophthalmology due to a lack of comprehensive safety and efficacy evaluation. This study evaluated the safety and efficacy of the novel antifibrotic compound, pirfenidone, in the prevention of canine corneal fibrosis using an in vitro model. Healthy donor canine corneas were collected and used to generate primary canine corneal fibroblasts (CCFs) by growing cultures in minimal essential medium supplemented with 10% fetal bovine serum. Canine corneal myofibroblasts (CCMs), used as a model of canine corneal fibrosis, were produced by growing CCF cultures in serum-free medium containing transforming growth factor β1 (1 ng/mL). Trypan blue viability assays were utilized to determine the optimal pirfenidone dose for this in vitro model. Trypan blue viability, phase contrast microscopy, and TUNEL assays were used to evaluate the cytotoxicity of pirfenidone. Scratch and MTT assays were used to evaluate the effect of pirfenidone on cellular migration and proliferation. Real-time PCR, immunoblot analysis, and immunocytochemistry were employed to determine the efficacy of pirfenidone to inhibit CCM formation in vitro. Treatment with 200 µg/mL pirfenidone significantly decreased αSMA expression when compared with the TGFβ1 control group ($P < 0.01$). Pirfenidone treatment ≤ 200 µg/mL did not affect CCF phenotype or cellular viability and did not result in significant cytotoxicity. Pirfenidone safely and effectively inhibits TGFβ1-induced myofibroblast proliferation in the canine cornea in vitro. In vivo studies are warranted.

P158 Safe and Effective Anesthesia of Terrestrial Arthropods

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As the use of traditional research animal models continues to elicit public concern, veterinarians and scientists are constantly pushing the limits of science in order to develop viable alternative models. While refining experimental procedures or reducing animal numbers are steps in the right direction, it is ultimately the replacement of sentient animals with nonsentient species (when possible) that will have the greatest effect on quelling opposition to animal research. To this end, invertebrate models are becoming increasingly prevalent, and present a useful alternative to mammals for studying human disease. In these animals, sedation is required for most procedures; yet little is known about how to effectively anesthetize them. A solid understanding of their anesthetic needs and establishment of a safe protocol is therefore paramount in the advancement of their care and use. We focused specifically on terrestrial arthropods, and included over 100 animals from 15 different representative species. The first experiment divided animals into groups by species and evaluated the efficacy of common injectable, volatile, oral, and topical anesthetics based on ease of administration, induction time, duration, side effects, and plane of anesthesia. This experiment determined that the ideal anesthetic method and agent for use in these species was temporary exposure to vaporized halothane. The study then went on to examine the effects of species, body size, and anesthetic variables (halothane and oxygen concentration, delivery method, temperature and exposure time) on the duration and quality of anesthesia. This was performed by randomly assigning animals to a spectrum of varied anesthetic conditions. These results showed that higher body weight, lower anesthetic gas concentration, and longer exposure time all increased the overall duration of anesthesia. Our work has revealed much about the pharmacodynamics of halogenated anesthetic gases in arthropods and our results provide a safe and reliable preliminary anesthetic protocol based on species, size, and desired duration of anesthesia.

P159 Stability of Tiletamine, Ketamine, Xylazine, and Zolazepam in Combination

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No FDA-approved drugs for anesthetizing pigs are commercially available in the United States. Tiletamine/zolazepam, ketamine, and xylazine are often combined (50 mg/mL of each) as "TKX" to anesthetize pigs in research and veterinary practice. Veterinarians extemporaneously compound TKX for extra-label use, as allowed under AMDUCA legislation, but data regarding safety, beyond-use date, and stability of the drug combination are limited. The purpose of this study was to evaluate the chemical stability of each constituent drug in TKX when stored at room and refrigeration temperature for up to 140 d. Commercially available, 500-mg vials of tiletamine/zolazepam powder were reconstituted with 2.5 mL of ketamine (100 mg/mL), and 2.5 mL of xylazine (100 mg/mL) to form a sterile TKX solution that contained 50 mg/mL of each drug. Using gas chromatography and mass spectrometry, mean recoveries for each of the 4 constituent drugs were determined at defined time points and compared using Kruskal–Wallis pairwise comparisons. In trial 1, recoveries on day 0, the day the drugs were compounded, were compared with those on days 4 and 14. Mean recoveries on day 0 ranged from 86.0% to 88.9% with SD from 0.7% to 1.5% and were no different after 4 or 14 d of storage ($P < 0.05$). In trials 2 and 3, recoveries on day 4 were compared with those on days 91 and 140, respectively. Although recoveries on days 91 and 140 were statistically lower than on day 4 ($P < 0.04$), the numerical differences were low (<7%). Recoveries of all drugs except zolazepam were slightly higher (0.9% to 2%) when TKX was stored at room temperature (24 °C) than at refrigeration temperature (4 °C) ($P < 0.02$). These preliminary empirical data suggest that the chemical stability of constituent drugs is practically retained for at least 140 d after compounding TKX and storing it at room or refrigeration temperature. Further testing is warranted to determine physical, toxicologic, and microbiologic stability and efficacy of TKX during storage.

P160 Corticosterone Plasma Levels of Rats after a Training Session of the Plus-Maze Discriminative Avoidance Task Compared with the Inhibitory Avoidance Task

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Despite several years of investigation, a complete and consensual notion about the neurobiologic mechanisms underlying learning and memory remains unclear. Phenomenologic approaches have been the most important focuses in the study of these processes, with behavioral animal models helping researchers to bridge the gap between emotional, molecular, and neurobiologic aspects of learning and memory. In this context, the inhibitory avoidance task (IAT), which involves punishment to the natural exploratory drive of rodents by a footshock, is the most widely used classic animal model of learning and memory. However, there is a strong ethical concern regarding this aversive task model, which involves a highly stressor factor as an operant-like conditioning component. On the other hand, the plus-maze discriminative avoidance task (PM-DAT) is a simple and ethically feasible animal model that simultaneously evaluates learning, memory, anxiety and motor function by the presentation of light and noise as aversive stimuli in a modified elevated plus-maze. Thus, the aim of the present study was to compare the corticosterone plasma levels of rats after the training session of either the IAT or the PM-DAT. Three-month-old Wistar male rats were submitted to the training protocol of one of the paradigms in separate rooms, while 2 other groups of animals remained in their home-cages, each in one of the rooms where the different tasks were being performed (CTRL IAT or CTRL PM-DAT groups). Twenty minutes later, rats were euthanized for blood samples collection and subsequent corticosterone quantification. The training session of both tasks significantly enhanced the plasma corticosterone levels in rats. However, the increase due to the training of the IAT was 55% higher than that observed after the training of the PM-DAT. In addition, rats that remained in the same environment where the IAT was being performed (CTRL IAT) did not significantly differ either from the PM-DAT or from the CTRL PM-DAT groups, suggesting a trend to present enhanced levels of corticosterone. In conclusion, our data reinforces with biologic proof that, beyond its methodological advantages, the PM-DAT is a less stressful rodent model than the IAT for the evaluation of learning and memory. Funding AFIP, FAPESP (#2011/12325-6) and CNPq.

P161 A Method for Training Bipedal Locomotion in a Rat Spinal Cord Injury Model

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Spinal cord injuries (SCI) are a prevalent complication, from which approximately 250,000 Americans suffer and with an estimated worldwide incidence of 10 to 40 cases per million people. Animal models have facilitated both the understanding of the pathophysiology of SCI and the development of interventions and treatment. Because spinal cord injury is readily induced in the rat model, the bipedally stepping rat has been widely used as a model for SCI. Most animals used in these studies were trained to step bipedally after injury. As it is important to understand the effects of spinal cord injury on physiologic measures within the animal, making physiologic measurements (kinematics, neuronal, EMG) on bipedally stepping rats preinjury is necessary. To develop bipedal locomotion pre spinal injury while minimizing stress on the animals, Long–Evans rats were trained through a 7-step protocol and evaluated at each step to assess progress. Factors taken into consideration for scoring included irritability and general walking style. Animals that did not reach criteria for each step were removed from the study to avoid excessive stress. To minimize stress, animals were first gentled (for example, handled daily and introduced to equipment used in training) 6 d prior to treadmill stepping. The animals were then supported on the treadmill during quadrupedal walking and progressed into bipedal movement. Body support during bipedal movement decreased over a period of 2 to 3 wk until 60% of their weight was supported by the apparatus. At this point, water restriction was used to encourage stepping, with water used as a reward for success. After successful training, the animals were implanted with EMG and neuronal electrodes. Training and data collection continued after 1 wk of recovery. The animals were then spinalized and training continued after recovery. Weight was maintained throughout the process, including during water restriction and surgery, suggesting that the rats handled stress relatively well. Using this technique, 60% to 70% of the animals entering the study achieved bipedal stepping. The rats exhibited few signs of stress during training, and this refined approach achieved a reduction in the number of animals used.

P162 Comparison of the Analgesic Effects of a Sustained-Release Formulation of Buprenorphine with Buprenorphine Hydrochloride in a Rat Laparotomy Model

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The aim of this study was to determine if a single dose of a sustained-release formulation of buprenorphine (buprenorphine-SR) would reduce stress and discomfort due to breakthrough pain, from a laparotomy, as well as the additional restraint required for readministration of the shorter-acting buprenorphine hydrochloride (buprenorphine-HCl) formulation. Rats were randomly divided into 6 groups, with 6 male Sprague–Dawley rats in each group: group A (no surgery, no anesthesia, no analgesia); group B (no surgery, anesthesia, buprenorphine-HCl); Group C (no surgery, anesthesia, buprenorphine-SR); group D (surgery, anesthesia, saline injection); group E (surgery, anesthesia, buprenorphine-HCl); group F (surgery, anesthesia, buprenorphine-SR). The surgical groups received a 3-cm midline laparotomy, during which the viscera were gently manipulated by the surgeon's index finger for 2 min. At 24 h (T24), T48, and T72, each rat was weighed and food and water consumption measured. Rats receiving buprenorphine-HCl had significantly greater mean bodyweight loss compared with rats receiving either buprenorphine-SR or no buprenorphine. Rats in group B consumed significantly more water than the rats in group A. Rats in group D consumed significantly less water compared with groups E and F. Rats not receiving either formulation of buprenorphine had a significantly higher mean food consumption compared with rats receiving either buprenorphine formulation. Each rat had its behavior monitored and evaluated with a behavioral ethogram at T4, T8, T12, T24, T36, T48, and T72 after administration of either their formulation of buprenorphine or saline. There was no significant difference between pain scores for any of the groups at any time points. The decrease in food consumption and decrease in bodyweight is likely due to the administration of buprenorphine. The lack of any statistically significant difference in pain scores between any of the groups may be due to small population size, observer inexperience, and/or the laparotomy model not eliciting a significant pain response.

P163 Pharmacokinetics of Transdermal Buprenorphine in Göttingen Minipigs (*Sus scrofa domestica*)

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Buprenorphine is an opioid that has been shown to provide adequate postoperative analgesia in both companion and laboratory animals. However, its use is still hindered by the need for multiple parenteral injections to achieve continuous analgesia. The purpose of the current study was to conduct a pharmacokinetic analysis of a long-acting formulation of buprenorphine in healthy Göttingen

minipigs using liquid chromatography- electrospray ionization-tandem mass spectrometry. Administration of a 30 µg/h transdermal buprenorphine (TDB) patch resulted in an $AUC_{0\text{-}Tlast}$ of 25.2 ± 3.9 ng × h/mL, in comparison to 9.7 ± 1.4 ng × h/mL for 0.02 mg/kg IV buprenorphine. Using a hypothesized therapeutic plasma buprenorphine concentration threshold of 0.1 ng/mL, therapeutic concentrations were achieved at the first study time point (5 min) and lasted an average of 8.0 ± 1.3 h for intravenous buprenorphine. TDB achieved therapeutic concentrations in 12 to 24 h after patch application and lasted until the patch was removed at 72 h. The results of this study suggest that TDB may be a long acting alternative for pain management and its use could decrease animal handling and stress, thus simplifying pain management and improving animal welfare in laboratory swine.

P164 Analgesic Effect of Voluntary Ingested Buprenorphine on Thermal and Postoperative Pain in Rats

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Voluntary ingestion of buprenorphine is a convenient and noninvasive method for oral administration of postoperative analgesia to laboratory rodents, with reduced stress and longer-lasting serum concentrations of the drug as shown in previous studies. However, it has been questioned whether voluntary ingestion of buprenorphine actually provides a sufficient analgesic effect. We hypothesized that voluntary ingestion of buprenorphine does provide analgesic effect similar to that of the traditional subcutaneous administration of the drug, in the hot-plate test as well as in acute postoperative pain after plantar incision. In total, 98 Sprague–Dawley rats were used in the study. In the first part, 50 male rats were divided into 5 groups and treated with buprenorphine administered via subcutaneous injection (0.1 mg/kg body weight [bw]) or by voluntary ingestion (0.5, 1 or 2 mg/kg bw), and tested in the hot-plate test before treatment and then at 0.5, 1, 2, 4, 6, and 8 h after treatment. The treated animals were compared with a control group receiving only vehicle. In the second part, 24 male and 24 female rats were divided into 4 groups and allowed to voluntary ingest buprenorphine in (0.4 or1.0 mg/kg bw) prior to a surgical incision in the hind-paw plantar skin and muscles during isoflurane anesthesia, and the postoperative pain was assessed at 1, 6 and 24 h after surgery by measuring the withdrawal threshold in an electronic von Frey test. The orally treated animals were compared with animals receiving subcutaneously buprenorphine (0.05 mg/kg) or no buprenorphine. A 2-way ANOVA showed that buprenorphine had an analgesic effect in the hot-plate test, both after oral and subcutaneous treatment, and that the effect lasted for at least 4 h. In the incisional model, there was a similar analgesic effect after oral administration to that after subcutaneous injection at 1 and 6 h after surgery. Thus, to some extent, the present data support the hypothesis that voluntary ingestion of buprenorphine has a sufficient analgesic effect. Taken together with its known stress-reducing effects, the method may be applied as a routine treatment in connection to invasive procedures in laboratory rats. However, further studies on beneficial as well as possible adverse effects need to be carried out before the method can be fully implemented.

P165 Occurrence of Gut Parasites and Anthelmintic Treatments in a Laboratory Colony of Wild-Caught Gambian Pouched Rats (*Cricetomys* spp.)

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Gambian pouched rats (*Cricetomys* spp.) are large rodents native to subSaharan Africa. Fifty-one pouched rats were wild-caught in Tanzania and Ghana and imported to Oklahoma State University for use in ordnance detection studies. Their use in a laboratory setting is confounded due to the potential for horizontal and zoonotic transmission of parasites which may be present in wild populations of the genus. A survey of gastrointestinal parasitism via fecal flotation revealed the presence of multiple parasites in the colony, including *Nippostrongylus brasiliensis*, *Heterakis spumosa*, *Trichuris muris*, *Hymenolepis nana*, *Eimeria* spp., and *Strongyloides ratti*. Little data exist regarding effective treatment options for these rodents; recommendations are based only on similar species. Several common treatments were administered to determine their efficacy in treating gastrointestinal parasitism in the colony; serial fecal flotations were used to determine continued presence of parasite ova. Oral self-administered fenbendazole 150 ppm, topical moxidectin 2 mg/kg, oral pyrantel pamoate 15 mg/kg, oral piperazine 100 mg/kg, and injectable ivermectin 0.25 mg/kg were utilized. Pyrantel pamoate and piperazine were easily administered and resulted in a significant reduction in parasite load through the study (84% and 91%, respectively); moxidectin and ivermectin were ineffective at reducing fecal egg shedding. Fenbendazole was most effective at clearing infection with *T. muris*. Given the varied response of each parasite to different medications, a combination treatment may be necessary to successfully treat all parasites present in any given animal.

P166 Dexmedetomidine as an Alternative to Xylazine for Anesthetic Induction in Swine

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Xylazine is an α_2 agonist frequently used in combination with tiletamine HCl and zolazepam HCl (tiletamine/zolazepam) for anesthetic induction in swine. Dexmedetomidine is frequently used as an alternative to xylazine in veterinary medicine, and there are published doses for this drug in many species. However, there is little information on the use of dexmedetomidine in swine. Shortages of veterinary drugs are not uncommon and can create challenges in providing veterinary care. In response to a shortage of xylazine, we evaluated the use of dexmedetomidine in combination with tiletamine/zolazepam as an alternative for the anesthetic induction of swine. Seventeen 40-kg Yorkshire-Landrace cross swine that were being used in acute surgical training received either our standard combination of tiletamine/zolazepam at 4.4 mg/kg + xylazine at 2.2 mg/kg (TX), tiletamine/zolazepam at 6.6 mg/kg + 0.0325 mg/kg dexmedetomidine (TD), or tiletamine/zolazepam at 6.6 mg/kg + dexmedetomidine at 0.02 mg/kg and butorphanol at 0.2 mg/kg (TDB) for anesthetic induction. No significant difference was seen in the time to recumbency ($P = 0.054$), the time to intubation ($P = 0.945$), heart rate ($P = 0.904$), respiratory rate ($P = 0.607$), or $\%SpO_2$ ($P = 0.163$) between the 3 groups. No animals in the TD group required additional anesthesia in order to intubate them, whereas both the TX group and the TDB group had animals that needed additional anesthesia to facilitate intubation. The results of this study indicate that dexmedetomidine at 0.0325 mg/kg can be used in combination with tiletamine/zolazepam for successful anesthetic induction in swine, and may serve as an alternative to xylazine.

P167 Comparing Newer Methods for Detection of Common Rodent Parasites: Serum IgE of Sentinels Compared with PCR of Rack Exhaust Dust

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Rodent fur mites and pinworms are still widespread in research rodent colonies, likely because traditional diagnostic methods are labor-intensive and prone to false negatives. Serum IgE testing of sentinels has been proposed as a means to detect parasite infestations because it increases in response to parasite infestation and testing can

use existing sentinel surveillance procedures. However sentinels themselves do not reliably become infested with fur mites or pinworms via soiled bedding exposure, and it is not known if there is an IgE response without active infestation. Therefore we tested serum IgE and infestation status of sentinels exposed to soiled bedding from 3 rodent colonies with longstanding monoinfestations of *Syphacia muris* (rats), *Aspiculuris tetraptera* (mice) and *Myocoptes musculinus* (mice). We used 5 sentinel cages per colony. Each of the 3 infested colonies was housed on a separate ventilated rack in quarantine and used solely for this project. For each infested colony, soiled bedding from all colony cages was mixed and 240 mL placed in each of the 5 sentinel cages every 2 wk for 3 mo. At 30, 60, and 90 d, we tested sentinels by microscopy and for serum IgE, and tested rack exhaust dust by PCR. None of the mouse sentinels exposed to the fur mite or pinworm colonies were positive microscopically or by serum IgE titer at any time point, whereas 2 rat sentinels were positive microscopically for pinworms and one was positive for serum IgE, but only at 60 d. Infested controls from the 3 colonies were all IgE-positive. Rack PCR tests were positive for all parasites at all time points. We conclude that serum IgE from soiled bedding sentinels is not an improvement over traditional microscopy because although IgE in positive controls increased in response to infestation, there was no IgE response when the sentinels did not become infested. However rack exhaust PCR is an early and efficient detector of common rodent parasite infestations.

P168 Generation of Chimeric Mice: Comparison of the Effects of Holding Conditions of Blastocysts Prior to Embryonic Stem Cell Injection

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Generation of chimeric mice from mutated embryonic stem cells (ES cells) injected into blastocysts to study gene function is a commonly used method worldwide. Here we looked at how holding of blastocysts prior to ES cell injection in fresh harvested, cryo-thawed and chilled conditions affected key parameters such as chimera formation and germline transmission (GLT). We aim to achieve GLT in the most efficient and effective manner as it impacts on the available resources within research facilities and also on the number of animals used in experiments (3Rs). Ten to 25 mutated ES cells from 41 ES cell clones were injected into blastocysts from the C57BL/6Brd-Tyr strain and transferred to the uterus of pseudopregnant recipient female mice. From the resulting mice born postembryo transfer we observed the following differences: In the chilled blastocyst group we found an increase in birth rates 31% compared with 22% and 15.5%, respectively from freshly harvested and cryo-thawed blastocysts. Chimera formation was the same in both the fresh and chilled group (9%) but lower in the cryo-thawed group (5%). Chilling of the blastocysts also gave differences in the sex ratios of these chimeras with fresh giving 74% male:26% female, Chilled 40% male:60% female and cryo-thawed 63% male:37% female. Results showed GLT rates were noticeably different amongst the 3 groups with fresh (27%), chilled (3.5%) and cryo-thawed (0%). In conclusion our results suggest that the preinjection condition of the blastocysts may influence the outcome of pregnancy rates, chimera formation, sex ratios in chimeras and their ability to give GLT.

P169 A Simple Solution to Prevent the Abdominal Migration of Temperature Loggers, and to Facilitate Their Smooth Retrieval Poststudy in Macaques

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Before being infected with an Influenza virus, 12 cynomolgus monkeys (*Macaca fascicularis*) were implanted with temperature data loggers. These devices are small implantable recorders that measure temperature with a high degree of accuracy, and store the data in their internal memory. All measurements are in real time, and can be accessed after the logger has been retrieved at the end of the study. After retrieval of the data, the logger can be reprogrammed and reused for as long as the battery lasts. The transmitters' upper surface is very smooth, and has no ridge or other affixing possibility to attach the logger to the abdominal wall. In previous experiments, we experienced that such loggers migrated through the entire abdomen, which made surgery to retrieve the loggers difficult, necessitating almost an explorative laparotomy. In order to refine this retrieval surgery, a simple homemade solution was devised: a knot of nonresorbable suture material with needle was created around the logger. After surgery, the abdomen and the skin incision were closed and 5 mo after insertion, the temperature loggers were removed from all 12 animals. All loggers were still fixed in the position where they had initially been stitched. The retrieval surgery was scored as a minor discomfort. All measured data was uploaded successfully into a computer. The implanted loggers did not have an adverse effect on the animal's health, which was checked daily. After retrieval, all animals were alive. The knot of nonresorbable suture material with needle around the logger showed to be a simple solution to prevent the abdominal migration of temperature loggers, and to facilitate their smooth retrieval poststudy in macaques.

P170 Effects of PTEN and PI3 Kinase Inhibitors on Superovulation in A/J Mice

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Strain/individual differences in superovulation efficiency with gonadotropins constitute a serious problem in mouse reproduction. Since PI3K signaling pathway is involved in ovarian folliculogenesis, effects of inhibitors of phosphatase and tensin homolog deleted from chromosome 10 (PTEN) and PI3 kinase on superovulation were examined in one of the low-responder strains, A/J mice. Superovulation was induced by peritoneal injections of equine chorionic gonadotropin (eCG, 5 IU) at 28 d of age and, 48 h later, human chorionic gonadotropin (hCG, 5 IU). In the PTEN inhibitor-treated groups, 30 µg of dipotassium bisperoxo (picolinato) oxovanadate (V) (bpV(pic)) in Ringer solution were intraperitoneally injected on various days before, on, or after eCG injections. In the PI3 kinase inhibitor-treated group, 0.1 mg/kg of LY294002 in Ringer solution with 5% DMSO were intraperitoneally injected 1 d after eCG injections. Ovulated oocytes were collected 16 h after hCG injections. The numbers of ovulated oocytes in the control and bpV(pic)-treated groups, respectively, were 9.4 ± 1.0 and 7.2 ± 1.9 when bpV(pic) was administered 2 d before eCG (mean ± SEM, $n = 5$); 9.8 ± 1.6 and 11.2 ± 1.0 when administered 1 day before eCG ($n = 5$); 12.6 ± 3.7 and 21.2 ± 2.0 when administered on the same day as eCG ($n = 5$); and 15.4 ± 2.0, 10.0 ± 0.7 and 11.6 ± 1.9 when administered after eCG (control, 1 and 2 d after eCG, respectively; $n = 5$). Although no significant difference was found by ANOVA, the number of ovulated oocytes tended to be highest when bpV(pic) was injected on the same day as the eCG injection. Inhibition of PTEN, that is, activation of PI3K signaling pathway, 1 d after eCG tended to decrease the ovulation rate, suggesting that PI3K signaling pathway should be suppressed 1 d after eCG for better superovulation. This is also suggested because the number of ovulated oocytes in LY294002-treated group tended to be higher than that in vehicle control group (12.4 ± 2.1 and 10.2 ± 5.7, $n = 5$). This novel technique using a PTEN inhibitor and gonadotropins may be useful for efficient superovulation in mice, although the optimal timing for modulations of PI3K signaling pathway should be determined.

P171 Development and Progression of Renal Functional Decline in the ZSF1 Rat Model of Diabetic Nephropathy

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CT

The ZSF1 obese rat closely approximates the pathophysiologic conditions of human diabetic nephropathy (DN), including hypertension, proteinuria, and hyperglycemia. We sought to further characterize disease progression in this model with a particular focus on glomerular filtration rate (GFR) and renal biomarkers. Furthermore we sought to test the effect of losartan on these endpoints. Male ZSF1 obese rats and their age-matched lean controls were studied from 11 through 41 wk of age for the development and progression of DN. Starting from week 25, losartan was administered at 3 doses (3, 10, 30 mg/kg once a day) for 16 wk. Mean arterial pressure (MAP) and heart rate (HR) were assessed from week 25 onwards. Weekly measurements of proteinuria were performed. True GFR measurements in the conscious and anesthetized rats were performed using the inulin clearance method. Additionally, histologic quantification of glomerular and interstitial lesions were performed at select ages on a subset of rats (11, 25, and 41 wk) and specific biomarkers were assessed using biomarkers for mesangial proliferation (PCNA, Ki67), myofibroblast activation (α-SMA), macrophage infiltration (ED-1), podocyte (Glepp-1) and tubular damage (Kim-1) by immunohistochemistry. Proteinuria developed by 16 wk of age (Lean: 25 ± 1; Obese: 61 ± 6 mg/d) and continues to increase with age. This coincided with increased incidence of glomerular lesions and (Lean: 1 ±0.1; Obese: 20 ±0.6 %) and interstitial lesions (Lean: 0; Obese: 77 ± 3.2 total number foci). Furthermore, there was an increase in ED-1 (Lean: 0.2; Obese: 2 ± 0.1%), KIM-1 (Lean: 0; Obese: 4 ± 0.4%), SMA (Lean: 0.4; Obese: 2.9 ± 0.3%) and a decrease in GLEPP1 (Lean: 37 ± 1.0; Obese: 19 ± 0.7%). Losartan dose-dependently reduced proteinuria and MAP. In summary, the ZSF1 obese rat model displays key hallmarks of early DN and progresses towards overt diabetic nephropathy over the course of 11 to 41 wk of age.

P172 Vascular Catheter Locking Solutions in Rats: Sodium Citrate as an Alternative to Heparin

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Pharmacokinetic studies in rats are conducted using a chronically implanted catheter that allows for repeated blood sampling; however, maintaining continuous patency sets practical limits on its uses. Catheter patency is affected by factors including flushing regimen, catheter material, and choice of locking solutions. In this study, a recently introduced nonheparin-based locking solution containing 4% sodium citrate is compared with traditional heparinized locking solutions with respect to their ability to maintain patency of indwelling polyurethane vascular catheters in rats. Locking solutions of heparinized (500 IU/mL) 50% dextrose (LOCK 1) and heparinized (500 IU/mL) glycerol (LOCK 2) were obtained from SAI infusion technologies. Sodium citrate (4%) with 30% glycerol (LOCK 3) pH adjusted to 6.2 (range 6.0 to 6.5) with citric acid. In this IACUC-approved study, 60 adult male 200- to 225-g CD rats (Crl:CD (SD)IGSBR) were randomly allocated into 3 groups of 20 each for LOCK1, LOCK2, and LOCK3. Standard feed, bedding and water were provided ad libitum. Rats were anesthetized and a polyurethane catheter was inserted into the femoral vein as previously described. LOCK1, LOCK2, or LOCK3 was applied, the catheter was sealed with metal plug and the extravascular portion was extended subcutaneously, exiting at the interscapula region. Patency of the catheter was checked for 5 animals per time point within each lock solution group at 7, 14, 21, and 28 d postimplantation. Catheter was considered fully patent if withdraw of blood was successful with first or second attempt. LOCK1 and LOCK2 groups (heparinized) retained 100% patency to day 21. Patency rates decreased to 40% and 25% per group (respectively) at day 28, confirming earlier findings. 80% of LOCK3 group retained patency to day 7, decreasing to 40% to 60% at day 14, 21, and 28. These findings support heparinized catheter locking solutions to maintain patency; however, sodium citrate locking solution may be used as an alternative, at a lower patency rate, where heparin is contraindicated or unavailable.

P173 Short-Term Cultivation of Murine Gut Microbe Segmented Filamentous Bacteria on a Human Cell Line

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The use of animal models in biomedical research is pivotal to advancing the field of both human and animal medicine. Unfortunately, researchers encounter changes or loss of model phenotypes associated with differences in environment or vendors. One explanation for such changes is potential differences in gut microbiota. Segmented filamentous bacteria (SFB) are anaerobic, spore-forming gram-positive bacteria found in the intestinal tracts of several species, including humans. Once thought to be a nonpathogenic commensal microbiota, SFB has been shown to modulate the development and maturation of the mucosal immune system. More importantly, the presence or absence of SFB can alter many mouse model phenotypes ranging from intestinal to systemic disease models. However, the inability to culture SFB in vitro is a hindrance to studying the mechanisms of these changes. The purpose of this study was to establish a reproducible method to culture SFB in vitro. We hypothesized that SFB requires a low oxygen environment and cells to facilitate growth outside of the host. We tested 2 candidate cell lines: a human colonic carcinoma line and a mouse fibroblast line paired with several incubation conditions including aerobic, anaerobic, and 2 microaerophilic environments. Cell lines were inoculated with either serial dilutions of mouse ileal scrapes with or without chloroform treatment or limiting dilutions of antibiotic combinations. We found that inoculation of ileal scrapes from SFB-positive mice treated with antibiotics onto an established monolayer of human colonic carcinoma cells while providing a low oxygen environment for incubation resulted in survival in culture for 5 d. These data describe a cell culture technique that shows promise for in vitro isolation and propagation of SFB for use in studies of intestinal and systemic diseases.

P174 Development of a Multiplex Bead-Based Array Protocol for Determination of Positive Sera for Select Feline Viruses

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The most frequent vaccination protocols for feline companion animals include those for feline herpes virus 1 (FHV), feline calicivirus (FCV), and feline panleukopenia virus (FPV). Vaccination against these 3 viruses is part of a minimum recommended protocol by the American Association of Feline Practitioners (AAFP). Until post vaccination sarcomas began being recognized in cats in the early 1990s, veterinarians were recommending that these vaccinations be repeated annually based on pharmaceutical labeling of the vaccines. Current recommendations appear arbitrary because few studies have determined the duration of protective immunity. The purpose of this study was to design a multiplex assay to assess antibody titers of vaccinated cats in an effort to determine vaccination booster frequency. Viral cultures of FHV, FCV, and FPV were purified and bound to fluorescent beads. The concentration of protein bound to beads, secondary antibody and serum were optimized for each antigen using serum samples from cats with monovalent antibodies to FHV, FCV, and FPV. Optimal parameters were identified as 12.5 µg of viral protein, a 1:200 dilution of CY3-labeled secondary antibody and a 1:200 dilution of stock sera. The results demonstrated specific antibody recognition of each antigen:bead combination without cross reactivity in individual testing of FHV, FCV, and FPV monovalent control sera. When performed in a multiplex fashion with trivalent

sera there was specific recognition of each antigen:bead combination without apparent cross reactivity. This suggests that a multiplex serologic assay could be used to assess antibody titers in vaccinated cats to determine the appropriate frequency of booster vaccinations and aid in the identifying SPF cats.

P175 A Novel and Sensitive Method for Detection of Mouse Fur Mites: Big Tapes

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Despite the availability of commercial fur swab PCR as a highly sensitive indicator of mouse fur mite infestations, inhouse testing is still widely used. PCR is slower and more expensive than inhouse testing, plus the occasional false positive on PCR dictates confirmation by visualization of mites or their eggs. Microscopy of fur pluck samples is commonly used for detection of fur mites in live mice; however, sparse fur mite infestations in adult mice frequently result in false negative fur pluck tests. We hypothesized that testing a larger fur sample would increase diagnostic sensitivity. We compared the sensitivity of 'big tapes' (96-well plate covers) compared with fur plucks for detection of mites in a colony infested with *Myocoptes musculinus*. Multiple cages comprising young, old, and breeding mice were each tested twice a month apart for a total of 52 cage tests. One mouse per cage was randomly selected and tested first by fur pluck then by big tape. Fur pluck samples were taken from 3 sites (scruff, tail-base and inguinal region), adhered to clear cellophane tape on microscope slides, and evaluated at 40×. Big tapes were applied directly to the dorsum, the ventrum, and one side of the mouse and evaluated under a dissecting microscope at 10× using both incident and transmitted light. Cages testing negative by both big tape and fur pluck were evaluated by PCR of fur swabs: cages testing positive on PCR were considered positive. Of 52 total cage tests, 33 were positive on fur pluck, 47 were positive on big tape, and one additional test that was negative on both tape tests was positive on PCR. This resulted in a sensitivity of 77% for fur pluck testing and 98% for big tape testing. We conclude that microscopy of large fur samples obtained by direct application of 96-well plate covers to the mouse is a highly sensitive method for in vivo fur mite diagnosis.

P176 Benefits of 21% O_2 Compared with 100% O_2 for Isoflurane Delivery to Mice

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At research institutions, isoflurane delivered by precision vaporizer to a face mask is becoming standard for rodent surgery and for procedures with a duration of more than a few minutes. At our institution, the current rodent surgical guidelines "strongly recommend" against the use of gas mixtures other than 100% oxygen, such as room air, for rodent anesthesia. This is in spite of documented complications from long-term 100% oxygen use in human medicine and the known occupational safety risks associated with pure oxygen. Therefore, we conducted studies to examine the effect of anesthetic delivery gas on physiologic parameters in mice. Animals were anesthetized for 60 min via a nose cone with isoflurane delivered in either 21% or 100% oxygen. Femoral artery catheters were placed, and physiologic data and arterial blood were collected at the end of the 60-min anesthetic period. Our results demonstrated no differences in physiologic parameters, including body temperature, respiratory rate, mean arterial pressure, surgical recovery time, pH, or P_aCO_2. However, blood gas analysis did reveal evidence of a ventilation/perfusion mismatch suggestive of significant atelectasis in the 100% oxygen group. We confirmed the increased atelectasis in the 100% oxygen group (compared with 21% oxygen) with respiratory function testing to measure lung compliance and with histomorphometric analysis to quantitate the percent open airspace in the lungs. Taken together, this information can be used to make mouse isoflurane carrier gas recommendations based solidly on physiologic data. Our data suggest that both 100% and 21% oxygen are acceptable for delivery of isoflurane to mice. However, mice being anesthetized for studies focused on lung physiology or architecture would likely benefit from delivery of isoflurane in 21% oxygen in order to reduce alveolar atelectasis and the potential associated downstream inflammatory effects.

P177 Development of a Rabbit Model for Comprehensive Vital System Monitoring

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Improving the quality of physiologic data collected from research animals in toxicology studies is paramount. This is most easily accomplished by collecting as much data as possible from a single animal, thereby reducing animal use and error associated with satellite groups. The present study investigates the feasibility of applying 2 implantable telemetry devices capable of providing data on animal activity, core body temperature, blood pressure (BP), electrocardiogram (ECG), electroencephalogram (EEG), and impedance-based respiratory parameters in the rabbit. Six New Zealand white rabbits were implanted with 2 telemetric devices to accomplish several tasks. The first task was to develop an optimal implantation technique that yields calibrated tidal volume (Vt) measurements that are within 10% of those obtained simultaneously from a pnemotachograph (PNT), low noise ECG, stable BP, and regular EEG. The second task was to challenge with a known respiratory stimulant and a seizure inducer (doxapram HCl, 5.0 mg/kg IV and bicuculline HCl, 0.2 mg/kg IV, respectively) to assess linearity of the calibration across a range of Vt and confirm EEG electrode placement captures generalized seizure activity. Of the 3 impedance electrode placements attempted, only one resulted in calibrations consistently under 10% error. Optimal impedance electrode placement results in calibrated Vt measurements within 1.7% (± 1.6%) of those obtained from a PNT during normal tidal breathing, 6.0% (± 3.6%) following doxapram HCl injection and 7.3% (± 4.4%) following saline injection. Vt range for normal tidal breathing and saline injection was 9 to 15 mL, and following doxapram injection was 25 to 30 mL. Similar error ranges were associated with derived flow parameters. Increases in mean BP of 25.0 ± 6.82 mm Hg and decreases in heart rate of 56.3 ± 6.82 bpm were associated with doxapram injection only. In all cases, the 2-electrode EEG placement was able to detect generalized seizure activity induced by bicuculline administration. No departure from normal body temperature was observed in any group. The development of this model offers a solution to monitoring vital organ system function the conscious rabbit.

P178 Effects of Perioperative Analgesia and Anesthesia on Postoperative Pain Following an Excisional Wounding Procedure in C57BL/6 Mice

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Experimental research procedures using animal models have the potential to cause pain, and it is imperative this be alleviated when possible to ensure humane use of animals in research studies. Previously, analgesics were not used for wound healing studies due to their association with immunomodulation, potentially obscuring research studies at the molecular level. However, current animal research approaches require that analgesia be used unless proven unnecessary or detrimental to obtaining data. The goal of this project was to evaluate the effects of perioperative analgesia in the mouse used in an excisional dermal wound healing study. Four groups of 8-wk-old C57BL/6 male mice underwent isoflurane anesthesia and

received perioperative subcutaneous treatments (0.003 mg buprenorphine, 0.125 mg bupivacaine, 0.003 mg buprenorphine + 0.125 mg bupivacaine [B+B], or 0.15 mL saline) prior to receiving 2 6.0 mm full-thickness excisional wounds. Behavioral assessments including nest building, exploratory activity, and hyperanalgesia were used to assess wellbeing at 4, 8, and 24 h, followed by euthanasia and tissue collection for histopathologic analysis. Nest complexity scoring (NCS) revealed a significant decrease in nesting behavior for all treatment groups. Saline-treated and bupivacaine groups had significantly higher NCS than buprenorphine and B+B treated mice. Exploratory behavior was assessed by open field testing; mice receiving buprenorphine and B+B had increased centerfield passes compared with other treatment groups. All analgesic-treated mice had significantly increased rearing behavior compared with saline-control mice. Hyperanalgesia developing in response to pain is assessed using von Frey hairs. There was no difference between treatment groups at any of the timepoints. Buprenorphine and buprenorphine/bupivacaine treated mice displayed more exploratory behavior compared with other groups, although both had impaired nesting behavior, potentially due to sedative effects of the drug. Bupivacaine-alone treatment did not alter behavior assessments compared with the saline-control.

P179 Repopulation of Rat Whole Lung Cells on a Decellularized Rat Lung: Determining the Influence of the Extracellular Matrix

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A large number of lung diseases result in airway and vascular remodeling. Pulmonary hypertension (PH) is characterized by inflammatory cell recruitment, altered inflammatory cytokine profiles and pulmonary vascular remodeling. A better understanding of the complexities of the extracellular matrix remodeling, especially in chronic inflammatory disease, is woefully needed. The mechanical properties of extracellular environments are tissue specific and influence cellular behavior. We hypothesized that the extracellular matrix shapes the phenotypes of cells in lungs with PH. To begin to test our hypothesis, our lab perfected a technique to decellularize rat lung, and then repopulate and grow rat whole lung cells on the residual lung matrix. Decellularization was achieved using a treatment regimen with detergents, salts, and DNase followed by thorough washing with medium. We were able to repeat successful repopulation of fluorescently labeled whole lung rat cells on $n = 5$ decellularized rat lungs. This methodology is expected to allow our laboratory to investigate the importance of matrix biochemical makeup, stiffness, porosity, mechanical stress changes, etc., all concerns of change within the PH human lung. We believe using our model to better understand the role of the extracellular matrix in PH will lead to newer therapies aimed at restoring normal lung structure and function.

P180 Assessment of Different Fixative Protocols for Ex Vivo Ocular Globes Intended for Ophthalmology Training

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The purpose of this project was to assess different tissue fixation protocols for safely and effectively preserving ex vivo ocular globes. Our goal was to create a suitable ophthalmology training model for novice research personnel. Isolated eyeballs were collected from 4 euthanized cynomolgus macaques after death was confirmed. For each pair of eyeballs, one received 0.1 mL intravitreal injection of 3% glutaraldehyde fixative solution, while the other one did not. The isolated eyeballs were then cooled in three different cryopreservation media, including 10% PEG400 solution, 20% sucrose solution, and 1:9 volume ratio of DMSO: fetal bovine serum, before cryostorage at −20 °C. After 7 d of cryostorage, all of the isolated eyeballs were thawed using a room temperature water bath and examined using slit lamp biomicroscope and indirect ophthalmoscope. Examination results indicated that eyeballs frozen in 10% PEG400 had the best outcome for allowing assessment of the cornea and the lens under slit lamp biomicroscope. However, the fundus could not be sufficiently evaluated using indirect ophthalmoscopy. The intravitreal injection of 3% glutaraldehyde solution preserved the anatomic structures of the uvea and the retina. The cilliary body and zonule fibers kept the lens in place, and the retinal vasculature, the optic disc, and the macula lutea could be visualized under magnification after dissection. Without intravitreal fixation, all eyeballs showed signs of autolysis, including liquefaction of the vitreous body, softening of the tissues, and shrinkage of the ocular globe. While the effects of longer term storage on the physical condition of isolated ocular globes still warrant further investigation, this project demonstrated that it is possible to salvage unique tissues from euthanized animals to create teaching tools. Ocular globes from other animal species, as well as those with lesions, may be collected and preserved for specific technical and anatomic training at a later time.

P181 Monitoring and Maintenance of an Azotemic Environment in an Acute Renal Failure Model in Swine

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Uremia is a clinical syndrome in humans associated with chronic kidney disease or acute kidney injury if loss of renal function is severe. Progression of uremia is characterized by fluid, electrolyte, and hormone imbalances and metabolic abnormalities, resulting from retention in the bloodstream of waste products normally excreted in the urine. In an acute renal failure model in swine, we sought to establish and postoperatively maintain an azotemic environment, accumulation of abnormally large amounts of nitrogenous waste products in blood similar to that observed in uremia, for preclinical evaluation of novel therapeutic devices. Seven female Yorkshire swine underwent laparoscopic bilateral nephrectomy on day 0. Blood was collected immediately prior to surgery, and at 8 h and approximately 24 h postsurgery for blood gas, hematology, and clinical chemistry analysis. Increases in average baseline levels for blood urea nitrogen and serum creatinine were 82% ($P = 1 \times 10^{-6}$) and 190% ($P = 2 \times 10^{-5}$), respectively, at 8 h after nephrectomy, which further escalated to 239% ($P = 4 \times 10^{-6}$) and 417% ($P = 4 \times 10^{-9}$), respectively, at approximately 24 h after nephrectomy. Animals also became hyperkalemic the day after surgery. Serum potassium levels at 8 h after nephrectomy were similar to baseline levels; however, at approximately 24 h after nephrectomy potassium levels significantly increased on average 45% ($P = 0.006$) from baseline. The most severe example of hyperkalemia occurred in the first animal to undergo bilateral nephrectomy. Sudden cardiac arrest occurred under anesthesia during therapeutic evaluation 24 h after nephrectomy. The animal was revived several times and treated with lidocaine, atropine, phenylephrine, calcium chloride/gluconate, and 50% dextrose. For subsequent animals, a successful plan using insulin and 50% dextrose was devised to proactively combat hyperkalemia. This acute renal failure model using swine has been established with consistent and repeatable elevation of serum blood urea nitrogen and creatinine levels. Postoperative electrolyte imbalances can successfully be combated to allow for this model to be maintained in an azotemic environment for preclinical development and evaluation of novel therapeutic devices.

P182 Comparison of MALDI-TOF Mass Spectrometry to Phenotypic and Genotypic Methods for Identification of Bacteria Isolated from Research Animals

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As part of health surveillance, diagnostic laboratories routinely screen animals for pathogenic bacteria by culturing the respiratory and gastrointestinal tracts on cell-free media. Isolated colonies of interest based on colonial and cellular morphology, are phenotypically characterized most often using multitest biochemical systems as well as serotyping. Phenotypic methods, however, may be imprecise or inconsistent and hence, need to be corroborated by genotypic tests including PCR and 16S rDNA sequencing. We routinely use PCR to verify biochemical identifications of *Pasteurella pneumotropica* and *Corynebacterium bovis*, common in genetically engineered and athymic nude mice, respectively. Matrix-assisted laser desorption ionization time-of-flight mass spectrometry (MALDI-TOF MS) identifies bacterial isolates by their unique peptidic spectra primarily of ribosomal and other housekeeping proteins expressed at high levels. It is much more rapid and has a lower cost per identification than biochemical test systems, and is reportedly more accurate. Therefore, we evaluated MALDI-TOF MS to determine its suitability as an alternative to biochemical and corroborative PCR testing. Of 28 bacterial species, representing 20 genera (but excluding *P. pneumotropica*), IDs by phenotypic methods matched those of MALDI-TOF MS for 22 (79%) and of 16S rDNA sequencing for 18 (64%). Most mismatches were for the 5 isolates of *Salmonella*, *Shigella*, or *Escherichia*; excluding those, the correspondence of phenotypic IDs to those MALDI-TOF MS and 16S rDNA sequencing was 91% and 78%, respectively. Of 20 isolates phenotypically identified as *P. pneumotropica,* matching IDs were obtained for 17 (85%) by PCR and 16 (80%) by MALDI-TOF MS; the correspondence between PCR and MALDI-TOF MS IDs was 85%. These results provide preliminary evidence that MALDI-TOF MS is a suitable alternative to phenotypic methods for identification of bacterial isolates, and may reduce the need for confirmatory PCR testing.

P183 Pharmacokinetics and Excreta Recovery of [^{14}C]Erioglaucine to Determine the Impact of Solid-Bottom Compared with Wire-Bottom Caging in Sprague–Dawley Rats

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The *Guide* recommends the use of solid-bottom caging rather than wire-bottom caging in rodents for many reasons, including reduction in environmental stress, allowance for species-typical behaviors, and decreasing the incidence of pododermatitis and other types of foot lesions. In toxicologic facilities, there often has been a concern for reexposure to test article due to coprophagia when rodents are housed in solid-bottom caging, which is used as a scientific justification for the use of wire-bottom caging. Our IACUC questioned whether housing rats in solid-bottom caging rather than wire-bottom caging would interfere with data interpretation on toxicologic studies. Coprophagia is a normal behavior in rodents that occurs as the fecal pellet is being expelled from the anus rather than while lying on cage bottom floor. Nutritional studies that require complete elimination of coprophagia must use special collars or fecal cups, as wire bottom caging is not sufficient to control the behavior. Furthermore, approximately 10% to 50% of feces are reingested, so even with a test article that is excreted 100% in active form in the feces, at most, this would only increase exposure by 50%. Different dose groups in toxicologic studies are often dosed at 5- to 10-fold dosing intervals, which would likely obscure any variability introduced by reingesting of test article in the feces. To determine the extent to which animals housed in solid-bottom caging are exposed to reingestion of test article compared with animals housed in wire-bottom cages, 2 groups of rats were housed individually in either wire-bottom or solid-bottom, bedded cages. All animals were administered a single dose of [^{14}C]Erioglaucine (FD&C Blue Dye #1) via oral gavage. This compound was chosen because it is largely excreted in the feces unchanged within 36 h of administration. After dose, serial plasma samples and feces samples were collected for radioanalysis. After the final excreta sample collection at 72 h, all animals were euthanized and the residual carcasses were solubilized and analyzed for total radioactivity. Results showed no appreciable difference in either the plasma levels or total carcass recovery of the [^{14}C]Erioglaucine between the 2 groups, indicating that there was no net increase in plasma exposure, or in the amount of test article retained in the gastrointestinal tract from reingested feces for rats housed in solid-bottom, bedded cages compared with those housed in wire-bottom cages. Based on the results from our study, solid-bottom caging has a minimal effect on test article reexposure. Therefore, animals can be housed in solid-bottom caging to provide them with the highest quality husbandry while maintaining scientific integrity. This study did not consider the wide variety of classes of test articles and the different ways in which these test articles may be metabolized. It may be important to consider these variables when designing future studies.

P184 Do Singly Housed Male Mice Get Lonely? Evidence of Depressive States after Short-Term Single Housing of BALB/c and C57BL/6 Mice

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Through behavioral and preference studies we know that mice prefer company. Laboratory mice should thus never be housed alone, if a viable option exists. Yet single housing is a fairly common practice, in particular with male mice, because an experimental protocol requires it or because groups exhibiting aggression need to be broken apart. But what are the negative consequences of single housing, if indeed there are any? Does the singly housed male mouse get lonely? Having found inconsistencies in results obtained from singly housed mice in welfare studies we hypothesized that single housing of male mice is capable of inducing depressive states. We also hypothesized that this is demonstrable through challenges with serotonin receptor agonists. The agonist 8-OH-DPAT induces a hypothermic response where the degree of hypothermia can be related to the density/activity of the target serotonin receptor 5-HT$_{1A}$. The 5-HT$_{1A}$ activity is, in this paradigm, a well-consolidated proxy measure of depression. In a proof-of-concept study, mice were injected subcutaneously with 40 µg of the agonist and the core body temperatures were recorded before and 30 min after the injection. We were able to demonstrate that 3 wk of single housing was enough to induce a significantly exacerbated hypothermic response ($F_{4,31} = 3.50$, $P = 0.015$) in 11-wk-old BALB/c mice ($n = 8$) when compared with age-matched group housed controls ($n = 8$). By further screening a cohort of 215 male breeders on a C57BL/6 background—group housed and singly housed—selected at random from 2 breeding facilities, we were able to demonstrate that this effect was ubiquitous. Accounting for age, weight, substrain, and differences in baseline body temperatures, a singly housed C57BL/6 would present with a 0.5 °C (95% CI: 0.3 to 0.7 °C) attenuated hypothermic response for every 10 d of single housing when challenged with 8-OH-DPAT. Our findings indicate that even shorter durations of single housing of male mice may constitute a welfare concern meriting investigation. Furthermore, as antidepressant drug candidates are often first screened in mice, our results suggest that the housing conditions may be of utmost concern in order to obtain reliable results in these studies.

P185 Use of Vinyl Nonadhesive Tape as an Alternative for Positioning Rodents in Small Animal Imaging

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Specific positioning for different imaging modalities is essential in our small animal imaging core. While we have different animal holders designed to position the animal inside the MRI, additional securing of the animal to the holder is needed. Adjustments also have to be made to the body position of each animal imaged to ensure that the correct body part or organ is in the center of the coil and that the rest of the anesthetized animal is secure in the closed-bore MRI. In the interest of refinement of technique, we discovered a type of nonadhesive "tape" made of vinyl that overcame several drawbacks of the other restrainers that were used to position and secure our animals, including residue after tape removal, adhesive sticking to and removing fur, skin sensitivities to the adhesive, tape sticking to and ripping gloves, tape being easily soiled and rendered unusable, and tediousness of adhesive removal. The nonadhesive vinyl is waterproof and can be disinfected and reused making it very economical. Our imaging core has been using it for over a year with no loss of function noticed. It only sticks to itself, providing an adjustable strong hold without sticking to fur, sensitive skin, or gloves. There is no residue left behind because the tape does not use adhesive. The vinyl tape does not stick to gloves making it easy to apply. It is easy and fast to apply and remove, allowing us to accomplish more work in a shorter time frame when imaging batches of animals. In conclusion, the addition of the vinyl tape has increased the safety of our animals, decreased the amount of time used to position each animal, and alleviated some of the frustrations that accompanies the use adhesive tape.

P186 Effects of Renal-Specific Downregulation of Dopamine D2 Receptors and Renal Denervation on Proinflammatory Factors and Blood Pressure in Mice

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Lack of dopamine D2 receptor (D2R) function in mice increases blood pressure and vulnerability to renal inflammation. To study the effects of renal downregulation of D2R function in mice, we selectively downregulated D2R by unilateral subcapsular infusion of D2R siRNA (3 µg/d; 28 d). D2R silencing increased systolic blood pressure (preinfusion: 99 ± 3; postinfusion: 125 ± 1.5 mm Hg; $n = 5$, $P < 0.02$) but not in mice infused with nonsilencing siRNA (preinfusion: 100 ± 2; postinfusion: 103 ± 3 mm Hg; $n = 5$, P = NS). QRT-PCR showed that D2R expression was decreased by 60% in D2R siRNA-infused kidneys but not in contralateral kidneys. Western blotting showed that TNFα (26%; $P < 0.05$), TNFβ (59%; $P < 0.03$), MCP-1 (98%; $P < 0.02$), and IL6 (70%; $P < 0.04$) were increased in the D2R siRNA-infused kidney but not in the noninfused kidney. Similarly, the expression of the injury markers Lcn2, Fn1, and Col1a were increased only in the infused kidney. These results indicate that the intact kidney is unable to compensate for the long-term loss of D2R function in the other kidney. We then hypothesized that the renal nerves may be involved in the blood pressure increase in this experimental model. To test our hypothesis, we performed renal denervation on day 1 of infusion, measured blood pressure, and determined the expression of inflammatory and injury markers. We found that the increase in systolic blood pressure in response to the infusion of D2R siRNA was similar in mice with intact renal innervation, mice with denervation of the intact kidney, mice with denervation of the infused kidney, or mice with bilateral denervation indicating that renal nerves do not significantly contribute to the blood pressure increase. In mice treated with D2R siRNA, denervation of the intact kidney blunted the increase in the expression of TNFα, Fn1, and Col1a in the treated kidney. In contrast, denervation of the treated kidney enhanced the increase in expression of TNFα, Fn1, and Col1a, suggesting that renal innervation restrains the inflammatory response. These results indicate a protective role of the D2R on renal inflammation and development of hypertension. Renal innervation, although it modulates the expression of inflammatory and injury markers, does not affect blood pressure in this model.

P187 Determining the Volume of Isoflurane Liquid Needed for Effective Euthanasia of Mice via the Drop Method

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While CO_2 asphyxiation is our facility's preferred primary method of euthanasia for adult mice, in some circumstances another form of euthanasia may be a more viable option. One alternative is an overdose of the inhalant anesthetic isoflurane. The new AVMA Guidelines for the Euthanasia of Animals lists isoflurane as the preferred inhalant anesthetic for euthanasia of small animals. Since mice have an aversion to isoflurane, high concentrations resulting in rapid loss of consciousness is preferred. Although the volume and exposure time required to reach a level of deep anesthesia is well-documented, there are no concrete parameters detailing the volume and length of exposure required to ensure euthanasia using isoflurane. A closed container, open drop method was used to determine the lowest volume of isoflurane needed for sufficient and humane euthanasia. Based on the findings from a pilot study our current experiments use groups of 13 mice. A 1-L glass jar is set up with a modified 50-cc conical tube inside. Isoflurane is added to the conical tube in specific amounts, starting with 33%, in order to produce the desired concentration. Each mouse is individually added to the jar and observed until 1 min after their last observed breath, with a 10-min cutoff and observed after removal to ensure complete euthanasia. The percent isoflurane for each successive group is refined based on the previous group's results. The desired endpoint is the concentration that reliably obtains death in all mice within 10 min. Preliminary results suggest that 10% Isoflurane vapor is optimal for euthanasia with this method.

P188 Verification of the Congenital Nephropathy Resistant Locus Using *tensin2* Mutant Congenic Mice

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Chronic kidney disease (CKD), which is increasingly common in the world irrespective of developed or developing countries, is an important public-health problem that is strongly linked to diabetes, hypertension, and cardiovascular disease. The ICGN mouse is a CKD model that presents common symptoms (for example, renal anemia) and pathologic changes (for example, tubulointerstitial fibrosis) associated with a variety of kidney diseases. Previously, we found that a deletion mutation of the *tensin2* gene ($Tns2^{nph}$) leading to the proteinuria in ICGN mice. We have also shown that congenic strains carrying the $Tns2^{nph}$ mutation on a C57BL/6J (B6) or 129X1/SvJ genetic background exhibit milder phenotypes than do ICGN mice, indicating the presence of several modifier genes controlling the disease phenotype. Recently, we performed quantitative trait loci (QTL) analysis using backcross progenies from susceptible ICGN and resistant B6 mice, and identified genetic loci investing resistance to CKD progression on chromosomes 2 and 13. To prove the existence of the CKD-resistant locus on chromosome 2, which linked to all tested parameters for CKD, we generated ICGN congenic mice introgressed QTL on chromosome 2 from the B6 mouse (ICGN.B6-(*D2Mit1-D2Mit164*)) using marker-assisted congenic strategy. The progression of CKD was then evaluated by a blood test and kidney histology in 16-wk-old mice. Neither renal anemia nor tubulointerstitial fibrosis was observed in the congenic mice, although severe albuminuria was developed as well as wildtype ICGN mice. The pathologic changes in the congenic glomeruli, such as expansion of the mesangial matrix were milder when compared with wildtype ICGN mice. In conclusion, our results confirm the existence of

CKD-resistant locus on chromosome 2, and suggest that this resistant effect is attributed to antifibrotic actions.

P189 Development of Patient-Derived Xenograft Standard of Care Models of Lung Cancer for Preclinical Testing

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Lung cancer is the most common cancer in men and the fifth most common in women, with more cancer-related deaths worldwide than breast cancer. Patient-derived xenografts (PDX) are presently being used to discover and validate new cancer therapeutics. We are interested in validating the usefulness of PDX lung cancer models by testing in them the therapeutic standard of care (SOC) used in the clinic. In an effort to create standard of care treatment models for lung cancer, a study using common chemotherapeutics was conducted to determine their efficacy at inhibiting tumor growth. Cisplatin and Docetaxel were used to determine the optimal frequency and dosage for maximum tumor inhibition. In this study, lung tumors from 4 human patients were used; 3 of the tumors originated from patients with squamous cell carcinoma and the fourth from a patient with adenocarcinoma. Each tumor model was selected for a different patient tumor background, which results in a wider spectrum of available PDX models for use in future testing. Tumor fragments were subcutaneously implanted in NOD.Cg-Prkdc^{scid} Il2rg^{tm1Wjl}/SzJ (NSG) mice, harvested, and passaged into 45 NSG mice in each study. Once the tumors achieved a volume of 70 to 300 mm^3, mice were randomized into treatment groups. Each study consisted of a control group with vehicle administration and two treatment groups of cisplatin or docetaxel. Cisplatin (1.5 mg/kg) was intravenously administered on days 1, 2, 3, 14, 15, and 16 while docetaxel (20 mg/kg) was intravenously administered once a week. Body weight and tumor volumes were measured twice weekly. Cisplatin and docetaxel consistently inhibited tumor growth. Using cisplatin and docetaxel, a standard of care treatment for each of the 4 lung tumor models has been developed and has created a baseline of comparison available for preclinical trial research.

P190 A Study of Continuous Supply of Uniform Solutions in Rat Reproductive Engineering Technologies

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Genetic modifications and strain preservation of rats are frequently carried out, and stable reproductive engineering technologies have been developed. However, the solutions required for use in the experiments are not uniform. Therefore, we proposed preparing and storing such solutions in large quantities to allow uniform solutions to be used by multiple research institutes. In this experiment, mR1ECM, used during in vitro culture of embryos, and P10 (10% propylene glycol in PB1) and PEPeS (10% propylene glycol, 30% ethylene glycol, 20% percoll, 0.3 mol sucrose in PB1), which are used for the vitrification of embryos, were prepared in large quantities and stored at 4 °C in ampoules. Collection of 2-cell stage embryos for the experiments was done using superovulated female Wistar rats. Experiment 1 involved assessing the integrity of the solutions stored for long periods of time. A solution from the same lot was prepared and used after 1 wk and after 2 y. There were no significant differences in the developmental rates to blastocyst of fresh embryo (89% and 90%, respectively). The embryo survival rates after vitrification were 98% and 99%, and in vitro developmental rates of vitrified embryos were 74% and 67%, respectively. These differences were not significant. Experiment 2 involved validating the solution in the ampoule through practical application. The 8 organizations included in the study carried out tests using the same embryo manipulation method. The survival rate of the vitrified embryos was over 90% and there were no significant differences among the results of any of the organizations. Furthermore, in vivo and in vitro developmental rates for embryos that survived were 41% to 57% and 47% to 81%, respectively. These results indicate that the developmental rates showed significant differences among the various research institutes. However, it was thought the solution in ampoules were sufficient for rat embryo manipulation use. These results suggest that uniform solutions stored in ampoules can be used continuously over long periods. Furthermore, the same type of technology can be used across multiple research institutes.

P191 Intrafemoral Artery Perfusion in Mice: A Practical Method for Cell Delivery into Skeletal Muscles

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Stem cell therapies are promising approaches for treating muscle disorders such as muscular dystrophies. To test the efficiency of these new therapies in mice models of muscular dystrophies, the arterial route of delivery is advantageous as it provides uniform muscle exposure to the therapeutic agents and avoids lung barrier. However there is no in depth methodologic description of this technique and its downstream muscle perfusion efficiency. This study is designed to develop a reproducible and practical method for intrafemoral artery perfusion in the mouse. We have compared 2 common sites of femoral artery canulation for their efficacies in downstream muscle perfusion. Ten 4-mo-old NSG-mdx4cv mice were divided into 2 groups (n = 5 mice per group). The femoral artery was canulated in group 1 proximal and in group 2 distal to superficial caudal epigastric artery. A 1-cm incision was made at the inguinal region on the right hindlimb parallel to the femoral vascular bundle. After visualization of the branches the inguinal fat tissue was carefully dissected from the neurovascular bundle. The femoral nerve was isolated from the bundle before separation of the artery from the vein and a 6-0 silk suture was used proximal to each cannulation site for the temporary ligation of the artery. After making a partial incision in the artery wall, the 32-gauge intrathecal catheter was guided into the artery and advanced followed by the catheter. At this stage the distal suture was placed over the cannulated artery to secure the catheter inside the artery. Then the guide wire was completely removed and a 1-mL insulin syringe containing the fluorescent dye or cells was attached to the catheter. For muscle perfusion visualization, fluorescent dye was injected and evaluated using near infrared fluorescent imaging. Different muscle compartments were dissected and analyzed for individual muscle evaluation. Additional human cells were perfused with the same perfusion rate and analyzed by immunohistochemistry. Our results describe a detailed anatomic description of the femoral artery and its branches as well as a step by step technical guide to perform this delicate method in mice. It also indicates huge differences in skeletal muscle perfusion among these anatomic sites. While the proximal cannulation site provides robust and uniform perfusion of different muscle compartments including tibialis anterior (TA), gastrocnemius (GC), extensor digitorum longus (EDL), medial and lateral thigh muscle groups in the hindlimb, the distal site, which is only few millimeters distal, fails to perfuse the major muscle groups. Furthermore the vessels of the cell perfused muscles were filled with human derived cells and therefore demonstrated the efficiency of this method to deliver any therapeutic agents into the hindlimb skeletal muscle. This data describes a step-by-step guide for femoral artery perfusion in mice and confirms the importance of anatomic site of cannulation for robust hindlimb muscle perfusion.

P192 One Mouse, One Pharmacokinetic Profile: Quantitative Whole Blood Serial Sampling for Biotherapeutics

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Pharmacokinetics data provide a time course of drug concentrations in circulation, and are important in evaluation of new biotherapeutics in drug development. Mice are chosen for these studies because of their small body size and requirement for less drug although their size imposes physiologic restrictions on blood sampling volume. Traditional composite sampling is often used, where time points across a dosing regimen are taken from different animals and different sites. Composite sampling could lead to increased variability in measured analyte concentrations. More importantly this approach requires greater numbers of animals thus requiring greater amounts of drug material with associated resource costs to support a study. The purpose of this study was to validate the approach of serial sampling from one mouse through a novel ligand binding assay. Ligand binding assays enable detection and quantification of biotherapeutic in a diluted whole blood sample to derive a pharmacokinetic (PK) profile, for example, plate-based enzyme linked immunosorbent assay (ELISA). The ligand binding assay used here was on a platform that is a nano scale immunoassay run on a compact disc using the work station. This investigation has compared PK parameters obtained using serial and composite sampling methods following administration of a human IgG monoclonal antibody. The serial sampling technique was established by collecting a small sample volume of 10 µL of blood via tail vein at each time point following IgG administration. Blood was immediately diluted into buffer followed by analyte quantitation using the platform to measure drug concentrations. Additional studies were conducted to understand matrix and sampling site effects on the drug concentrations. The drug concentration profiles, irrespective of biologic matrix, and PK parameters using both serial and composite sampling data were not significantly different ($P > 0.05$). There were no sampling site effects on drug concentration measurements except that concentrations were slightly lower in sodium citrated plasma than other matrices. We, therefore, recommend the application of mouse serial sampling for PK evaluations, particularly in cases of limiting drug supply or specialized animal models. Overall the efficiencies gained by serial sampling were 40% to 80% savings in study cost, animal usage, study length, and drug conservation while reducing intersubject variability across PK parameters to less than 30%.

P193 Concentration Impacts Analgesic Efficacy of Tramadol in Mice

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Effectiveness of tramadol in mouse models of postoperative pain has not been reported in the literature. Previous studies in our lab suggested some analgesia with tramadol when given subcutaneously at 80 mg/kg in a concentration of 16 mg/mL. However, hyperactivity was documented and may have confounded behavioral observations. Literature using higher doses (up to 100 mg/kg) did not indicate adverse side effects but reported volumes of administration ranged from 0.1 to 0.4 mL. This led to our hypothesis that effectiveness of 80 mg/kg tramadol might be demonstrated when administered at a lower concentration and larger volume of 1.0 mL than in a higher concentration and smaller volume of 0.15 mL subcutaneously. Male C57BL/6 mice received saline or tramadol 80 mg/kg in high (HC) or low (LC) concentrations preoperatively as well as every 12 h for a total of 3 doses. Animals underwent aseptic typhlectomy and were assessed for pain by blinded observers using 3 methods. The first method was a general pain scoring system (GPS), which measured posture, coat condition, relation to other mice, activity, and breathing pattern. A higher GPS score signified increased abnormality indicating increased pain. The second assessment quantified the number of times a mouse stretched up (SU) over a 3-min period. A higher SU score represented return to normal activity and indicated decreased pain. GPS and SU were measured 10 h after the second injection of tramadol or saline. Finally, von Frey fibers were applied periincisionally 5 h after the third tramadol or saline doses, and animals were monitored for abdominal retraction, licking or scratching, and jumping. Von Frey fibers showed no significant difference between tramadol HC, tramadol LC, and saline. However, tramadol LC mice had significantly lower GPS scores and significantly higher SU scores than mice that received HC or saline. These findings suggest that tramadol 80 mg/kg LC provides better analgesia with fewer side effects documented 24 h after surgery. This could be due to prolonged absorption of tramadol in the higher volume or lower side effects confounding the pain assessments. When dosing analgesics, concentration and/or volume of the compound may impact perceived analgesic effects.

P194 Establishment of In Vivo ADCC Activity Model Using the hIL2 Tg NOG Mice

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We investigated whether antibody-dependent-cell-cytotoxicity (ADCC) activity is effective for antitumor therapy using human interleukin 2 (hIL2) transgenic (Tg) NOD/Shi-*scid*, IL2Rg^{null} (NOG) mice. Natural killer (NK) cells were obtained by culturing peripheral blood mononuclear cells (PBMCs) derived from human subjects who gave informed consent for this study with an original culture method that expands NK cells abundantly. Twenty five female hIL2 Tg NOG mice (8 wk of age) were divided into vehicle-treated (C), NK-treated (T1), Herceptin-treated (T2) and NK+ Herceptin-treated groups (T3). All mice were irradiated and subcutaneously transplanted NCI-N87 tumor cells, which are overexpress human epidermal growth factor receptor type2 (HER2). One week later, each treatment was started (day 0). Vehicle or NK cells (1.0×10^7 cells per mouse) were administrated intravenously to C, T1, T2, and T3 on days 0, 3, 5, 28, 31, and 33. Herceptin was intraperitoneally administrated to T2 and T3 on days 0, 5, 7, 28, 33, and 35. Body weight and tumor size were measured twice a week. Antitumor efficacy was evaluated by T/C %, which defined as the ratio of mean tumor volume in treated group (T) and control group (C). Reconstitution of administrated NK cells were analyzed using flow cytometry. Antitumor efficacy was confirmed at day 35 and thereafter. T/C percentages at day 84 were 38.2, 67.6, and 10.1 in T1, T2 and T3, respectively. The ratio of human NK cells in T1 or T3 was approximately 35 to 80 or 25% to 80 % during the observation period, respectively. Significant antitumor efficacy due to in vivo ADCC activity was observed. The data indicates that this model would be useful to evaluate the efficacy of antibody candidate drugs in preclinical studies.

P195 Impact of Blood Sampling Procedures on the Welfare of Laboratory Mice

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A previous study showed that blood sampling either automatically, through a catheter, or by puncture of the tail vein is less stressful than facial vein phlebotomy in mice. We decided to investigate how facial vein phlebotomy would affect animal welfare compared with retroorbital blood sampling, which is also commonly used for blood sampling in mice. The study was conducted in male C57BL/6 mice to compare facial vein phlebotomy with retroorbital puncture. Blood was obtained at 2 time points, 1600 and 1800, from either the retroorbital plexus ($n = 12$), the facial vein ($n = 12$) or from control

mice by decapitation (n = 8). No anesthesia was used for any of the blood sampling methods. The samples were analyzed for plasma corticosterone using an enzyme-linked immunosorbent assay. Body weights were recorded pre- and postprocedure, and the food consumption was recorded automatically during 24 h after blood sampling. Cheeks and orbital regions were subjected to histopathology. The hypothesis was that mice subjected to facial vein phlebotomy would express equal or higher stress levels in relation to blood sampling than mice subjected to retroorbital puncture. Acute inflammation was expected in the tissues of both groups of blood sampled mice. Normally distributed data sets were analyzed with analysis of variance and for body weight data repeated measures were taken into account. Data sets that did not follow a Gaussian distribution were analyzed with Kruskal–Wallis H test or a Mann–Whitney *U* test. Mice sampled by facial vein phlebotomy had elevated plasma corticosterone levels at both time points; whereas mice subjected to retroorbital puncture had not. Both these groups lost weight following blood sampling, but the body weight loss was higher in mice subjected to facial vein phlebotomy. The food consumption was not significantly different between the 2 groups of mice. Subcutaneous hematomas and extensive tissue trauma were found after both blood sampling methods. This study supports the preliminary finding that blood sampling from the facial vein of mice induces the highest stress response compared with other routine blood sampling methods. However, both retroorbital puncture and facial vein phlebotomy had considerable impacts on animal welfare, which must be considered whenever blood samples are obtained.

P196 Identification of and Surveillance for *Syphacia* Pinworms in a Colony of Golden Hamsters (*Mesocricetus auratus*)

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It may be necessary in a multispecies facility to cohouse different rodent species free from adventitious infections. Goals of this study were to speciate pinworms found in a closed golden hamster colony and to assess if a dirty bedding surveillance program can detect presence of this pinworm. For speciation, we retrieved worms from the cecum of euthanized hamsters and fixed some either in 5% formalin for morphologic analysis or 90% ethanol for DNA sequencing. For surveillance, we transferred 1 tsp of dirty bedding with feces from pinworm-infected hamsters to 3 sentinel cages weekly. Sentinels were 2 female Hsdhan:AURA hamsters and 3 female 129.*Ifnar1*^{tm1Agt}, *IFNγr1*^{tm1Agt} and 5 C57BL/6.129 *Ifnar1*^{tm1Agt}, *IFNγr1*^{tm1Agt} mice, immunodeficient strains with a double knockout for Type I and II interferon receptors. We conducted weekly perineal cellophane tape tests plus we sent perineal swab samples to a commercial lab for PCR. Morphologically, the worms are consistent with *Syphacia mesocriceti*. However, worm dimensions are larger overall than in previously published descriptions. One-thousand bases of the 28S rDNA locus were sequenced from 4 worms and all 4 were identical. A BLAST search revealed that the closest match to the sequences is *S. agraria* at 86% identity, followed by *S. ohtaorum* and *S. obvelata*. No evidence of pinworms was detected by tape tests or perineal swab PCR at 1 and 2 wk after bedding transfer. Ova was observed on hamster cellophane tapes in weeks 3, 4, and 5 while no ova was seen in any of the mouse cellophane tapes. Hamster swab PCR was positive for generic pinworm in week 3 and mouse swab PCR was equivocal in week 5. Our data suggest this hamster colony is infected with *S. mesocriceti* and that this pinworm is nontransferable to the immunodeficient mouse strains tested by dirty bedding.

P197 Protection Against Dietary Fat-Induced DNA Damage by the Fanconi Anemia Pathway

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Fanconi anemia (FA) is a genomic instability syndrome affecting 1 in 131,000 people in the US. FA phenotypes include developmental defects, bone marrow failure, cancer predisposition, and metabolic disorders. The FA pathway becomes activated by DNA replication stresses and plays a major role in responding to interstrand DNA crosslinks (ICLs). Though it is known that FA patients are hypersensitive to exogenous genotoxins, the endogenous sources of damage repaired by the pathway remain poorly characterized. Our objective was to test the hypothesis that the Fanconi Anemia pathway protects against DNA damage caused by lipid metabolism using a mouse model based on targeted disruption of *FancD2*, which encodes a central component of the pathway. *Fancd2*$^{-/-}$ and wildtype (WT) mice were either continued on standard diet (SD) or challenged with a high-fat, high-cholesterol diet (HFD) at weaning, which led to hepatic steatosis and hepatitis within weeks. In a long term HFD trial, *Fancd2*$^{-/-}$ mice had decreased survival compared with WT mice (P = 0.01; log rank). In *Fancd2*$^{-/-}$ mice, HFD feeding for 10 wk resulted in an increase in hepatic pathology relative to WT controls, including bile duct hyperplasia, neutrophil infiltration, and lipogranuloma formation. This trend toward increased pathologic changes in *Fancd2*$^{-/-}$ mice suggests that the FA pathway plays a role in protecting against HFD induced damage. Consistent with this concept, *Fancd2*$^{-/-}$ mice on HFD also had greater hepatocellular apoptosis vs WT mice on HFD as assessed by TUNEL staining (P = 0.004; Student t test). We propose that reactive oxygen species and lipid peroxidation products, which can result in DNA damage including ICLs, are a source of endogenous damage repaired by the FA pathway. We will test this hypothesis by determining how *Fancd2* inactivation affects HFD-induced oxidative DNA damage and associated DNA damage responses. The endogenous stresses to which the FA pathway responds is an unanswered question of fundamental significance to our understanding of genome maintenance and disease pathogenesis in FA patients. The HFD hypersensitivity described here in *Fancd2*-deficient mice provides a powerful opportunity to define the roles of the FA pathway in protecting against HFD-induced DNA damage.

P198 A Novel Touchpad Application as a Functional Tool for Gait Assessment: A Pilot Study Using a Murine Hindlimb Ischemia Model

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Murine hindlimb ischemia models are used to study a variety of diseases including peripheral artery disease and diabetic vasculopathies. Often these studies include the Tarlov score, a functional gait assessment, and Doppler flow imaging. There is great variability in the degree of ischemia and functional alterations appreciated depending on surgery type, surgical proficiency, and anatomic variation in collateral circulation. The purpose of this project was to use a variety of methods to sensitively detect the onset of decreased limb function postischemic event. To evaluate and quantify mobility post high femoral artery ligation and excision, surgery was performed on C57BL/6 male mice (n = 4) and data collected at specified time points: pre- and postoperatively at day 1, 2, 3, 5, 7, and 14. Data gathered included body weight, rearing behavior, the Tarlov Score, and the Modified Ischemia Score (a scoring scheme for ascending necrosis secondary to vascular compromise). A novel touchpad application was also used to also gather quantitative open field data over a 10-min observation period including distance traveled, velocity, number of touches, and movement time. No significant difference from baseline was appreciated in Modified Ischemia Score, velocity, movement time or number of touches. However, body weight, Tarlov score, rearing events, and total distance traveled were all significantly depressed from baseline at 48 h. The Tarlov score was also significantly depressed from baseline at 24 and 72 h postoperatively. Based on this preliminary data, overall limb function and

mobility appears to nadir at 48 h postoperatively. Use of this data will guide future experiments to assess for the presence or absence of pain during the identified limb function nadir using the traditional nociceptive methods, von Frey testing, as well as evaluation of response to analgesia using the parameters described. With this data, an evidence-based approach for loss of limb function and potentially presence of pain may lead to refinement of this model.

P199 Targeted Suppression of Midbrain α-Synuclein mRNA Levels for Parkinson Disease Treatment

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Prior studies support a role for the protein α-synuclein in the pathogenesis of Parkinson disease (PD). Thus, therapeutic approaches aimed at reducing the expression of α-synuclein in the substantia nigra would be expected to slow the progression of PD. Here, we delivered varying concentrations of siRNA constructs directed against α-synuclein into the subtantia nigra of 18 adult female rhesus macaques (n = 6 per concentration) for 1 mo using a pump and catheter system. Tolerability of α-synuclein inhibition was assessed by monitoring clinical observations and body weights as well as by neurochemical and histopathologic examinations of the striatum and midbrain regions. Histopathologic evaluations indicated that the catheter tip was placed in or near the subtantia nigra pars compacta region in all animals. Molecular analyses of mRNA levels from midbrain tissue punches indicated silencing of α-synuclein expression ranging from 85% to 90% at a dose of 6 mg/mL and 90% to 98% at a dose of 18 mg/mL after a 1 mo infusion at a rate of 0.1 µL/min (or 144 µL/d). There were no indications of toxicity as noted in clinical observations and body weight values. There were no adverse test article-related neurochemical findings in the putamen and no test article-related pathology in the midbrain. There was no evidence of necrotic neurons in any of the midbrain sections evaluated and no microscopic changes in cerebrum/neocortex, ventricular system, or limbic system/hippocampus. Microscopic changes in the midbrain were attributed to catheter placement, including slight/minimal bleeding, astrocytic and microglial cell reaction, extracellular fluid and local tissue loss. The microscopic changes noted would not be expected to result in clinical signs. Our data support further testing of direct midbrain delivery of siRNAs designed to suppress α-synuclein expression as a potential therapy for PD.

P200 Antimicrobial Peptides Are Induced in the Lower Bowel after *Helicobacter hepaticus* Infection

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Antimicrobial peptides (AMPs) play an essential role in innate immunity to enteric bacterial infection. Previous studies have demonstrated higher expression of the antimicrobial peptides RegIIIβ and RegIIIγ in the colon of azoxymethane (AOM) treated 129 RAG-deficient mice 12 wk after *Helicobacter hepaticus* (*Hh*) infection. We and others have previously shown that *Hh* induces expression of IL22, a known initiator of antimicrobial peptide production in the colon. Treatment with an antiIL22 blocking antibody interferes with the induction of AMPs. These data indicate that AMP expression in the colon of AOM treated mice chronically infected with *Hh* depends on IL22. However, whether *Hh* infection alone acutely induces expression of AMPs, and whether the ability of *Hh* to induce AMP expression varies in the different regions of the large bowel has not been evaluated. To examine these issues, sex-mixed 129 $Rag2^{-/-}$ mice were infected with *Hh* and expression of AMPs was examined in anatomic regions of the lower bowel 2 and 6 wk postinfection. Surprisingly, we found that while AMP expression at baseline was markedly lower in the cecum than the colon, AMP expression in the cecum was strongly induced within 2 wk of *Hh* infection. This was associated with higher expression of IL22, and cecal tissue infiltration by neutrophils and monocytes. In contrast, infection with *Hh* markedly inhibited expression of IL25 mRNA in both the cecum and colon. Interestingly, there was no difference in the ability of *Hh* to induce AMP expression in RAG-deficient mice that also lacked IL10, suggesting that AMP expression is not regulated by production of IL10 within the innate immune system. We hypothesize that *Hh* mediated induction of AMP expression may lead to alterations in the cecal microbiome. Further, we propose that this rapid model of *Hh*-induced AMP expression will be useful for delineating key cellular and molecular pathways that regulate intestinal innate inflammatory pathways.

P201 Monitoring Body Temperature of Anaesthetized Mice with Leukemia during Optical Bioluminescence Imaging

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Diagnostic imaging has many potential 3Rs benefits when applied to disease studies involving laboratory mice. Longitudinal monitoring of disease progression can be carried out on the same anaesthetized animals reducing the number of animals used. Imaging is also likely to have less of an impact on the welfare of laboratory animals than alternatives such as serial blood sampling if refined anesthetic techniques are used and if care is taken so that mice do not become cold. To determine if mice with cancer get cold during optical bioluminescence imaging, body temperature was monitored in mice involved in an ongoing program of work. Leukemia was engrafted in 6 female immune compromised NOD SCID Gamma (NSG) mice at 8 wk of age. Mice were injected intrafemorally with acute lymphoblastic leukemia (ALL) luciferase expressing patient derived xenograft cells under a brief isoflurane anesthetic with carprofen (5 mg/kg) administered as analgesic. Temperature sensitive, glass encapsulated, passive radiofrequency identification (RFID) transponders (2.2 × 1.4 mm; 0.12 g) were injected subcutaneously approximately 2 cm from the base of the tail under the same anesthetic. From 3 weeks post tumor implantation, mice were imaged weekly in 3 stages: 1) intraperitoneal luciferin (bioluminescence substrate) injection, 2) isoflurane induction then 3) examination of anaesthetized animals in the optical bioluminescence imaging system. Body temperature was monitored over a 5-min period during each of these 3 stages by placing an automated RFID reader system directly adjacent to the animals' home cage or induction box. Changes in body temperature differed between the 3 stages ($F(2,16)=37.6$, $P < 0.0001$) with temperature falling over 5 min of anesthetic induction but not following luciferin injection or during optical imaging. As cancer progressed, decrease in body temperature during anesthetic induction was positively correlated with disease progression ($R^2 = 0.73$, $P = 0.03$). In summary, we found that body temperature was maintained in anaesthetized mice during the process of optical bioluminescence imaging. However, this pilot study suggests that care should be taken during anesthetic induction as mice lost heat during this period and the drop in temperature was enhanced in animals with advanced disease.

P202 Pharmacokinetics of Ceftiofur Crystalline-Free Acid in Clinically Healthy Dogs (*Canis lupus familiaris*)

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Economical and injectable antibiotics are beneficial when clinical manifestations of disease in research animals prevent the use of oral antibiotics. Ceftiofur crystalline-free acid (CCFA) is an injectable, sustained release form of ceftiofur, a third generation cephalosporin. Administered subcutaneous or intramuscular, it is currently approved for use against susceptible respiratory tract pathogens in swine, cattle, and horses, and infectious pododermatitis in cattle. Since CCFA is an economical, injectable antibiotic that could be of value for use in research dogs, the objective of this study was to determine the pharmacokinetic properties of CCFA in apparently healthy dogs. Five, adult dogs (24.7 to 26.9 kg) were accepted to be apparently healthy after no abnormalities were found on physical exam, complete blood count, and chemistry panel. Dogs were given 5.0 mg/kg of CCFA subcutaneously, and blood samples were collected prior to drug administration and 1, 4, 8, 12, 24, 36, 48, 72, 96, 120, 144, 168, 192, 216, and 240 h after injection. The plasma ceftiofur and desfuroylceftiofur-related metabolites were derivitized to desfuroylceftiofur acetamide (DCA) and measured by using mass spectrometry. The plasma DCA concentration compared with time data were analyzed based on noncompartmental pharmacokinetics using computer software. The C_{max} was 1.98 ± 0.40 µg/mL, the T_{max} was reached at 22.3 ± 8.9 h, the half-life was 56.6 ± 16.9 h, and the $AUC_{0\text{-last}}$ was 124.98 ± 18.45 µg-h/mL. Based upon MICs from common veterinary bacterial isolates cultured by our institution's veterinary diagnostic laboratory, 54% of respiratory pathogens, 79% of skin and wound pathogens, and 86% of the urinary tract pathogens would be susceptible to a single dose of CCFA. Based upon multiple-dose pharmacokinetic predictions, CCFA would be effective against 54% of respiratory isolates re-dosing every 72 h, 75% of skin and wound infections redosing every 96 h, and 84% of urinary tract isolates redosing every 168 h. In summary, CCFA is a cost effective antibiotic for the treatment of skin, wound, and urinary tract infections in laboratory dogs.

P203 Pharmacokinetic Comparisons of Intravenous Bolus Dosing and Serial Blood Sampling from Different Sites in Rats

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The conduct of a simple pharmacokinetic study involving intravenous bolus injection followed by serial blood sampling can be carried out differently in terms of how animals are actually manipulated by research technicians. This project compared the pharmacokinetic profile of a high clearance drug, metoprolol, in Sprague–Dawley rats under 4 different drug administration and blood sampling approaches. There are 3 animals in each group. Group 1 and group 2 animals received metoprolol via tail vein injection followed by serial blood sampling from their jugular cannula (JVC) and their carotid cannula (CAC), respectively. Group 3 and group 4 animals received an IV bolus injection of the compound through a jugular cannula followed by serial blood sampling from a carotid cannula and a tail vein, respectively. The PK profile and parameters (AUC, CL and $T_{1/2}$) of groups 1, 3, and 4 were not significant different. The CL of group 2 was significantly lower than those of the other three groups. This study not only confirmed the importance of keeping consistency in compound dosing and blood sampling techniques during a study, this information should also be disclosed in order to ensure reproducibility of a study in the future. Based on our results, a similar PK profile and parameters were obtained for group 1 approach compared with classic lab approach (group 3). The classic approach needs preparative surgery work of dual-cannulation (JVC and CAC) which may result in organ lesion of animal. In addition, tail vein bleeding is not recommended due to potential contamination and vein damage. Therefore, we recommend the combination of tail vein administration and sampling through jugular vein cannula is to be the best approach for intravenous pharmacokinetic studies.

P204 Presence of Nesting Material Does Not Prevent Ability to Accurately Identify Sick or Dead Mice During Routine Health Checking

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Whether nesting material assists or hinders the ability to identify sick mice during routine health check is a widely debated topic. Currently, our large and diverse academic research institution is exploring the possibility of providing nesting material to all of our mice. In preparing for this change, we conducted an epidemiologic study to evaluate if there was evidence of a disparity in the ability to identify sick or dead mice when nesting material was present or absent in the cage. We hypothesized that provision of nesting material would not result in an inability to identify sick mice and, therefore, would not cause an increased incidence of found dead or severely ill mice. Over a 6-mo period we collected Animal Treatment Reports (ATRs) from 14 rooms, in 5 different animal facilities, balanced across a variety of research and breeding uses either requiring or prohibiting nesting materials. The nesting materials used include nesting squares, crinkled paper strips, cardboard tubes, and cardboard or plastic huts. The data included the clinical condition of the animals on initial veterinary exam and the quality of the nest and nesting material (if present). We also evaluated the death logs from the same rooms to assess the relationship between providing nesting materials and identifying mice that had spontaneously expired in the cage. The data gathered included 800 ATRs and more than 450 death incidences evenly distributed between cages with and without nesting material. The results demonstrate that nesting material does not cause a significant increase in severity of the clinical condition at the time the animal is first reported for veterinary evaluation. Among those cages with nests, there was not a significant difference in clinical condition between the different types of nesting material. Similarly, there was not a significant difference between the number of moribund mice nor numbers of mice found dead in cages under active veterinary monitoring with or without nesting, indicating the ability to identify dying or dead mice is equivalent whether or not nesting material is present. When nesting material was present, significantly more mice were found dead outside the nest rather than in the nest. In summary, nesting material does not hinder the ability to identify sick mice and did not negatively affect the ability to identify mice that expired spontaneously in the cage.

P205 Screening of Nonhuman Primate Records for Bacterial Pathogens

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Nonhuman primates (NHPs) are known to carry various bacterial pathogens, some of which are zoonotic. It is common for paper brought into the NHPs environment, such as animal health records, to have evidence of gross contamination with blood or feces. Hence, there is a possibility that these paper records could act as a fomite for microbial contamination. While there is anecdotal evidence to support the possibility of microbial contamination of animal records, no scientific data exists to either support or refute this. Animal records often come in direct contact with the animals or indirectly contaminated surfaces. We tested animal records in an effort to ascertain whether paper records with exposure to NHP material could be contaminated with bacteria. Sixty animal health records were pooled 10 per group on days 0, 14, and 30 following animal contact and tested via PCR. Sixty records that had not been in contact with animals were used as controls. Additionally, fecal samples from

40 animals were collected for 3 consecutive d, pooled 4 per group, and tested via culture. All health record samples in the test groups on day 0, 14, and 30 were significantly positive ($P < 0.05$) for *Campylobacter*, *Helicobacter*, and *Shigella* when compared with controls. None of the samples tested positive for *Salmonella* or *Yersinia* via PCR. All fecal cultures tested negative for these pathogens. Data from this study shows that direct or indirect exposure of paper products to NHPs may result in prolonged bacterial contamination, even if the animals are not clinically ill. Because it is common for records to be brought into office areas where they are handled without appropriate personal protective equipment, it is possible that zoonotic contamination could occur. Further work needs to be conducted to ascertain the extent of this contamination.

P206 Reproductive and Developmental Toxicology Assessment of Oral Dietary Calcium Formate In Yucatan Miniature Swine

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Calcium Formate (CaF) is being considered as a dietary calcium supplement because of superior bioavailability. We assessed the reproductive/developmental toxicity of CaF in miniature pigs when administered orally in diet. No evidence of reproductive or developmental toxicity was recorded for oral CaF in miniswine. Sixty (30 male and 30 female) sexually mature Yucatan miniature swine were gender paired for breeding and randomized into 3 groups of 10 pairs receiving untreated control, low dose (2.25% CaF), and high dose (4.5% CaF) in the daily diet. Standard reproductive and developmental variables were assessed, including clinical, gross, and microscopic pathology (parents and piglets). There were no significant findings of developmental/reproductive toxicity related to dietary exposure to CaF. Female fertility index/group ranged from 80% to 100%. Total fetuses delivered were 174 with 170 livebirths. Piglet viability was good. Length of gestation, birth weights and body weights were comparable. Litter size was robust (range 5.4 to 7.1) for all groups. There were no statistical differences between groups for these endpoints. CaF supplementation was associated with decreased food intake in both parents in the high dose group and with decreased body weight in low and high dose parents during the premating phase of the study. These between group differences persisted through the gestational phase for the females, but there was not a statistically significant increase in the magnitude of these differences over time. CaF supplementation was also associated with increased serum calcium levels in both parents of the high dose group. Although statistically significant, the magnitudes of these differences were small, and well within the normal physiologic ranges. These findings (decreased food intake and body weight, increased serum calcium) are expected responses to the administration of oral CaF supplementation. Decreased food intake (high dose), decreased body weight during premating phase (low and high dose), and increased serum calcium (high dose) were associated with oral dietary CaF administration. There were no significant findings of developmental/reproductive toxicity related to dietary exposure to CaF.

P207 Additional Use of the Rat Model to Study Liver Cirrhosis Regression

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Liver disease, however induced, eventually progresses to cirrhosis and liver failure. There is a pressing need for a suitable animal model to study all phases of liver disease, including regression of fibrosis. The model induced by dimethylnitrosamine (DMN model) thus far has been used to study the progression of liver disease until fibrosis, which corresponds experimentally to euthanasia of animals on completion of the fourth week of DMN injection. We have since shown that this model can also be used to study changes in the liver that is undergoing repair subsequent to cirrhosis. Eight Wistar rats 200 to 250 g in weight were given 10 mg/kg DMN intraperitoneally, for 3 consecutive days a week for 4 wk. Four rats of similar age and weight were control animals. All rats were given food and water ad libitum and monitored twice daily. Rats were weighed weekly and blood was collected at the start of the experiment, and every 2 wk thereafter. Body weight gain of rats decreased with each week of DMN administration. However, on cessation of DMN treatment, weight gain generally increased but to varying degrees in different rats. ALT and AST levels followed a similar trend of increase with DMN administration and decline on drug withdrawal. Rats were euthanized when they lost 20% body weight and exhibited other signs of being moribund. Five rats survived until euthanasia at 8 wk after completion of DMN administration. Liver fibrosis score of 3 rats which succumbed 2 to 3 wk after cessation of DMN was significantly higher than rats which survived. In addition, survivor rats had microscopic changes indicative of hepatic repair. These included areas of extinction of hepatocytes and replacement by regenerating hepatocytes, dissolution of collagenous septa and changes in hepatic architecture with changes in portal tract arrangement. This model presents opportunities for studies on the processes responsible for reversal of cirrhosis in addition to those for its development.

P208 Targeted Delivery of Magnevist to the Midbrain: A Proof of Concept Study for Parkinson Disease Treatment

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The protein α-synuclein has been implicated in the pathogenesis of Parkinson disease. Consequently, reducing the expression of α-synuclein in the substantia nigra would be expected to be therapeutic. However, targeted drug delivery to the midbrain region remains poorly studied. Here, we used an investigational needle-tip catheter and cranial anchor system coupled to implanted programmable pumps to continuously infuse 5 mM gadopentetate dimeglumine into the substantia nigra (SN) of female rhesus monkeys to lay the foundation for delivering drug therapies to the midbrain region. Flow rate tolerability of either 0.1 µL/min (144 µL/d) to the right SN for 7 d or 0.2 µL/min (288 µL/d) to the left SN for 7 days was assessed by monitoring clinical observations, body weights and food consumption measurements. Evaluation of postsurgical MRI indicated that the placement of each intranigral catheter was within a 2-mm radius from the intended surgical target and that all catheters were patent, as evidenced by the presence of Magnevist at the catheter tip. In addition, post-surgical MRI evaluation indicated that the volume of distribution achieved in the midbrain region with Magnevist infused at a rate of 0.2 µL/min was greater than that achieved at 0.1 µL/min by nearly 2-fold. There were no indications of toxicity as noted in clinical observations. Also, there was no difference between the two infusion rates with respect to changes in body weight values and no indication of adverse effects from either Magnevist flow rates on appetite (food consumption). Our data support that direct drug delivery to the midbrain maybe used for the treatment of Parkinson disease. Based on the data reported here, ongoing studies in nonhuman primates are evaluating the targeted delivery to the midbrain of an siRNA designed to suppress α-synuclein expression as a potential therapy for Parkinson disease.

P209 Efficacy of Various Analgesics on Shoulder Function and Rotator Cuff Tendon-to-Bone Healing in a Rat (*Rattus norvegicus*) Model

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Although relief of postoperative pain is an imperative aspect of animal welfare, analgesics that do not interfere with the scientific goals of the study should be used. We compared the efficacy of different analgesic agents by using an established rat model of supraspinatus tendon healing. A custom-built image capturing walkway with serial force plates was used to measure gait parameters. Tendon tissue stress tests were performed on a mechanical test frame. We hypothesized that different analgesic agents would all provide pain relief in this model but would cause differences in tendon-to-bone healing and gait parameters. All collected data was statistically analyzed by two way ANOVA and follow up t tests for significance ($P \leq 0.05$). Buprenorphine, ibuprofen, tramadol-gabapentin, and acetaminophen were compared with a no-analgesia control group with 10 animals per group. Stride length and vertical force on the operative forelimb were decreased between the control group and both the buprenorphine (2 and 4 d postsurgery, $P < 0.001$ for all values) and ibuprofen (2 d postsurgery, $P < 0.001$ and $P < 0.008$, respectively) groups. Step length was decreased in the control group as compared with the tramadol-gabapentin (2 d postsurgery, $P < 0.007$)), buprenorphine (2 and 4 d postsurgery, $P < 0.001$), and ibuprofen (2 d postsurgery, $P < 0.001$) groups. Regarding tendon-to-bone healing, the ibuprofen group showed less stiffness ($P < 0.001$) at the insertion site; no other differences in tendon-to-bone healing were detected. In summary, the analgesics evaluated were associated with differences in both animal gait and tendon-to-bone healing. This information will be useful for improving the management of postsurgical pain without adversely affecting tissue healing. Given its ability to improve gait without impeding healing, we recommend use of buprenorphine for postsurgical pain management in rats. In addition, our gait-analysis system can be used to evaluate new analgesics.

P210 Effects of Breeding Configuration on Behavior, Reproduction, and Pathology in the Laboratory Mouse (*Mus musculus*)

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While numerous published studies have evaluated the effect of housing density on the wellbeing of laboratory mice, little is known on the effect of breeding configuration. The eighth edition of the *Guide for the Care and Use of Laboratory Animals* lists the recommended minimum floor area/animal for a female mouse and her litter as 51 in.^{2} Additional consideration is needed for other breeding configurations such that there is sufficient space for litters to develop to weaning without resulting in "detrimental effects for the mother or the litter." For institutional housing density policies that deviate from the recommendations of the *Guide*, the 8th edition of the *Guide* encourages the use of performance indices to help determine appropriate space allocation. We present preliminary data studying the effects of 4 different breeding configurations (pair, trio with litter culled to ≤ 10, trio with nonculled litters, and harem) on environmental, behavioral, and pathologic changes in both C57BL/6 and BALB/c mice. Our hypothesis was that a pair breeding configuration is the least stressful for both the dam and litter. Additionally, we hypothesize that overcrowding will have a similar behavioral and pathologic effect as seen in mouse models of acute and chronic stress. We measured ammonia levels at the cage level, growth curves, and mothering behavior. A series of behavioral tests, including the elevated plus maze and tail suspension test (TST) were performed on weanling mice. Growth rates were slower in BALB/c harem-raised mice, however positive maternal behavior was most prevalent in the harem breeding configuration. Ammonia levels tended to be approximately 100 to 150 ppm over the latrine area by 7-d cage change interval, however, levels measured at the middle of the cage rarely reached > 50 ppm. Weanlings raised in harems tended to be immobile longer on the TST and there were no significant differences found on EPM. In summary, there appear to be both benefits and detriments to all examined breeding configurations.

P211 Comparison of the Physiologic Effects of Hypothermia, Isoflurane and Sevoflurane Anesthesia in Neonatal Rats

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Information regarding humane and effective anesthetic regimens for neonatal rodents is limited. This study investigated the physiologic effects of 3 commonly used neonatal rodent anesthetics: hypothermia (immersion in an ice bath), isoflurane and sevoflurane in 4-d-old rat pups. Neonates were randomly assigned to one of 6 anesthetic treatments ($n = 12$): hypothermia survival, hypothermia nonsurvival, isoflurane survival, isoflurane nonsurvival, sevoflurane survival or sevoflurane nonsurvival. Upon confirmation of a surgical plane of anesthesia, a skin incision was made on the right lateral thigh. Parameters monitored included heart rate, respiratory rate, oxygen saturation, righting reflex, and pedal withdrawal. Weight, corticosterone and glucose were sampled for the nonsurvival group 5 min postprocedure and for the survival group at 24 h postprocedure. All neonates in the survival group were accepted by the dam. Hypothermia-treated neonates became hypoxic, underwent tachycardiac arrest and experienced a longer recovery time when compared with inhalant anesthesia; however, all anesthetic techniques were determined to be safe. No significant differences were seen between experimental groups in corticosterone, glucose, or weight gain measurements. We conclude that hypothermia, isoflurane and sevoflurane anesthesia are sufficient for maintaining neonatal rodents at a surgical plane; however, the long term effect of the hypothermia induced hypoxia and asystole remains unknown. The availability of equipment, experience of personnel, and physiologic effects of hypothermia should be considered when choosing an anesthetic method.

P212 Refining Blood Collection Techniques to Improve Animal Welfare and Sample Quality

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An effort to refine rodent blood collection techniques resulted in an examination of alternative methodologies to improve animal welfare and sample quality. Disease model or repeated sample collection resulted in inadequate sample volume, occasional hemolysis, and complications including bruising, scarring, and necrosis of sample collection sites. A study was designed to determine whether the use of a vibrating device in comparison to standard straight stick methods would decrease health complications as well as improve sample quality and quantity. C57BL/6 mice were used as a control strain for both tail and saphenous venipuncture. NOD mice were used for the diseased tail stick groups. Atherosclerosis induced ApoB mice were used as a disease model for saphenous venipuncture. Tail sticks were performed twice daily for 2 mo and saphenous bleeds were performed biweekly for 2 mo to mimic study schedules. Sample volume increased with the use of the vibrating device and blood collection was achieved without the use of heat, unlike the standard direct venipuncture method. Comparison of the 2 bleeding methods in the ApoB strain showed that we were able to collect complete volume in 73% of samples via the standard method compared with 84% with the use of the vibrating needle. Full sample volumes were collected with single sticks in 38% of attempts of the standard method compared with 70% of attempts with vibrating needle in ApoB mice. An incidence of bruising was noted at 36% with standard method compared with 12% with the use of vibrating needle. Tail circumference at the site of standard venipuncture of C57Bl/6 mice averaged 13.2 mm; this was a 3-mm increase from both the control

and vibrating needle groups which averaged 10-mm tail circumference. This work demonstrates that, as compared with standard blood collection methods, the vibrating needle allows for increased sampling compliance while decreasing associated trauma.

P213 Moving Evidence into Practice: Assessment of Foraging Devices as a Model for Decision-Making in Nonhuman Primate Environmental Enrichment

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Continued progress to move evidence-based best practices into community and regulatory animal welfare standards depends in part on developing common metrics to assess cost, benefit, and relative value. Here we describe a model approach to evidence-based evaluation and an example of cost:benefit assessment for a common element of environmental enrichment plans for laboratory-housed nonhuman primates. Foraging devices encourage a species-typical activity that dominates the time budget of primates outside captivity and provide inherent cognitive challenges, physical activity demands, and multisensory stimulation. Cost, device selection, frequency of rotation, and nutritional concerns also serve as practical challenges to standardized implementation. We used a comprehensive cost:benefit analysis to directly compare: 1) monkeys' engagement with different foraging devices and 2) the comprehensive cost of implementing foraging opportunities. Benefit was operationalized as manipulation and engagement with devices. We recorded adult male cynomolgus monkeys' (n = 14) interaction with 7 types of devices filled with a range of enrichment foods. An additional 200 animals were evaluated with one foraging device. Behavioral observation occurred at initial device presentation and at 1, 2, and 24 h following. All devices elicited foraging behavior, but there were significant differences between them both initially and over subsequent observations. Devices that afforded opportunity for extraction of small food items elicited greater manipulation. Relatively little foraging behavior was observed after 2 h. Costs were relatively similar beyond the initial investment to purchase or construct devices. Comprehensive analysis demonstrated that the cost of providing a foraging opportunity to a single monkey is roughly US$1.00, with approximately 80% attributable to labor. To our knowledge, this is the first study to perform a rigorous cost:benefit analysis and comparison of commonly used foraging devices included in environmental enrichment. Its broader significance lies in its contribution to development of methods to facilitate improvement in evidence-based practices and common standards to enhance laboratory animal welfare.

P214 Is Liposomal Bupivicaine an Effective Analgesic for Tail Biopsy in Mice?

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Tail biopsy for genotyping of genetically modified mice is a common procedure in mouse colonies. Mice older than 21 d need to have anesthesia/analgesics for the tail biopsy procedure, as the vertebral endplates have matured in many strains of mice. The best anesthesia/analgesia is widely debated in laboratory animal circles. Liposomal bupivicaine is a new formulation of bupivicaine that has 2-peaks of distribution. We proposed that the use of liposomal bupivicaine with topical ethyl chloride spray will prove to be more effective than other anesthesia/analgesia formulations currently used to perform tail biopsies in adult mice. The experimental groups (n = 20) used were: sham biopsy (mouse is restrained and tail is touched, no cut is made); no analgesia/anesthesia; ethyl chloride spray alone before cut; tail dipped in liposomal bupivicaine before cut only; tail dipped in normal bupivicane after cut; tail dipped in liposomal bupivicaine after cut; isoflurane (that is, mouse is anesthetized during entire procedure); ethyl chloride spray applied before cut, tail dipped in normal bupivicane after cut; isoflurane + ethyl chloride spray applied before the cut; ethyl chloride spray applied before cut, tail dipped in liposomal bupivicane after cut; ethyl chloride spray applied before cut, lidocaine gel applied after cut. We assessed the effectiveness of 9 different anesthesia/analgesia combinations using a Pain Scoring Chart that measures the mouse's interest in its tail (tail bothering), respiration, mentation, haircoat, activity level, and also incorporates the Nest Complexity Scoring Scale. Our score sheet found that there was interobserver bias for respiration (P = 0.0402) and haircoat (P = 0.0025) scores. There were significant differences as well as sex differences in the Nest Complexity Scoring data and the tail bothering data. The liposomal bupivicaine added no benefit to either the tail bothering score or the Nest Complexity Score, despite being applied before the biopsy or after the biopsy. The ethyl chloride showed an immediate effect and mitigated pain, regardless of what anesthesia/analgesia it was coupled with; however, histopathology showed significant osteonecrosis up to 20 d after biopsy. This study provides insight into the effects of analgesics and anesthetics used for the biopsy in adult mice, as well as data on the usefulness of different evaluation parameters. This information can be used as a stepping stone for studies on recuts in adult mice, and also tail biopsies in preweanling mice (10 to 14 d old).

P215 Serial Sampling in Mice: An Alternative to Retroorbital Bleeding

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Pharmacokinetic studies in mice using composite blood sampling techniques often have more variable data due to the inability of taking all of the time points from one animal. Composite studies also require larger supplies of test compounds. Additionally, the commonly used method of retroorbital bleeding requires an animal to be anesthetized multiple times over the study duration, and may add to the variability. Serial samples are difficult to obtain in mice due to the limited volume of blood that can be sampled and the constraints of reliable sampling methods. Therefore, the additional compound requirements and variability discussed above, combined with the increased scrutiny with using retroorbital bleeding as a sampling technique, have driven us to find an alternative reliable method for obtaining serial samples in mice. The identified technique involves a collection device that is easily assembled, allows for rapid collection and ease of manipulation during sampling, and provides a blood volume that is useful for multiple types of analysis. Mice are dosed (n = 4) either via intravenous bolus or orally. First, mice are warmed under a heat lamp for approximately 3 min and then placed in a restrainer. A 27-gauge butterfly catheter with a 44.7-µL EDTA-coated capillary tube attached is then used to obtain a sample from the opposite lateral tail vein that was used for dosing. Samples are obtained at 3 and 30 min, 1, 3, 5, and 7 h in order to achieve an accurate pharmacokinetic curve. For an oral study, the 3-min time point is substituted for a 15-min time point. Less than 20% of the animal's circulating blood volume is removed over a 24-h period. Plasma (20 µL) is used from each time point for analysis via mass spectrometry. The pharmacokinetic results obtained from tail vein serial sampling in mice show excellent correlation with established composite study sampling designs. Pharmacokinetic data can accurately be obtained by performing tail vein serial sampling in mice.

P216 Removal of Dura Mater Prevents Bone Recalcification over Mouse Brain

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Chronic cranial window surgeries are routinely performed in mice being imaged under a 2-photon microscope allowing for visualization of deep cortical structures. Over time we began to notice recalcification over cranial windows within a few weeks postoperatively preventing clear imaging of the desired cortical structure. Recalcification causes light from the microscope to scatter prohibiting a clear image from being obtained. Furthermore, we noticed in mice where the dura mater had been unintentionally removed over the cranial window that no recalcification occurred. We set out to determine if performing a durotomy over a cranial window is a viable option for preventing this artifact. In a series of experiments conducted, the dura mater was removed in 10 mice receiving a cranial window. A control group of 10 mice received a cranial window with the dura mater left intact. All mice were closely monitored postoperatively and given a 2-wk recovery period before imaging commenced. The control group experienced recalcification within 2 to 3 wk postoperatively requiring an additional surgery to remove the artifact over the cranial window. On the other hand, mice without dura mater experienced no recalcification for up to 6 mo allowing for continuous imaging during this time. Based on these results, a durotomy is an effective and viable method for preventing recalcification over cranial windows in mice allowing for uninterrupted imaging under a 2-photon microscope.

P217 Effect of Changes to Social Environment on Platelet Activation and Function in Pigtailed Macaques

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The *Guide* states that social housing is considered the default method of housing social species. However, there is limited research on the health implications of social housing and changes to housing environment in laboratory animals. Changes to the housing environment of laboratory animals have the potential to cause stress, compromise animal health and confound research results. Increased platelet activation and heightened platelet reactivity to stimuli have been previously demonstrated in humans in response to stress, and activated platelets contribute to cardiovascular disease and thromboses that can adversely affect health and research results. We hypothesized that separation and/or relocation of pigtailed macaques induces transient platelet activation and heightened reactivity to stimulus. Moreover, we hypothesized that an effect on platelet function persists after platelet activation has returned to baseline levels. We compared platelet activation and function in macaques that have been separated from their compatible cagemate, relocated to a novel environment with their cagemate, or separated coupled with relocation, with unmanipulated pair-housed controls. Flow cytometry was used to assess platelet activation longitudinally. Preliminary results show that platelets are transiently activated 7 to 10 d after relocation and separation from cagemate with increased expression of P-selectin (27.1% more P-selectin+ platelets than unmanipulated controls), PAC-1 (30.9% more), and HLA-ABC (46.4% more). We saw a similar effect in some animals that were relocated and remained pair-housed, but not in those animals that were not relocated, regardless of pair-housing status. Platelet activation returns to baseline in all animals by day 20. Additionally, 10 d after animals are separated and relocated, platelets show a polarized response to different stimuli, with heightened reactivity to inflammatory stimulus (LPS) (78.1% more P-selectin+ and 77.7% more HLA-ABC+ platelets than unstimulated blood) and attenuated reactivity to hemostatic stimulus (collagen) (20.1% fewer P-selectin+ and 12.1% fewer HLA-ABC+ platelets). Conversely, control animals show heightened reactivity to collagen (96.3% more P-selectin+ and 97.2% more HLA-ABC+) and attenuated reactivity to LPS (0.6% fewer P-selectin+ and 1.0% more HLA-ABC+). In conclusion, these preliminary data show transient activation of platelets and heightened reactivity to inflammatory stimulus following separation and relocation of macaques.

P218 Mouse Tail Venipuncture with Vibrating Needle Increases Collection Success and Blood Amount, while Requiring Fewer Needle Insertions

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Blood sampling is an often required procedure in biomedical research, but successful mastery of techniques can be difficult, leading to stress for the research subject and experimenter. Sampling stress, including painful needle punctures, can increase stress hormone levels and variability in research subjects. To help address challenges of blood sampling, a vibrating device designed to reduce the penetration force of the venipuncture needle was evaluated in mice. Previous work in serial rat tail blood sampling showed less animal vocalization and movement, and yielded lower corticosterone (CORT) levels. In the present study, in vivo serial blood sampling was completed on 47 male C57BL/6J mice divided into 2 groups: vibration turned ON (n = 24) and OFF (n = 23). For each mouse, 3 consecutive blood samples were collected from the ventral tail artery every 30 min and this procedure was repeated once a week for 3 wk (n = 9 collections per mouse). Data recorded included number of needle insertions required during each collection, total blood mass obtained, circulating glucose concentration, animal handling time, and plasma CORT concentration. Blood samples obtained with device ON compared with OFF yielded an overall higher success rate (84% compared with 74%; $P < 0.05$). In addition, when the device was ON the experimenter required fewer needle insertions to collect a full blood sample: comparing ON compared with OFF groups, 51% compared with 38% ($P < 0.005$) of all trials required only one needle insertion, and 34% compared with 44% ($P < 0.05$) of all trials required 3+ needle insertions. Finally, average blood mass collected per trial was 30% to 40% higher for the ON compared with OFF group ($P < 0.05$), and there was a trend for lower and less variable CORT in the ON compared with OFF group. In summary, the vibrating device improved blood sampling outcomes with mice, for both novice and experienced experimenters. These results, along with prior study results, indicate that the vibrating needle device will benefit studies conducted with either rats or mice.

P219 Analysis of Fertility and Mating Behavior in BL6CBAF1/J Mice Treated with Intratesticular Injection of Zinc Gluconate

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A number of different chemical castration formulations have been used to sterilize various nonrodent species in the past. Given the lab animal community's call to refine methodologies whenever possible to reduce potential pain and distress, we decided to test a pharmaceutical-grade zinc gluconate sterilization solution in mice as a potential alternative to surgical sterilization by vasectomy. Male BL6CBAF1/J mice were treated with an intratesticular injection of either zinc gluconate (n = 7) or sterile saline (n = 3). They were mated with superovulated females at various time points before and up until 11.5 wk after injection to assess fertilization rates. At 12 wk postinjection, males were euthanized and testicles and accessory sex glands were collected for histopathology. Blood was also collected before and at various time points after treatment for future testosterone analysis. Fertility in the zinc gluconate-treated mice dropped significantly (to 14.7 %) by 2.5 wk after treatment with almost complete loss of fertility by 3.5 wk postinjection. By 11.5 wk, zinc gluconate treated males had an average fertilization rate of 4.3 % compared with 90.0 % in saline treated animals. No signs of pain or adverse reactions at the injection site were noted, and sexual

behavior in zinc gluconate treated males was similar to those injected with saline. Histopathologic analysis of the testicles revealed few seminiferous tubules remained in the zinc gluconate-treated testes and most of these were acellular and contained amphophilic debris and varying numbers of mature spermatids. Rare tubules contained a disorganized mixture of germ cells at various stages of maturation. Interstitial connective tissue was prominent and contained clusters of Leydig cells. Spermatozoa were not present in tubules of the epididymides from zinc gluconate injected mice. Seminal vesicles and prostate gland of zinc gluconate injected mice were morphologically unremarkable. Our results indicate that zinc gluconate may be a possible alternative to surgical vasectomy of males used in transgenic facilities to produce pseudopregnant females for embryo transfer.

P220 Cadaveric Avian Wing Model Complements Live Rat Model in Microsurgical Simulation Training for Neurosurgical Residents: Technical Aspects

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Training of surgical residents based on the traditional Halstedian model is becoming increasingly scrutinized. The emergence of competency-based training has put pressure on training programs to provide high-fidelity simulation sessions that compliment residents' training in the operating room. Microvascular anastomosis of the rat femoral artery is a well-established training model for microsurgery. Here we present a novel combination of perfused cadaveric avian wing model in conjunction with live rats for neurosurgical resident training. Our objective is to reduce the number of rats necessary to reach the learning plateau via the use of avian wings. This is in keeping with the replacement, reduction, and refinement tenet regarding the ethical use of animals. The microsurgical curriculum has been previously described in a pilot study. Residents performed 15 anastomoses on femoral arteries and veins in live rats in a distributed fashion over a maximum of 2 y. For comparison, cadaveric duck wings were obtained from a local poultry slaughterhouse and repurposed for microsurgical training. The brachial artery was cannulated and connected to a roller pump. The duck wings were perfused while residents performed microvascular anastomoses of the brachial and ulnar arteries. This took place prior to live rat modules. The duck wing brachial artery diameter measured 1.5 to 2.0 mm, similar to the proximal middle cerebral artery in humans. The ulnar artery diameter measured 1.0 to 1.5 mm, similar to the cortical vessels. The duck wing vessels compared favorable to what's typically seen in neurosurgery. Eight to 10 interrupted sutures were placed during anastomosis using 10-0 nylon. Residents were also able to successfully perform running sutures on the duck wing. Quality of the anastomoses was verified with the perfusion pump. Residents who performed the duck wing module felt more comfortable when they moved onto the live rat model. The perfused cadaveric avian wing model provides intermediate- to high-fidelity simulation that complements the live rat model well. The number of rats needed for neurosurgical simulation training could be reduced via the use of avian wings.

P221 Detection of Amphibian Chytridiomycosis before and after Formalin Fixation

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Batrachochytrium dendrobatis (*Bd*) is a fungal pathogen of amphibians and is the causative agent of chytridiomycosis, which is responsible for worldwide amphibian population declines and extinctions. *Bd* has been identified in amphibian samples collected from the wild and the pet trade, as well as the laboratory setting. Characterization of this pathogen has included the use of archived, formalin-fixed amphibian samples to determine its origin and spread. Analysis of these samples historically has relied on histology. However, recently, quantitative PCR (QPCR) has replaced histology as the gold standard for *Bd* detection. To determine the effect of formalin fixation on *Bd* detection by QPCR, *Bd* load was quantified by QPCR before and after formalin fixation of five species of frog (n = 19 total) and odds ratios were calculated to determine the probability of detection post fixation for a given pre fixation load. Analysis of 114 swabs by QPCR identified that samples with less than 100 zoospores per swab as detected by QPCR prior to formalin fixation were 160 times less likely to be detectable by QPCR after fixation. To examine the effect of swab location of *Bd* load and frequency of detection, a one-way analysis of variance (ANOVA) was performed which identified that there was no statistical difference in the frequency of *Bd* detection or in *Bd* load between swabs collected from the dorsum, ventrum, inner thigh, and toe web. Histologic evaluation identified characteristic lesions of chytridiomycosis (presence of zoosporangium and zoospores within stratum corneum, acanthosis, hyperkeratosis, and epithelial sloughing) and was less sensitive than QPCR alone, however when combined with post-fixation QPCR increased overall post-fixation detection. Based on these findings, archival samples should be analyzed by a combination of QPCR and histology in order to maximize sensitivity and when possible swabs should be collected and stored prior to formalin fixation of specimens. Furthermore, when collecting swabs, anatomic location has minimal impact on the outcome of QPCR.

P222 Cobalamin (Vitamin B12) Deficiency in Rhesus and Pig-tailed Macaques with Chronic Diarrhea

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Chronic diarrhea is the most frequently encountered clinical problem in nonhuman primates and is responsible for high levels of morbidity and mortality within captive macaque colonies. In a large proportion of cats, dogs, and humans affected by chronic gastrointestinal disease, a deficiency in cobalamin (vitamin B12) has been demonstrated and identified as a risk factor for negative outcomes. In addition, supplementation with cobalamin has been shown to improve clinical outcomes in these species. However, no research has been conducted to identify the presence of a cobalamin deficiency in macaques with chronic diarrhea. We hypothesized that macaques with chronic diarrhea would have significantly lower serum cobalamin levels than healthy controls. We measured serum cobalamin levels in rhesus and pig-tailed macaques with chronic diarrhea and compared them to those of healthy controls. Additional data obtained at the time of sample collection included age, weight, body condition score, complete blood count, serum chemistry, and fecal culture. Our results show that there is no difference in serum cobalamin levels in either rhesus or pig-tailed macaques with chronic diarrhea as compared with healthy controls. Initial inclusion criteria for both diarrhea and control groups included a negative fecal culture; however, results show no difference in serum cobalamin levels in culture positive animals as compared with culture negative animals. In both rhesus and pig-tailed macaques, animals with diarrhea had significantly higher platelet counts as compared with normal animals ($P < 0.01$ and $P < 0.001$, respectively). This study is the first to our knowledge to examine cobalamin levels in macaques with chronic diarrhea. Based on the clinical implications of such a deficiency and its use as a diagnostic and therapeutic tool in other species, it is critical to determine whether or not this is a problem that exists in macaques as well. The negative results that we have obtained address this question, and indicate that serum cobalamin level is not a useful component to the clinical workup and treatment of macaques with chronic diarrhea.

P223 Methods for the Implantation and Maintenance of Separate Intrathecal Catheters for Dose Administration and CSF Sampling in Cynomolgus Monkeys for Advanced Administration and Sampling Regimens

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Intrathecal implantation of catheters equipped with subcutaneous access ports provides the means for repeated access to the CSF space without the need for chemical sedation but can be subject to loss of catheter patency and to postsurgical complications. Factors such as catheter position or location in the intrathecal space can also influence long term patency as well as postsurgical complications. Implanting a single catheter and port for both dosing and sampling increases the risk of failure and precludes the ability for concurrent sampling and dosing. Intrathecal implantation of two access port-equipped catheters provides capability to dose and sample from separate lines as well as backup in case of failure of either dosing or sampling patency in either of the lines. For this study 3 cynomolgus monkeys were implanted with dual lumbar catheters (L3-4 and L4-5). One catheter was advanced to the vicinity of the cercivothoracic junction and the second to the thoracolumbar junction. Postsurgical radiography was performed following radiopaque infusion into the intrathecal space via the ports. Distance of the catheter in the intrathecal space was determined by a combination of measuring remaining catheter postsurgery and viewing the post radiopaque X-ray. Postsurgery catheter maintenance was performed once weekly for 4 w unsedated to verify patency of the ports and to assess the overall health and recovery of the animals. Retention of capability to sample and infuse from the ports for up to a month postsurgery has been demonstrated and suggests that this technique is both viable and useful in that it allows for separate paths for dosing and sampling accessing two different locations within the intrathecal space.

P224 In Vitro Culture of Mouse (*Mus musculus*) Tetraploid Embryos with Cumulus Cells

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The introduction of genetically manipulated mouse embryonic stem cells (ES cells) into blastocyst embryos is a powerful technique used in research involving the genetic basis of disease. The use of tetraploid host embryos (created by electrofusion of 2 diploid embryos) for microinjection of ES cells results in the production of offspring that develop from the ES cells alone, as tetraploid embryos contribute only to extraembryonic tissues during development. This technique has potential to be more time and cost efficient than diploid embryo microinjection. ES cell quality and pluripotency remain important factors in determining survival of ES cell-derived embryos, however in vitro culture conditions can either support or detract from embryo survival. Previous studies have demonstrated that cumulus cells produce beneficial growth factors and metabolize harmful reactive oxygen species when added to in vitro culture media. The purpose of this study was to investigate if the addition of mouse cumulus cells (CC) from same-strain donors to in vitro culture media could improve the quality of tetraploid embryos, and ultimately increase the percentage of fetuses produced after ES-cell injection and embryo transfer. CCs were collected from cumulus-oocyte complexes from 4-wk-old superovulated Crl:CD1(ICR) female mice, separated from oocytes via incubation with hyaluronidase-enriched media, and cultured overnight. Diploid embryos were collected the following day at the 2-cell stage (from 4-wk-old superovulated Crl:CD1(ICR) females), and an electrofusion device was used to generate tetraploid embryos (86% fusion rate). Tetraploid embryos were cultured in KSOM-aa medium with and without CCs for 48 or 72 h, then injected with ES cells prior to embryo transfer (12 embryos per female (n = 12)). Cesaerean sections were performed at day 18 of gestation, and the number of developing fetuses was assessed. More fetuses developed from the group of embryos that had been cultured with CCs in KSOM (12 fetuses) for 72 h than those cultured without CCs (0 fetuses)(P = 0.0001). Ongoing experiments seek to further define if cumulus cells have a positive effect on embryo development in vitro prior to embryo transfer.

P225 A Comparison of Soiled Bedding Densities and Stress in Sentinel Mice

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Sentinel animals are used to monitor the health status of rodents used in research. It is important to screen for potentially pathogenic viruses, parasites, and bacterial organisms to detect and rapidly respond to unwanted pathogens that may adversely affect research outcomes. Soiled bedding exposure is the most common method of sentinel surveillance. However, standards and protocols for bedding transfer vary widely. In an effort to standardize surveillance programs with global colleagues, we proposed a change from our standard 50% soiled bedding protocol to 100% soiled bedding. At the request of the IACUC, a pilot study was conducted to investigate whether mice housed on 100% soiled bedding for a duration of 12 wk experienced greater stress when compared with those on 50% soiled bedding. Twelve adult CD1 female mice were housed in 2 groups (6 per group) and exposed to either bedding concentration. Body weights and clinical observations were recorded weekly. At weeks 0, 1, 4, 8, and 12 blood samples were collected for serum corticosterone analysis. At the completion of the study, 3 mice per group were euthanized and tissues submitted for histopathologic review. At week 12, all the mice in both study cohorts had continued to increase in body weight. No animals were reported for any clinical abnormalities, including abnormal behavior. Changes in serum corticosterone levels were not statistically significant. Histologic analysis did not reveal gross or microscopic changes in the mice from either cohort. Based on the results of this study we conclude that housing mice on 100% soiled bedding does not result in increased stress and does not induce pathologic or clinical abnormalities when compared with animals housed on 50% dirty bedding for a duration of 12 wk.

P226 Effects of Needle Gauge on Blood Quality and Potential for Bruising with Repeated Blood Collections in Cynomolgus Macaques

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Obtaining quality blood samples while minimizing animal trauma related to venipuncture continues to be an important area of focus within the laboratory animal environment as it relates to both animal welfare and sample integrity. A review of the literature suggested that in humans, needle gauge can affect the quality of blood samples, and has implications for both the aversiveness of the sampling procedure acutely, as well as the number of samples which ultimately may need to be collected in a given experiment. Based on these basic principles, we hypothesized that there is a positive correlation between needle size and the magnitude of clinical bruising, but an inverse relationship between needle size and blood sample quality based on hemolysis and clotting incidence rates. Twenty-seven cynomolgus macaques (*M. fascicularis*) were divided into three groups (n = 9 per group) based on body weight (< 3 kg, 3 to 5 kg, and > 5 kg) and 3 animals in each group were bled using one of 3 size needles (21, 23, and 25 gauge). Animals in each group were bled at 6 time points over 24 h, mimicking a typical toxicokinetic study. Blood samples were evaluated for hemolysis, clotting, traditional hematology endpoints, and C-reactive protein (CRP), an acute phase inflammatory biomarker. Clinical measures included evaluation of

injection sites for bruising. Results confirmed a positive correlation between needle size and the severity of bruising; however, no meaningful difference in sample quality, based on hemolysis and clotting incidence rates, hematology results, or CRP values were observed as a function of needle size. These results support the conclusions that increased needle size may cause increased clinical bruising, and hence an animal welfare concern, but does not impact overall blood sample quality.

P227 Behavioral Ethogram as a Health Assessment Tool in a Feline Vaccine Study

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For infectious disease studies, assessment of feline health is traditionally limited to metrics such as body temperature (BT), change in body weight (BW), and clinical signs (CS). Recently, behavioral ethograms designed for assessment of rabbits and rodents were demonstrated to serve as sensitive indicators of pain or illness. Similarly, we hypothesized that feline behaviors, such as play and resting behaviors, evaluated before and after viral challenge may complement traditional measures (T, BW, CS) as means to identify animals with disease. We evaluated 21 cats, divided between 3 vaccine treatment groups (control, intranasal (IN), and intramuscular (IM)) at baseline (day before viral challenge) through 11 d postviral challenge (PVC). In addition to measuring daily BW and T, remote video data was collected and analyzed over 60-s increments according to a novel ethogram. The behaviors assessed via ethogram demonstrated significant changes in the PVC period including changes in play, rest, and consumption (eating/drinking). Results across the different treatment groups indicate that all cats developed elevated BT 4 d PVC. Concurrently, a significant decrease in the control and IN groups BW was first achieved on day 4 PVC. In contrast, the IM groups BW decline was not significant until day 9 PVC. Play and consumptive behaviors decreased significantly at 2 d PVC in the control and IN groups, respectively, compared with the IM group. Rest behavior was significantly elevated in IN and control groups from d 6 and 7, respectively, until d 9 PVC. The IM group had no significant difference in rest behavior throughout the study. In summary, our novel ethogram identified changes in play and consumptive behaviors 2 d before changes in BT and BW were evident. Additionally, increases in rest behavior were evident in both IN and control groups, but not the IM group. The ethogram findings provided significant clinical information and a more sensitive means for the detection of cats with viral disease than some of the standard metrics. Therefore, we propose that future feline infectious disease studies incorporate such an ethogram.

P228 Anesthetic Effects of a 3-Drugs Mixture and Antagonistic Effects of Atipamezole in Mice

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The anesthetic mixture of medetomidine (MED), midazolam (MID), and butorphanol (BUT) has been used for intraperitoneal (IP) injection in mice. However, other administrative routes may cause different anesthetic effects. We examined anesthetic effects of the anesthetic mixture by subcutaneous (SC) and intravenous (IV) compared with IP injection (experiment 1). Atipamezole (ATI) is an antagonist of MED. After injection of the anesthetic mixture, administration of ATI causes mice to rapidly recover from anesthesia. However, an appropriate dosage, an injection route or an optimum injection timing of ATI after administration of the anesthetic mixture are not clear. Then, we examined how different dosages, routes, and injection timing of ATI affected the recovery from anesthesia (experiment 2). We used 8 mice per each injection group. The mice were used repeatedly and allowed at least 2 d of rest after experimental use. In experiment 1, we injected the anesthetic mixture by SC, IV, and IP routes and measured anesthetic depth using anesthetic scores. In experiment 2, we administered 0.3 mg/kg or 1.5 mg/kg of ATI by IP and SC routes at 10 and 30 min after injection of the anesthetic mixture and then measured anesthetic scores. To measure vital signs such as oxygen-saturation, heart rate, and respiratory rate during anesthesia, we used a pulse oximeter. Statistical analysis was conducted by analysis of variance followed by Scheffe test. A p value less than 0.05 was considered to be statistically significant. There were no significant differences of anesthetic duration among the 3 injective routes. Antagonistic effects of ATI: 0.3 mg/kg and ATI: 1.5 mg/kg by IP injection worked equally when administered at 30 min after the anesthetic mixture. The antagonistic effect of ATI: 1.5 mg/kg was strongest at 10 min after the anesthetic mixture. Antagonistic effects of ATI by SC injection was weaker than that by IP injection. In summary, a mixture of MED, MID, and BUT produced almost same anesthetic duration by IP, SC, and IV injection in ICR mice. This anesthetic mixture is a useful drug to have an antagonist as ATI which helps mice quickly recover from anesthesia. These results may contribute to the welfare of laboratory animals.

P229 Experimental Feasibility of Treadmill Exercise for Chinese Bama Pigs

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In recent years, exercise has been advocated for preventing cardiovascular diseases. In 1989, Yucatan miniature swines were trained to study the effects of exercise training on coronary transport capacity. The training program for Yucatan miniature swine has been widely used in the research of cardiovascular system since then. However, there is scarce literature on exercise training on pig breeds that are available in China. There are abundant resources of Chinese experimental miniature pigs, which include about 6 or more sub-species. Among them, Bama pigs have the following characteristics: stable transmissibility, fecund species, lighter weight of about 30 kg for 12-mo-old swines, and large area of body surface covered with white hairs. This study investigated whether treadmill exercise for Bama pigs would be practical. The exercise training consisted of a warmup, a sprint run, an endurance run, and a cool down, once a day for 4 consecutive wk. Eight 9-mo-old, castrated-male Bama pigs were enrolled in the study, and the mean weight, body height, abdominal girth, and hip breadth were 53.46 ± 2.52 kg, 79.75 ± 3.77 cm, 105.88 ± 1.89 cm, and 21.13 ± 1.36 cm, respectively. In the process of treadmill exercises, the daily maximum speed, longest duration, and maximum distance were 7.5 km/h, 1585 s, 2.31 km, respectively; which were less than the reported speeds of 9 to 13 km/h and training bouts of 85 min. The above parameters in the first week were 3.55 ± 1.02 km/h, 854.97 ± 244.47 s, 0.65 ± 0.32 km, respectively; with that in the fourth week of 2.92 ± 0.85 km/h, 701.80 ± 323.80 s, 0.46 ± 0.27 km, respectively; showing significant differences ($P = 0.002$, $P = 0.020$, $P = 0.003$, respectively). The temperature, heart and respiratory rates before exercise were 40.25 ± 0.85 ℃, 120.70 ± 28.28 times/min, 47.92 ± 20.58 times/min, respectively, differing significantly from that of 40.94 ± 1.15 ℃, 135.33 ± 26.20 times/min, 107.58 ± 38.74 times/min after exercise (for all $P = 0.000$), respectively. This study shows that Bama pigs are feasible for treadmill exercise. Nevertheless, their exercise tolerance may be less than that of the Yucatan swines.

P230 Histologic Artifacts in Large Animals Adversely Affecting Microscopic Pathology Assessment

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Histologic artifacts may be introduced during several phases post necropsy: at sample collection, during tissue processing, or during sectioning. To identify and characterize tissue artifacts, modifications in sectioning of the standard operating procedures were made in order to reproduce some of these effects. The liver, spleen, and kidney of dogs and nonhuman primates were introduced with artifacts at sectioning. Induced artifacts included the creation of knife marks on tissues, the introduction of human cells into the water bath, and the cutting of dry blocks. All tissues were preserved for 48 h in formalin before trimming. Tissues were processed, embedded in paraffin wax, sectioned, and stained with hematoxylin and eosin. Tissues were then examined microscopically by a pathologist. The evaluation confirmed artifacts such as those induced in this study can interfere with accurate histologic examination, ultimately confounding pathology interpretation. These results also indicate artifacts created during tissue sectioning can make it difficult for pathologists to evaluate submissions in a timely manner and interpret results correctly. Our results emphasize the importance of sectioning practices to ensure a consistently high quality of tissue specimens.

P231 Cationic Liposome-Oligonucleotide Complexes as an Alternative Adjuvant for Polyclonal Antibody Production in Rabbits

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Rabbits are routinely used for polyclonal antibody production and the most common adjuvants used are Freund and gold adjuvant. While Freund adjuvants induce robust antibody responses there are some animal welfare concerns related to the granulomas complete Freund adjuvant causes. Gold adjuvant is an alternative adjuvant to induce antibody response without animal welfare concerns, but the antibody response may not be as robust as Freund adjuvant. Cationic liposome-oligonucleotide complexes (CLDC) are potent activators of the immune response without reported animal welfare concerns. We assessed the antibody response to CLDC compared with Freund adjuvant and gold adjuvant. Eight rabbits were immunized subcutaneously with ovalbulin in either complete Freund adjuvant (CFA, n = 2), gold adjuvant (n = 3) or CLDC (n = 3). Two weeks after immunization, blood was collected for antibody responses and rabbits were given a booster immunization with incomplete Freund adjuvant, gold adjuvant or CLDC. Additional blood samples were collected 2 wk after the booster immunization. ELISA was performed to assess antibody responses based on optical density values. Although there was no statistical differences, CFA resulted in a higher antibody response than gold adjuvant and CLDC at 2 wk after immunization, while CLDC resulted in a higher antibody response following the booster immunization. Injection site swellings were noted in the CFA groups, and mild bruising in the gold adjuvant and CLDC groups. One drawback to the use of CLDC is the ability to reconstitute the antigen in an appropriate volume for injection which resulted in a 5-mL immunization for the rabbits due to the charge of the antigen. These results suggest that CLDC may be used as an alternative adjuvant to produce polyclonal antibodies with mild clinical side effects similar to gold adjuvant; however, antigens with negative charges are more easily formulated with CLDC.

P232 Probiotic Bacteria Inhibit Age-Related Obesity by Preserving Thyroid Function in Aged Mice

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During studies of obesity in mice, we found that feeding of a purified probiotic microbe, *Lactobacillus reuteri*, forestalled typical old age-associated weight gain and lethargy, and instead conveyed physical features of much younger mice. We hypothesized that these features may be related to increased thyroid gland activity. Using 5 to 15 female CD1 mice per experiment with 2 repetitions, we discovered elevated levels of serum T4 and larger thyroid glands in 1-y-old recipients of probiotic microbes, when compared with their age-matched obese control subjects. Mice were fed experimental control diet with subgroups fed *L. reuteri*. Mice consuming oral probiotics were found to be significantly slimmer than their age-matched controls, while both groups of mice had similar caloric consumption. Mice ingesting *L. reutieri* had significantly increased baseline activity levels. Baseline activity was measured using the line crossing method with manual analysis. Mice were videoed for 30 min at the same time daily for 3 wk. Activity was measured from the video with a line crossing being defined as all 4 paws crossing a gridline. This shows that a shift from fat storage to activity was seen with the addition of the probiotic. Oral *L. reuteri* treatment also preserved thyroid follicle epithelial height, a key histologic feature of thyroid gland activity, which relied mechanistically upon bacteria-triggered antiinflammatory CD25+ regulatory T cells. CD25+ cells were depleted using an antiCDcd25 antibody and confirmed by comparing sham-dosed animals using flow cytometry for the absence of FoxP3+ cells in the spleen. Statistical analysis was determined with the Mann–Whitney U test for body weight, diet, calorie consumption and histomorphometry with $P < 0.05$ being significant. These data from animal models suggest that probiotic microbe supplementation may be used to stimulate beneficial host immune interactions with improved thyroid function.

P233 Pharmacokinetics of Intravenous Compared with Oral Carprofen in Mice

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Carprofen is a commonly used nonsteroidal antiinflammatory drug with potent analgesic effects in many animals; however, there is conflicting information regarding efficacious doses and dosing intervals when treating mice. Recent studies have suggested that only doses at or exceeding 20 mg/kg are effective for relieving postoperative pain in mice undergoing laparotomy. To determine accurate and safe dosing intervals of medications it is imperative that pharmacokinetic studies be conducted comparing intravenous compared with oral blood drug levels and these formal pharmacokinetic studies are missing for carprofen in mice. We evaluated the pharmacokinetics of carprofen in C57BL/6 male mice following a single intravenous injection of 20 mg/kg and compared with oral gavage (20 mg/kg), oral administration in the drinking water, and in a preformulated soft gel diet to determine whether similar drug exposures can be achieved with oral dosing methods. Blood was collected at 0, 5, 15, 30, and 60 min, and 2, 4, 8, 12, 24, 36, and 48 h after dosing. Postmortem and histopathology analyses were collected on mice at 36 h to evaluate dosing tolerance. Serum was analyzed by LC-MS/MS. C_{max} for carprofen was 5 min (intravenous) and 2 h (gavage), followed by a rapid decline. Pharmacokinetic parameters (AUC, Vd, $t_{½}$, and CL) were calculated for each paradigm. Oral bioavailability in fed mice was determined to be high (76%). Mice readily consumed water or gel containing carprofen. C_{max} occurred after 8 and 12 h of exposure to carprofen in gel and water, respectively, values were similar for both, but were markedly lower than those obtained for intravenous or oral gavage. Values fluctuated between dark and light phases but were stable within a phase out to 48 h. No lesions were detected in any mouse for any administration method. In summary, carprofen is well tolerated at 20 mg/kg and has high oral bioavailability in mice.

P234 Measurement of Blood Volume in Rhesus Macaques

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A calculated estimation of total blood volume (TBV) in rhesus macaques (*Macaca mulatta*) is often the basis for determining institutional limits of benign blood withdrawal for experimental purposes in this species. TBV is typically calculated using a fixed ratio of blood volume to body weight. However, the assumption that blood volume is a linear function of body weight is likely erroneous as body condition and obesity have profound effects on TBV per kg body weight in humans. To test the validity of this assumption, a pilot study was undertaken in which TBV was calculated from plasma volume and hematocrit in 20 animals. Plasma volumes were determined by diluting a known amount of two measurable "tracer" substances in the circulatory compartments of each anesthetized subject. ^{125}I-labeled rhesus monkey serum albumin (^{125}I-RSA) and fluorescent-labeled hydroxyethyl starch (FITC-HES) were administered simultaneously for side-by-side comparison of the results. The degree of dilution of the tracers within the circulatory compartment of each subject was measured in blood samples taken at t = 12, 18, 24, 30 and 36 min after injection. Time-of-injection plasma concentrations were then determined by linear regression of the serial plasma concentrations to t = 0. The accuracies of the in vivo methods were compared with dilution curves in vitro performed at the same time as the in vivo tests. Our results show that the FITC-HES technique yields TBVs that are generally lower than those calculated using the ^{125}I-RSA technique with no systematic trend. In addition, subject body composition, specifically body fat percentage, has a significant effect on TBV ($P < 0.0001$) that is not captured by the fixed ratio of blood volume per kg body weight formulas currently in use.

P235 Postmortem Analysis of Blood Glucose and Electrolytes, and Validation of a New Glucose Meter in New Zealand White Rabbits

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Recently, our institution experienced 2 spontaneous deaths of research rabbits under anesthesia with marked postmortem hyperglycemia, which had been premedicated with ketamine/xylazine. α_2-Adrenergic receptor agonists, such as xylazine and dexmedetomidine have been used widely in human and veterinary medicine for their sedative and analgesic properties. While these drugs have numerous advantageous properties, concerns focus on their cardiovascular side effects. A less obvious effect is hyperglycemia. The objectives of the current study were to characterize changes in blood electrolytes (Ca^{2+}, Cl^-, K^+, Mg^{2+}, and PO_4^-) and glucose in rabbits sedated with acepromazine/ketamine/xylazine and validate a new glucose monitoring system (GMS) for ante- and postmortem samples. Samples were collected antemortem and postmortem at 2 min by cardiac puncture and at 20, 40, and 60 min from the caudal vena cava. GMS measurements were paired to electrolyte and glucose concentrations determined by a clinical laboratory analyzer. Repeated measures ANOVA, linear regression, paired *t* test, and Bland–Altman analyses were performed. Postmortem samples exhibited a significant increase in glucose, K^+, and PO_4^-. The mean [SD] maximum observed glucose concentration (504.8 [294.6] mg/dL, $n = 7$) was observed at 20 min postmortem. Although the GMS results highly correlated ($R = 0.905$) with laboratory results, there was a significant ($P = 0.048$) difference in absolute values between the 2 methods. The GMS did not meet the ISO 15197:2013 acceptance criteria based on the Bland–Altman analysis. In summary, interpretation of postmortem blood may not adequately reflect levels at the time of death and postmortem hyperglycemia is variable between animals. In comparison to acepromazine and ketamine, which do not alter blood glucose, sedation with xylazine may influence hyperglycemia through its inhibitory effects on insulin. The data revealed inadequate GMS performance for ante- and postmortem samples. Further studies are needed to evaluate pharmacologic influences and to determine whether GMS devices yield reliable data in veterinary medicine.

P236 Ear Notching of Mouse Pups Does Not Require Analgesia as Assessed by the Mouse Grimace Scale and Behavioral Scoring

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Despite advances in pain recognition in adult animals, no methods have been validated for preweaned mice, although many potentially painful procedures are performed, such as tail tipping for genotyping and ear notching for identification. Our objectives were to evaluate and validate the mouse grimace scale (MGS) and behavioral scoring as tools to assess pain in preweaned mice, and to assess the efficacy of 10 mg/kg carprofen in drinking water. Ear notching was performed on 110 (14 litters) 19- to 28-d-old mice during routine colony management. Six litters received 10 mg/kg/d carprofen in drinking water beginning 24 h before ear notching, 6 negative control litters received no analgesic in the water, and 2 untreated control litters received no manipulations or treated water. Water consumption could not be quantitated as pups were housed with their dam; however, because pups were near or beyond common weaning ages, it was assumed that they were drinking most of their daily fluid as water. Litters were videorecorded immediately after ear notching for 5 h at the same time each day. For behavioral data, 21 behavioral categories were scored continuously for the first 10 min at 0, 0.5, 2, and 4 h by an observer blinded to treatment. For MGS scoring, 363 facial images were captured at 0, 2, and 4 h after ear notching and scored by 2 observers. Facial action units evaluated include orbital tightening, nose bulge, cheek bulge, and ear position and shape. MGS scores from untreated control pups were subtracted from scores from carprofen-treated or untreated pups. Data was analyzed using a linear mixed model ANOVA with post hoc Bonferroni tests. No litter-associated differences were noted in behavioral or MGS data and data was combined across litters. No differences were noted in behaviours of carprofen-treated compared with untreated mice except at 4 h, when untreated mice spent more time nursing ($P < 0.03$). There were no treatment differences in MGS scores. This indicates that ear-notching is not a highly painful or distressing procedure for mouse pups and that the MGS has utility for evaluating pain in preweaned mice. Provision of carprofen in water at approximately 10 mg/kg to mouse pups provided no additional benefit.

P237 Activated Mesenchymal Stem Cells Amplify Antibiotic Activity against Chronic *Staphylococcus aureus* Infection

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Antimicrobial resistance is one of the greatest challenges facing the medical community today and new interventions are needed. Recent studies have shown that mesenchymal stem cells (MSC) exhibit antimicrobial activity when activated by Toll-like receptor (TLR) ligands in vitro. We hypothesized that activated MSC could enhance the activity of conventional antibiotics in common wound infections. MSC derived from adipose tissue of mice were expanded in vitro and activated with TLR ligands to assess the effects on antimicrobial activity and production of ntimicrobial peptides (AMP). The effects of MSC treatment in vivo were assessed using a mouse model of chronic *S. aureus* biofilm infection. Mice were treated with a series of 4 MSC intravenous injections, with or without antibiotic treatment, and bacterial load assessed over 14 d via an in vivo imaging system

and bacterial culture of the wound site. Activation of MSC with TLR ligands significantly increased production of the AMP CXCL10 and inhibited bacterial growth in vitro. The most potent TLR agonist in vitro was the TLR3 agonist polyI:C. In the chronic *S. aureus* infection model, activation of MSC with polyI:C resulted in decreased bacterial counts. Treatment with activated MSC induced AMP production and enhanced antibiotic therapy in vivo. These results suggest that stem cell therapy using activated MSC may be an effective means to enhance antibiotic therapy for chronic infections.

P238 Zoonotic Enteric Pathogens in Research Swine Obtained from Commercial Vendors

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Zoonoses are diseases of animals transmissible to humans. While many animals currently maintained in a biomedical research setting are supplied by SPF vendors, potential for zoonotic disease transmission remains. Some research requires the use of animals that may not be available from SPF vendors, carrying the risk for introduction of zoonoses into the laboratory environment. Swine are reservoirs of various zoonotic bacteria, protozoa, and helminths, such as *Campylobacter*, *Yersinia*, *Balantidium*, *Giardia*, *Cryptosporidium*, and *Ascaris*. The use of swine in biomedical research is increasing; however, there is a paucity of literature on the prevalence of zoonotic swine enteric pathogens in the laboratory setting. Our objectives were to 1) determine the prevalence of zoonotic enteric pathogens in swine obtained from various commercial vendors and 2) develop a surveillance and prevention program to reduce disease transmission risk in the biomedical research environment. Based on our previous experience, we hypothesized that 1) zoonotic enteric pathogens are more prevalent in Yorkshires than miniature swine breeds and 2) Yorkshires harbor a greater variety of zoonotic enteric pathogens than miniature swine breeds. We utilized a fecal bacteria culture panel, fecal floatation, and direct wet mount techniques to detect zoonotic enteric pathogens within Yorkshire and miniature swine from multiple vendors. We found a high overall prevalence of *Campylobacter* (72%) and a lower prevalence of *Balantidium* and *Yersinia* (6% and 2%, respectively). All 3 enteric pathogens were detected in Yorkshires, while only *Campylobacter* and *Yersinia* were detected in miniature swine. Furthermore, we confirmed that miniature swine had a lower prevalence of *Campylobacter* than Yorkshires (61% and 74%, respectively). This study highlights the prevalence of zoonotic organisms in swine in the biomedical research environment and results serve as an educational tool for occupational health training of biomedical research staff. In addition, the data provides information about occupational risks associated with swine from various commercial vendors and assists with development of preventative medicine and occupational health programs for mitigating these risks.

P239 Carbon Dioxide-Induced Pulmonary Hemorrhage

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Carbon dioxide is the most commonly used method of euthanasia for rodents. AVMA Guidelines recommend CO_2 displacement rate of 10% to 30% per minute and recommend against placing conscious animals in prefilled chambers. An investigator reported pulmonary hemorrhagic lesions in Balb/c mice euthanized with slow-fill method that were not previously observed with prefilled method. This study aims to determine whether or not slow-fill CO_2 euthanasia method induces pulmonary lesions in Balb/c and C57Bl/6 mice compared with prefilled method. In a pilot study, 6-wk-old Balb/c mice (n = 6) and C57BL/6 (n = 4) were euthanized using either slow-fill method or prefilled method followed by cervical dislocation. All procedures were approved by the IACUC. Tissues (lung, heart, brain, liver, spleen, kidneys, nasal turbinate, muscle, and sexual organs) were collected for gross and histologic evaluation. Results showed that 3 Balb/c mice euthanized with slow-fill method had extensive pulmonary hemorrhage while no hemorrhage was noted in 3 Balb/c mice euthanized with prefilled method. One of 2 C57Bl/6 mice euthanized with slow-filled method had mild pulmonary hemorrhage while no hemorrhage was noted in 2 C57Bl/6 mice euthanized with prefilled method. Three Balb/c mice euthanized with slow-fill method had mild to moderate nasal hemorrhage while one of 3 Balb/c mice euthanized with prefilled method had mild nasal hemorrhage. Two C57Bl/6 euthanized with slow-fill method had mild to moderate nasal hemorrhage while one of 2 C57Bl/6 mice euthanized with prefilled method had mild nasal hemorrhage. No gross or histologic lesions were noted in other organs. Based on these findings, we have concluded that slow-fill CO_2 euthanasia method induces pulmonary and nasal hemorrhage in Balb/c mice compared with prefilled method. In C57Bl/6 mice, there were insignificant and inconsistent hemorrhagic responses. Further studies are planned to validate whether the lesions are attributable to chamber filling rate or due to strain differences.

P240 The Rat Grimace Scale and an Analgesic Intervention Threshold: Steps Towards Cage-Side Pain Assessment

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The use of species-specific facial expression scales in rodents have been recently introduced for pain assessment. These scales show promise in improving the predictive ability of pain models and meeting ethical obligations to provide analgesia. Study aims were to identify an analgesic intervention threshold and to assess reliability of the RGS in a novel environment (different laboratory, model and observers). Sixteen adult female Sprague–Dawley rats (284 to 420 g) scheduled for a surgical procedure as part of an unrelated study were randomized to receive one of 3 analgesic treatments: 1) 0.05 mg/kg SC buprenorphine, 2) 1mg/kg SC meloxicam, and 3) 0.2 mg/kg oral buprenorphine in jelly. Rats were video recorded for 30-min periods preoperatively and multiple time points up to 12 h postoperatively. Still images captured from each video recording were randomized, and then scored by 3 blinded observers. Five experts without RGS experience independently and blindly classified the images as either "pain" or "no pain." Interrater reliability for observers were assessed with an intraclass correlation coefficient (ICC). Determination of an analgesic intervention threshold was performed by receiver operating characteristics (ROC) curve analysis. Data are presented as percentage or coefficients with 95% confidence intervals (CI) and $P \leq 0.05$ considered significant. Eighty-seven images were collected and scored. Interrater reliability was "very good" with an ICC of 0.85 (0.78 to 0.90, 95% CI) for single measures. Fifty-three of the images were classified as "pain" and the remainder as "no pain". An analgesic intervention score of > 0.67 (0 to 2 scale range) was derived from the intersection between the greatest values of sensitivity (84.6% [71.9 to 93.1]) and specificity (84.6% [73.3 to 96.8]). These data suggest reliability of the RGS in a novelenvironment and its potential for use to guide analgesic intervention. This has important implications for the welfare of rats used in biomedical research.

P241 Saphenous Vein Blood Collection a Feasible Replacement for Retroorbital Blood Collection when Assessing Microfilaremia in Gerbils

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The NIH "Guidelines for Survival Bleeding of Mice and Rats" notes that retroorbital puncture has a greater potential for complications

when compared with other methods of blood collection, and that it should be performed under general anesthesia. Saphenous vein puncture has a low potential for complications and can be performed without anesthesia. Gerbils used for filarial parasite research at our institution require blood sampling to monitor the levels of microfilaremia, and the standard method has been retroorbital puncture. Our goal was to assess saphenous vein puncture as a feasible replacement for retroorbital puncture by comparing microfilaria levels in blood samples collected by both methods. We collected blood samples from the saphenous vein and the retroorbital sinus from 21 gerbils infected with the human filarial parasitic worm, *Brugia malayi*. Two slides were prepared from each sample, and technicians blinded with respect to method and gerbil provided a microfilaria count for each collection site by averaging the counts for each pair of slides. A paired *t* test was used to determine whether counts between the collection sites were significantly different. Saphenous vein counts were not significantly different from retroorbital counts at higher levels (over 50 mf/20 mL). However, at relatively low counts (under 50 mf/20 mL), saphenous vein counts were significantly lower than retroorbital counts. Saphenous vein puncture is a feasible standard for assessing microfilaria blood levels. It requires less training time and skill than retroorbital, does not require general anesthesia, and adequate volumes of blood can be collected for counting microfilaria. Our results indicate that at higher levels of microfilaremia, the lateral saphenous vein provides similar numbers as the retroorbital sinus. For assessment of lower numbers of microfilaria, the retroorbital sinus provides higher counts. While it has not been determined whether one site is truly representative of the actual microfilarial density, both sites allow assessment of microfilaremia, which is of use to filarial investigators. We established saphenous vein puncture as a feasible replacement to retroorbital puncture when microfilaria numbers are anticipated to be over 50 mf/20mL.

P242 Age-Related Differences in the Vaginal Microbiome of the Olive Baboon (*Papio anubis*)

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The composition of the vaginal microbiome affects fecundity and susceptibility to sexually transmitted infections. In order to better characterize the vaginal microbiome of the baboon, an excellent model for reproductive studies, vaginal swabs were collected from 49 wild-caught female olive baboons (*Papio anubis*) at a single time point. Estimated age and stage of menstrual cycle were recorded for each animal. Bacterial communities were characterized by 16S rRNA gene pyrosequencing. Sequence data sufficient for analysis were obtained from 38 of 49 animals. Three major bacterial phyla were identified: Bacteroidetes, Fusobacteria, and Firmicutes. Unlike the human vaginal microbiome, which is characterized by a preponderance of *Lactobacillus* spp., the baboon vaginal microbiome was polymicrobial with significant interindividual variation. Significant differences were seen in community diversity (Shannon diversity index) and Shannon estimator of evenness between adult and subadult group animals. Diversity is defined as the richness and evenness of a population where richness is the number of bacterial species and evenness is the distribution across species. Subadult baboons had greater community diversity and greater evenness than adults. Significant differences between vaginal microbial communities of adult and subadult animals were also found when compared by weighted Unifrac, which is a phylogeny-based measure of community dissimilarity that considers relative abundance of bacterial species. These findings suggest that there are age-related differences in the baboon vaginal microbiome. These differences may alter susceptibility to reproductive infections in natural disease outbreaks or in experimental use of baboons in reproductive infectious disease studies.

P243 The SR/CR Mouse Model of Cancer Resistance

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The aim of this study was to investigate if innate immune cells from SR/CR mice were able to induce regression of established tumors in wild type mice, and if SR/CR mice could resist challenges with other malignant cancer cell lines than the tested S180 cancer cell line. It was tested if SR/CR mice could resist cancer development after injections with other malignant cancer cells, groups of 6 wildtype and 6 SR/CR mice were used for each tested cancer cell line. In adoptive transfer settings we investigated if total leukocytes and leukocytes depleted of CD4+, CD8+ and B- cells could make established tumors in wild type mice regress. The experiments were performed on groups of 6 mice receiving adoptive transfer of leukocytes from wildtype mice and another group of 6 mice receiving adoptive transfer of leukocytes from SR/CR mice, and the same set-up was used when testing the effect of transferred innate immune cells on tumor growth in wildtype mice. In contrast to previous observations, the cancer resistance was limited to S180 sarcoma cancer cells. We were unable to confirm previous observations of resistance to EL-4 lymphoma cells and J774A.1 monocyte-macrophage cancer cells. The cancer resistance against S180 sarcoma cells could be transferred to susceptible nonresistant BALB/c mice as well as C57BL/6 mice after depletion of both CD4+/CD8+ leukocytes and B-cells from SR/CR mice. In the responding recipient mice the cancer disappeared gradually following infiltration of a large number of polymorphonuclear granulocytes and remarkably few lymphocytes in the remaining tumor tissues. This study confirmed that the in vivo growth and spread of cancer cells depend on a complex interplay between the cancer cells and the host organism. Here, hereditary components of the immune system, most likely the innate part, played a crucial role in this interplay and lead to resistance to a single experimental cancer type. The fact that leukocytes depleted of both CD4+/CD8+ and B cells from the cancer resistant donor mice could be transferred to inhibit S180 cancer cell growth in susceptible recipient mice support the vision of an efficient and adverse event free immunotherapy in future selected cancer types.