Journal of the American Association for Laboratory Animal Science
Vol 52, No 5
September 2013
Pages 608–700

Abstracts of Scientific Presentations

2013 AALAS National Meeting

Baltimore, Maryland

Platform Sessions

PS1 Food Enrichment of Laboratory Beagles: Behavioral Benefits and Durability Concerns

JK Willis*[1], JM Cavarra[2], LV Kendall[3]

[1]Biology, Colorado State University, Fort Collins, CO; [2]George Mason, Washington, DC; [3]Laboratory Animal Resources, Colorado State, Fort Collins, CO

Enrichment can be a key element in stress reduction in domestic dogs in both pet and kennel environments. However, providing enrichment to laboratory dogs can be a means for injury or ingestion of objects. Devices provided as enrichment may not be used as intended and may not effectively serve as enrichment at all. Our aim was to evaluate the activity of laboratory dogs provided food enrichment using commercially available activity feeder toys (2 types were used) and compared with a control condition without enrichment. We wanted to evaluate how much time dogs spent interacting with their food in each case, the efficacy and durability of devices, and if the presence of enrichment changed their behavior in other ways. All dogs were videotaped for 4 h, immediately following the presentation of either food alone or food in an activity feeder. Behaviors were scored using an ethogram and results were tabulated in amount of time spent performing different behaviors. Dogs spent more time engaged with their food when it was presented in both types of food enrichment when compared with control. Dogs spent as little as 5 min interacting with their food in the control condition and as much as 43 min interacting with their food when enrichment was provided. Dogs with enrichment did not stay consecutively engaged with the enrichment but rather exhibited an overall activity level increase, returning to devices repeatedly for short bursts of engagement. Dogs provided with enrichment were more active and used their space more when enrichment was provided. Enrichment altered the way in which laboratory dogs used their time with an increase in purposeful behavior and overall exercise. Commercially available activity feeder toys for pet dogs were not as durable as expected, with some being destroyed in as little as 15 min. We hope to design a similar, but more durable food enrichment device that could be used effectively for repeated use in the laboratory environment.

PS2 Colored Enrichment Devices Influence Behavior and Circadian Metabolism and Physiology in Sprague–Dawley Rats

MA Wren*[1], RT Dauchy[2], SM Hill[2], TG Ooms[3], LM Dupepe[1], DE Blask[2]

[1]Department of Comparative Medicine, [2]Department of Structural and Cellular Biology, Tulane University School of Medicine, New Orleans, LA; [3]Section of Laboratory Medicine, Illinois Institute of Technology, Chicago, IL

Enrichment devices and strategies, as currently endorsed by the *Guide*, are used to improve laboratory animal health and wellbeing. Many conclusions supporting this premise, however, are based primarily on observational studies with minimal consideration for circadian physiology and metabolism. Our previous studies demonstrated that spectral transmittance (color) of light passing through laboratory rodent caging impacts normal rhythms of physiology and metabolism in rats. Here, in conjunction with our *GLAS*-supported studies, we examined how the use of colored enrichment devices can influence laboratory rodents' daily circadian melatonin signal and alter temporal coordination of normal physiologic functions. Adult male Sprague–Dawley rats (Crl:SD; $n = 6$ per group) were maintained on a lighting regimen 12:12-h light:dark cycle (300 lx; 123.0 μW/cm^2; lights on 0600) in standard polycarbonate translucent clear rodent cages containing either polycarbonate translucent clear (C), amber (A), red (R), or PVC opaque (O) tubing (3 in. wide × 6 in. long; 1/8 in. wall thickness). After 1 wk acclimation, animals were assessed during light phase for time spent within the colored enrichment devices (CEDs) and for arterial plasma melatonin at 6 circadian time points (beginning at 0400). Results revealed no differences in dietary or water intake, or body growth rates among the groups. Time spent within the CED groups during light phase in week 2 of the study was significantly longer in duration ($P < 0.05$) at 40.3 ± 10.1% (R), 15.3 ± 3.8% (A), 9.4 ± 2.8% (O), and 1.0 ± 0.6% (C), respectively, compared with that of week 5 with R (5.3 ± 2.9%) > A (1.3 ± 1.2) > C (1.1 ± 0.6%) > O (1.0 ± 0.4%). Plasma melatonin levels in pg/mL (mean ± 1 SE) were low in the light phase (>10.0 ± 0.2) in all groups, but significantly higher ($P < 0.05$) during dark phase (0400) in group A (213 ± 74), compared with groups R (109 ± 21), C (92.0 ± 35.0), and O (64 ± 18). These findings indicate that use of colored enrichment devices impact behavior and circadian regulation of neuroendocrine parameters that influence laboratory animal health and wellbeing.

PS3 Put Your Environmental Enrichment Program on a Diet: Applying LEAN to Environmental Enrichment

CS Coke-Murphy*, JC Farley

Animal Care, Vanderbilt University Medical Center, Nashville, TN

Recently, LEAN Six Sigma (LSS) has gained favor in the laboratory animal science as a means of reducing waste and increasing operational efficiency. LSS is a managerial concept combining LEAN and Six Sigma that results in the reduction of waste. Waste is defined by LSS as nonvalue added tasks, process steps, and personnel practices that distract or take away from the essential functions of the organization. The LSS process includes: 1) sort, 2) set-in-order, 3) shine, 4) standardize, and 5) sustain. The Division of Animal Care decided to apply the LSS process to our Environmental Enrichment program (EE) in our large animal facility as a means to increase organization and efficiency of providing enrichment. The facility has the most species, enrichment devices, and historically has required the largest outlay of the EE budget. We applied LSS in the following manner: 1) sort: all of the EE devices were sorted by species and then tagged everything as "stay," "go," "broken," or "unknown;" 2) set-in-order: all of the EE devices tagged "stay" were placed in labeled bins, all "go" and "unknown" items were reevaluated either for use by another species or thrown away; 3) shine: guidelines for cleaning and storage of EE were created along with a "broken" bin; 4) standardize: bins and the pegboard were labeled; 5) sustain: a facility EE point person position was created to assist the EEC in managing the space and EE devices. As a result of the implementation of LSS we saw a dramatic decrease in the amount of "lost" EE devices as evident by a substantial decreasing in EE spending for that facility for example, ≥ 50% budget reduction. LSS implementation was a success in our large animal facility and we will now be implementing this process in our other animal facilities.

PS4 The Training of Many by a Few: A Hybrid Approach to Researcher Instruction in a Large Animal Care and Use Program

SM Boyd*, T Neubauer, CA Buckmaster

Center for Comparative Medicine, Baylor College of Medicine, Houston, TX

The animal program at our institution includes 8 vivaria with over 3,200 researchers covered by over 600 protocols and 2 dedicated train-

ers. Providing training and ensuring proficiency, prior to work with animals, on all requirements to every user in a program this large is very challenging for a small training staff, but is essential for preventing future animal welfare and compliance concerns. To meet this challenge, The Center for Comparative Medicine (CCM) established a hybrid approach to initial training that combines online lessons on all BCM and CCM requirements with hands-on technical instruction. By design, this format also establishes collaborative relationships between key CCM professionals and researchers to encourage lasting partnerships that promote a culture of support for all BCM studies. Researchers must complete customized AALAS Learning Library "tracks" in order to be listed on animal protocols. Once listed, access to CCM training modules is enabled automatically through a database interface. Certificates of passing are only generated for quiz scores of 100% and users are required to deliver their certificates to the CCM Access Agent for vivarium access. The agent then grants access and sends a template note to the relevant CCM manager instructing him/her to schedule a facility tour with the researcher within 2 business days. After the tour, the manager reviews technical training needs with the researcher and submits a digital "technical training requirement" form to the training office. After ensuring that the procedures listed are covered in the protocol, training personnel arrange for training and proficiency evaluations of the techniques specified with the new researcher. Nearly 100 researchers have participated in this training format since it was implemented in March 2013. Data is being collected currently to evaluate and quantify the success of this approach in reducing animal welfare and compliance concerns.

PS5 Can Seeds Help with the Daily Grind?

K Pritchett-Corning*[1], R Keefe[1], JP Garner[2], BN Gaskill[1]

[1]Research Models and Services, Charles River, Wilmington, MA; [2]Department of Comparative Medicine, Stanford University, Stanford, CA

Some laboratory mice gnaw food pellets without ingesting much of the gnawed material, resulting in the production of waste material called orts. The fact that this food grinding behavior is not seen in all individuals of a particular strain suggests that it might be abnormal, and thus indicate a welfare concern. Furthermore, the increased rate of feed consumption and cage soiling is undesirable from a husbandry perspective. To try to determine possible motivations for the behavior, and identify potential treatments, outbred Crl:CD1(Icr) mice exhibiting food grinding were selected for one of 3 treatments placed in the feeder: no enrichment, a chewing device, or sunflower seeds. Both enrichment groups showed a significant decrease ($P < 0.05$) in ort production when compared with baseline measurements, but only mice provided with sunflower seeds maintained the decreased rate of food wastage after the treatment was withdrawn. A relationship between body weight and ort production was also found, in that cages with greater average body weights had lower levels of ort production. This suggests that a simple need to gnaw alone cannot explain food grinding, and that a nutritional motivation may also be involved.

PS6 Building a Quality Assurance Program: Exploring the Pitfalls and the Peaks in the Process

E Vernasco-Price*, CJ Maute, L Steiner, J Lofgren

University of Michigan, Ann Arbor, MI

Routine animal room inspections are an essential part of any animal facility. A combination of factors at our facility made it increasingly difficult for area supervisors to perform consistent and timely inspections of the animal rooms. A quality assurance (QA) pilot program was designed with 2 goals in mind: 1) standardize inspections and 2) ensure inspections were conducted consistently. The pilot design was derived from several components; most notably feedback from an employee engagement survey and the desire to use the advanced skills of Animal Technician Seniors (ATS). Two ATS were selected as QA inspectors based on their desire to be involved with the pilot. Each QA inspector was responsible for inspecting one room per month per Animal Care Technician (ACT) for 2 mo using the standardized form created. Upon completion of the pilot, a survey was issued to the QA inspectors, the ACTs responsible for the inspected rooms, and the supervisors of the inspected areas. Eleven of 15 ACTs, 3 of 3 Animal Care Supervisors, and 2 of 2 QA inspectors completed the survey. The results of the survey are as follows: 82% of ACTs surveyed, reported a preference for supervisor inspection rather than QA inspection of their rooms. Many ACTs (82%) also stated that the QA inspection performed by another animal technician rather than a supervisor was very stressful because the area supervisor would have more area-specific knowledge and a QA inspector may not. Despite the senior status of the QA inspectors, ACTs perceived their QA inspector coworkers as unqualified and unable to give an unbiased assessment leaving them feeling vulnerable or disconcerted. In contrast, 100% of the supervisors felt it was helpful to have assistance with a task that had been increasingly challenging to complete on a consistent basis, fulfilling part 2 of our initial goals. All of the QA inspectors (100%) felt positive about the purpose of the program but negatively about the feedback from peers. All 3 groups agreed that a consistent, standardized QA program is necessary and wanted. This pilot study highlighted the collective desire for a comprehensive QA program as well as the importance in providing a clear, detailed message to the staff about the necessity and goals of the program.

PS7 Digital Signage Facilitates Communication with the Investigative Community

N Rogers*, K Kun, J Kilpatrick, SE Erdman, JG Fox

The Division of Comparative Medicine, MIT, Cambridge, MA

Our institution's Division of Comparative Medicine (DCM) has begun exploring new options for delivering key information to the investigative community. We are now debuting digital signage—a large television display hardwired to the internet—in the entryways and gowning areas of animal facilities. This way we are able to "push" essential and late-breaking information directly to investigators, in a convenient location that they already frequent. This approach aims to supplement rather than replace more traditional and slower methods of communication (for example, burdensome meetings, or email that may be overlooked). By placing the display in an area heavily trafficked by the research community, we take advantage of a captive audience that may be "standing around" (while gowning\waiting for an elevator or others in their party). Digital presentation of information allows us to reduce paper signage (associated "signage fatigue"), and also to update information regularly and instantaneously. Sample material presented includes: upcoming training dates, opportunities, and deadlines; new federal or MIT rules or regulations, timely information and/or problem areas (for example, "no shorts in animal facility," "facility renovations begin Monday," "please be sure to fill out postop monitoring cards"); names and faces of relevant DCM staff; and notification of various DCM services. A scrolling ticker at the bottom can also be implemented to display breaking news or emergency alerts. Ultimately, materials may be further custom-tailored to each individual animal handling area. Information is presented with eye-catching animations, audio, and video, and infused with humor where appropriate, as has been a theme in DCM's ever-growing training programs, to elevate potentially dry and tedious material to something more captivating and entertaining. Initial reception has been largely positive, but we are actively seeking feedback to determine if there is any objection to the proliferation of television screens in public spaces, or any feelings of intrusiveness or information overload. As we become more fully entrenched in the 21st century information age, DCM seeks to remain at the forefront of communication with the research community, keeping delivery of information fresh, modern, and convenient.

PS8 Development of a Database to Track and Trend Noncompliance for Use by IACUC Members and Office Staff

AL Reuter*

University of Texas Southwestern Medical Center, Dallas, TX

Institutions that receive awards from the Public Health Service (PHS) must promptly report to the Office of Laboratory Animal Welfare (OLAW) any serious or continuing noncompliance with the PHS Policy, any serious deviation from the provisions of the *Guide*, or any suspension of an activity by the IACUC. In addition, it is important

for IACUC members to be kept informed of investigations of alleged noncompliance. To ensure prompt reporting by our large academic institution, a database was developed to track allegations of noncompliance, document the outcome of the investigation, and maintain metrics of each case. Examples of tracked metrics include the general category of noncompliance, laboratory size, and the source of the report. These metrics allow the university to trend incidents and identify training opportunities. The database allows IACUC office staff to carefully document each case and follow its reporting status. The database is made available to IACUC members on a secure network, so that they can remain informed of each case between IACUC meetings. Furthermore, it is searchable, which allows IACUC members and staff to identify cases by their choice of query. The development of the incident database is a valuable tool for our university to track noncompliance investigations, allow for prompt reporting, trend cases to identify patterns, and keep our IACUC informed of investigation details.

PS9 Infrared Thermography (IRT): A New Noninvasive Tool for the Evaluation of Lab Animal Welfare

G Grignaschi*[1], F Luzi[2], G Marsella[1], L Buccarello[2], V Redaelli[2]

[1]Animal Care Unit, IRCCS Istituto di Ricerche Farmacologiche Mario Negri, Milano, Italy; [2]Dipartimento di Scienze Veterinarie per la Salute, a Produzione Animale e la Sicurezza Alimentare, Università degli Studi di Milano, Milano, Italy

It is widely recognized that the welfare of the animals used in biomedical experiments is crucial for the consistency of the results achieved. The welfare of laboratory animals is related not only to the experimental paradigm, but also to the housing conditions. Animals born and maintained in different cages may, in fact, behave in a slightly (but significant) different way, giving rise to experimental variability. The presence in the home cage of environmental enrichment can modify the behavioral and immune response of the animals to different stimuli. In these complex situations, it is important to determine animal welfare through easy and noninvasive methods that can be applied to wide range of different situations (that is, different cage types). Infrared thermography (IRT) is an innovative method to measure the skin temperature in order to identify stressful situations that could lead to a decrease in this parameter due to peripheral vasoconstriction in mammals. A recent study used IRT, to assess the effects of management systems and farming on the physiology and behavior of rabbits and another study, in nude mice, used IRT to detect the effects of anesthesia by assessing whether the skin temperature of the animals undergoing anesthesia quickly returned to a baseline level or could cause some type of stress to the animals. Since variations in surface temperature and alteration in blood flow are some of the most interesting indicators to evaluate a response to the presence of stress (peripheral vasoconstriction), this technique is a potential, noninvasive and objective method for the evaluation of welfare in laboratory animals. We will show data to support the argumentations.

PS10 Individually Ventilated Cages Impose Cold-Stress on Laboratory Mice: A Source of Systemic Experimental Variability

JM David*, D Stout

Molecular and Medical Pharmacology, David Geffen School of Medicine, University of California, Los Angeles, Los Angeles, CA

Individual ventilated cages (IVC) have recently been increasing in popularity. Based on the high rates of ventilation with IVCs, we developed 3 hypotheses: first, mice housed in IVCs experience significantly more cold-stress than mice housed in static cages; second, the additional cold-stress imposed by IVCs will affect the results of experiments using mice to model human disease, and third, if provided shelters, mice will behaviorally thermoregulate and thereby rescue the cold-stress effects of IVCs. To test the hypotheses, we housed mice in 3 different housing systems: an IVC, an IVC with a shelter, or a static cage. We quantified cold-stress of each housing system on mice with 2 previously established metrics: nonshivering thermogenesis with thermography and brown adipose vacuolation with histology. To test housing effects in a common, cold-sensitive murine model of human disease, we implanted the mice with subcutaneous epidermoid carcinoma (A431) cells and quantified tumor growth, tumor metabolism, and adrenal weights of mice housed in each system. Mice housed in IVCs had significantly higher nonshivering thermogenesis, exhibited signs of cold-stress on histology, had smaller subcutaneous tumors, lower tumor metabolism, and larger adrenal weights compared with mice housing in static cages. Shelters rescued IVC-induced nonshivering thermogenesis and adrenal enlargement and partially rescued the histologic morphology of brown adipose tissue, and tumor size. IVCs impose chronic cold-stress on mice, alter experimental results, and act as a source of systemic confounder throughout rodent-dependent research. By allowing behavioral thermoregulation to occur via seeking shelter, we can partially rescue the experimental altering effects of housing imposed cold-stress, improve physiologic uniformity across housing systems, and increase experimental reproducibility across housing systems.

PS11 Assessment of the Efficacy of Anesthetic and Analgesic Regimens Involving Rodents in Germany

K Herrmann*[1], P Flecknell[2]

[1]Department of Veterinary Medicine, Free University Berlin, Institute of Pharmacology and Toxicology, Berlin, Germany; [2]The Medical School, Newcastle University, Comparative Biology Centre, Newcastle upon Tyne, United Kingdom

According to Directive 2010/63/EU all Member States must "ensure refinement [...] of methods used in procedures, eliminating or reducing to the minimum any possible pain, suffering, distress or lasting harm to the animals" (Art. 4). This project was initiated to appraise the current situation of refinement in Germany in order to identify where improvements should be made. Animal research applications from all over Germany of 2010 were examined to assess the refinement methods used in laboratories throughout the country. The study focused on applications involving rats and mice as they are the most commonly used species. The project aim is to assess the efficacy of proposed anesthetic and analgesic regimens by examining applications in which rodents undergo surgical procedures. The study was carried out anonymously. Results of project applications reviewed to date show that isoflurane was the most widely used inhalant anesthetic in both rats (34%) and mice (32%). Isoflurane was either combined with an analgesic or used as the sole agent (rat: 30%; mouse: 24%). Ketamine/xylazine was the most frequently used injectable anesthetic combination (rat: 28%; mouse: 31%). Multimodal analgesic regimens were rare (9%) and were considered by experimenters only in exceptional cases following severe surgeries. In 46% of the applications the researcher's severity classification differed from the classification according to Annex VIII of Directive 2010/63/EU and other pain catalogues. Thus, the postoperative administration of analgesics did oftentimes not correlate with the severity of the experimental procedure, and the perioperative preventative use of analgesics was not consistently used.

PS12 Comparison of Immediate-Release and Sustained-Release Analgesics Using a Thermal Nociception Model and Plasma Concentrations in Mice

K Dorsey[1], S Kang[1], P Lunghofer[2], LV Kendall[2], R Hansen[2], DL Gustafson*[1]

[1]Laboratory Animal Resources, [2]Clinical Sciences, Colorado State University, Fort Collins, CO

Postoperative or postprocedural analgesia is imperative to eliminate undue pain or distress in murine models. Buprenorphine and carprofen are 2 of the most commonly used analgesics in mice and require dosing every 8 to 12 h, typically for the first 72 h after the procedure. Sustained-release formulations of analgesics require less frequent dosing, and thus, less handling and stress imposed on the animals, and steady state analgesia. To determine the efficacy of sustained-release analgesic formulations a preclinical evaluation of the pharmacokinetics and response to thermal nociception was evaluated for sustained-release (SR) formulations of buprenorphine (0.6 mg/kg), butorphanol (18 mg/kg), carprofen (15 mg/kg), fentanyl (3.5 mg/kg), and meloxicam (6 mg/kg) and compared with the immediate-release (IR) formulations of buprenorphine (0.5 mg/kg BID), carprofen (5 mg/kg SID), and meloxicam (1 mg/kg BID). Pharmacokinetics demonstrated plasma levels to spike

within the first 4 h, followed by a gradual decline which maintained plasma levels greater than immediate-release formulations for at least the first 48 h. There were no differences in the response to the thermal latency for the first 24 h of assessment. Based on these findings, the use of sustained-release formulations could be used to provide adequate analgesia in mouse models.

PS13 Comparison, Refinement, and Training of Mouse Endotracheal Intubation Methods

SW Baran*[1], MI Perret-Gentil[2], J Kehler[1]

[1]Veterinary Bioscience Institute, Harleysville, PA; [2]The University of Texas at San Antonio, San Antonio, PA

Safe and correct endotracheal intubation is used to mechanically ventilate rodents for various surgical and nonsurgical studies. Endotracheal intubation training is challenging because of the small size of rodents upper airways, and the fact that repeat intubation attempts lead to a high incidence of injury of the upper airways including hemorrhage and swelling. Such injury may result in undesirable research outcomes and can have a deleterious effect on the mice usually resulting in euthanasia. The goal of this study was to determine the number of times a mouse can be intubated per training session without resulting in severe trauma to the airway. Mice were assessed endoscopically after intubation and graded using the Kircher scale; erythema (score 1), excoriation (score, 2), and frank hemorrhage (score, 3). All mice demonstrated some degree of injury during the initial phase of training resulting in a recommendation of no more than 3 intubation attempts per mouse per training session, and one attempt per mouse in a study. We will discuss the study findings, compare the advantages and disadvantages of 5 intubation techniques, list equipment required to perform each of these techniques, and provide recommendations for intubation techniques for specific studies. Techniques will be presented with videos and images. After attending this session, attendees will be able to select reliable and expeditious methods for intubating rodents for specific studies.

PS14 Evaluation of the Sterility of an Alternative Rodent Surgical Drape Material

JC Smith*[1], D Mussmacher[2], CM Weiner[3]

[1]Veterinary Bioscience Institute, Winston Salem, NC; [2]Quality Assurance, [3]Surgical Services, Taconic Farms, Rensselaer, NY

Aseptic procedures, including draping, are an essential component to any surgical program and are increasingly important when working with rodents. A food-grade wrapping material has been suggested for use as rodent surgical drape. This study investigated the sterility and utility of this unique drape material. We hypothesized that this material would be sterilizable and used as a suitable alternative to current rodent surgical drape material. To test this hypothesis, 17 boxes, each containing 70 ft^2 of material around an inner cardboard tube, were obtained from a local supermarket. Each box was placed inside a sterilizing pouch and sterilized with ethylene oxide gas according to standard protocols. Sterility was confirmed for each pouch before use. Five of 17 of the boxes were held as controls and were swabbed only. The remaining 12 boxes were placed into use as rodent surgical drape according to standard protocols, and were swabbed after 1 wk of use at one of 6 different locations: 1) metal cutting edge, 2) first 2 in. of drape material, 3) next 2 ft of drape material, 4) next 2 ft of drape material, 5) next 2 ft of drape material, and 6) inner cardboard tube. All swabs were placed into sterile, liquid media and plated on TSA/B agar if turbidity was present after 24 to 72 h. Plates were evaluated at 16 to 24 h, and colony growth was identified by standard microbiologic techniques. Five of 5 of the control rolls had no organism growth at any of the 6 swab locations. Two of 12 rolls placed into use had growth for gram-positive, catalase-positive cocci (presumed *Staphylococcus* spp.) at location 1 only. These data show that once sterilized by ethylene oxide gas, this unique material can remain sterile down to the center cardboard tube. The only bacterial growth identified was at the metal cutting edge, which could be avoided using sterile handling techniques. In summary, this unique material can have utility for rodent surgical draping.

PS15 Assessment of Mandibular Nerve Block Using Bupivacaine in Yucatan Miniature Swine as a Model for Mandibular Condylectomy and Implant Surgery

JF Bova*[1], A da Cunha[2], RW Stout[1], S Bhumiratana[3], G Vunjak-Novakovic[3], SB Eisig[4], DM Alfi[5], MJ Lopez[2]

[1]Department of Pathobiologic Sciences, [2]Department of Veterinary Clinical Sciences, Louisiana State University School of Veterinary Medicine, Baton Rouge, LA; [3]Department of Biomedical Engineering, Columbia University, New York, NY; [4]Division of Oral and Maxillofacial Surgery, Columbia University College of Dental Medicine, New York, NY; [5]Oral and Maxillofacial Surgery Department, The Methodist Hospital, Houston, TX

Miniature swine models are commonly used for development of dental and oral maxillofacial surgical techniques. However, local nerve blocks used during standard human mandibular surgery are generally not incorporated into the models. We sought to establish a bupivacaine mandibular nerve block for analgesia in a Yucatan miniature pig condylectomy model. A mandibular nerve block should minimize pain during the surgical procedures, have residual analgesic effects after surgery, and may lower or eliminate the need for postoperative analgesics. We hypothesized that a bupivacaine mandibular nerve block would eliminate the need for systemic analgesics during and after surgery. Eight castrated male Yucatan miniature pigs underwent left mandibular condylectomy surgery as part of an implant study. Pigs were anesthetized with ketamine (10 mg/kg), midazolam HCl (0.2 mg/kg), and dexmedetomidine HCl (2 µg/kg) administered intramuscularly and maintained under anesthesia on isoflurane. Following intubation, pigs were randomized to receive a mandibular block with an equal volume of either bupivacaine (2 mg/kg, bupivacaine group, $n = 5$) or saline (control group, $n = 3$). A nerve stimulator was used for administration of the block with observation of masseter muscle twitch as indicator of proper placement. An arterial line was placed for monitoring of blood pressure (BP) and heart rate (HR). A rescue analgesic protocol consisting of fentanyl and lidocaine HCl was administered if HR or BP values increased 20% from baseline levels. Postoperative pain was quantified with a behavior scale consisting of HR, respiratory rate (RR), vocalization, mentation, nonpurposeful movement, ambulation, and feed consumption. All pigs required the fentanyl and lidocaine rescue. HR and BP were analyzed at 4 time points, baseline, before rescue, 10 min after rescue, and 20 min after rescue. No differences were seen between treatment groups for postoperative pain assessment or intraoperative time to rescue. Blood pressure fell significantly ($P < 0.01$) after rescue to near baseline levels in the bupivicaine group while no changes were observed in the saline group. On a percentage basis mean HR was significantly lower ($P < 0.05$) for the bupivacaine group than for the saline group. The mandibular nerve block provided improved analgesia during the intraoperative period but no beneficial effects were observed postoperatively. An anesthetic with a longer duration may improve postoperative pain management. The mandibular nerve block is a recommended addition to the anesthetic regimen in the miniature pig condylectomy model.

PS16 Determination of an Effective Tramadol Dose in a Murine Model of Abdominal Surgery and Inflammation

AM Wolfe*, L Kennedy, JA Nemzek

Unit for Laboratory Animal Medicine, University of Michigan, Ann Arbor, MI

Analgesic control is required in most rodent studies with the potential to cause pain. Thus, the search for effective, noncontrolled options has led to the consideration of tramadol for painful procedures in rodents. Although reported tramadol dosing for rodents has ranged from 20 to 80 mg/kg, there is no published dose for abdominal surgical pain and inflammation. Our hypothesis is that tramadol will provide effective analgesia in a model of abdominal surgery and inflammation in mice. For the abdominal surgical and inflammation model, male and female C57Bl/6 mice were anesthetized and maintained on isoflurane for aseptic cecal removal. Analgesic groups consisted of saline or tramadol at 20, 40, or 80 mg/kg administered once preoperatively and then every 12 h (3 total doses). Animals were observed daily for 3 d by 2 blinded

observers to assess general pain score (GPS) parameters including posture, coat condition, activity, and relation to other mice. A negative pain parameter was also assessed that consisted of the total number of times the mice stretched up to explore their surroundings within 3 min. Von Frey fibers ranging from 0.4 to 2.0 g(f) were applied daily for 3 d around the incision site with a blinded observer recording responses including jumping, licking or scratching, and abdominal retraction. The stretching up parameters showed changes pre- and postoperatively, but no differences were seen between treatment groups. The GPS observations show no differences between the saline or tramadol groups but there were observable differences between the sexes with females in the saline group demonstrating a significantly lower GPS mean of 1.5 compared with the males' GPS mean of 2.5. The female 80-mg/kg group also exhibited a significantly lower GPS mean of 0.36 compared with 2.5 in the males. Von Frey testing reveals a significant analgesic effect at the 80-mg/kg dose compared with the 20- and 40-mg/kg groups with a mean score of 4.3 reactions at 80 mg/kg compared with 6.4 and 7.2 for the 20- and 40-mg/kg groups, respectively. For abdominal surgical pain management in C57Bl/6 mice, tramadol can be effective at providing analgesia, but higher doses should be considered.

PS17 Development and Evaluation of a Novel Arterial Chronic Total Occlusion Model in Swine Coronary and Peripheral Arteries

A Dardenne*, A Peppas, C Gongora, A Carter, PA Mount, LV Anglin, M Morales, A Tellez, G Kaluza, J Granada

Skirball Center for Cardiovascular Research at the Cardiovascular Research Foundation, Orangeburg, NY

Chronic total occlusions (CTOs) remain a significant challenge for interventional cardiologists due to their complexity and tough fibrous caps, which prove to be problematic for successful guide wire and balloon passage. Further advancements in technologies are essential to the development of CTO treatment and recanalization of chronically occluded vessels. We aimed to evaluate a novel preclinical CTO model, simulating human coronary occlusions, to be used in the development of improved crossing devices. A total of 33 vessels (23 peripheral, 10 coronary) in 16 domestic swine were included in this model evaluation. The CTO model was created with the use of a plug, containing fat and calcium material. Quantitative vascular analysis was performed on each vessel prior to delivery, in order to determine size parameters and placement for the CTO plugs. Angiography was performed immediately following deployment to confirm successful placement and occlusion. Animals were euthanized at 2, 4, and 6 wk after deployment. At termination, vessels were evaluated angiographically for effective occlusions and harvested for histologic analysis. The CTO model successfully occluded blood flow in 29 vessels (19 peripheral, 83%; 10 coronary, 100%), as determined by angiography. Histologically, 2 wk after occlusion by the CTO model, vessels presented with small amounts of neointima, inflammatory changes and areas of calcification. Four-week occlusions had indications of granulation tissue, chronic inflammation, neoangiogenic vessels, giant cells, areas of calcification and areas of collagen with elastin fibers. Vessels evaluated at 6 wk were found to have fibrous connective tissue, calcified material surrounded by neutrophils, granulomatous inflammation, and perivascular fibrosis. We were successful in creating a novel CTO model in the porcine vasculature, using a method which is shown here to be reliable and easy to replicate. This is highly significant for the development of CTO therapies using preclinical models as a platform for future clinical implications.

PS18 Isoflurane Compared with Carbon Dioxide Anesthesia Disrupts Circadian Rhythms of Endocrine Physiology and Metabolism in Rats

MA Wren*[1,2], RT Dauchy[1], TG Ooms[3], SM Hill[1], LM Dupepe[2], DE Blask[1]

[1]Structural and Cellular Biology, [2]Department of Comparative Medicine, Tulane University School of Medicine, New Orleans, LA; [3]Section of Laboratory Animal Medicine, IIT Research Institute, Chicago, IL

An ongoing controversy exists over the use of CO_2 as an anesthetic. The new publication of the *Guide* highlights its use for euthanasia, but little research has focused on its use as an anesthetic. While it has been suggested that isoflurane may be a more suitable anesthetic for blood collection in laboratory animals than CO_2, evidence suggests that isoflurane significantly alters blood levels of corticosterone, glucose, lactate, and neuroendocrine hormones associated with normal metabolism and physiology. Here we examined the effects of isoflurane (4%) compared with brief CO_2 (70% CO_2, 30% air) anesthesia on circadian rhythms of endocrine physiology and metabolism in laboratory animals. Adult male Sprague–Dawley rats (Crl:SD; $n = 6$ per group) were maintained on a lighting regimen 12:12-h light:dark cycle (300 lx; 123.0 μW/cm^2; lights on 0600). After 1-wk acclimation, a series of 6 low-volume blood draws were collected over a 4-wk period via cardiocentesis (0400, 0800, 1200, 1600, 2000, and 2400) with 4 d between samplings using isoflurane (10-min procedure) compared with CO_2 (45-s procedure) anesthesia to assess arterial plasma melatonin, glucose, lactic acid, and corticosterone concentrations. Values for plasma corticosterone in isoflurane anesthetized animals at 2000 and 2400 were significantly higher than those anesthetized with brief CO_2 suggesting isoflurane may be a stressful stimulus. Plasma melatonin levels were low in the light phase (12 ± 1.0 pg/mL; mean ± SEM) in both groups, but significantly lower during the dark phase for isoflurane (48 ± 6.5 pg/mL) compared with normal dark phase levels in the CO_2 group (162 ± 18.5 pg/mL; $P < 0.05$). Arterial plasma circadian rhythms of glucose and lactic acid levels were significantly disturbed in isoflurane, compared with the CO_2 group. These findings demonstrate that circadian rhythms of endocrine physiology and metabolism normally observed under brief CO_2 anesthesia in rats are markedly disrupted using isoflurane anesthesia.

PS19 Validation of a Thermal Operant Pain Assay for the Evaluation of Centrally Acting Analgesics in Mice

HE Ramirez*[1], AH Battles[1], JK Neubert[2]

[1]Animal Care Services, [2]Department of Orthodontics, University of Florida, Gainesville, FL

Traditional methods for assessing analgesic efficacy in rodents include thermal (for example, tail flick test) and mechanical assays (for example, von Frey filament). These nonsurgically invasive methods, however, may not represent the best way to assess clinical analgesic efficacy. Postoperative behavioral-based pain assessment techniques have been used as a more comprehensive method for assessing analgesic efficacy in rats. However, they have been difficult to validate in mice. Here we present an automated, noninvasive operant behavioral approach for evaluating analgesic efficacy in mice. Male and female hairless mice (Crl:SKR-Hrhr, $n = 12$) were trained to reach a liquid reward while contacting the thermal elements with their cheeks. A solution of sweetened condensed milk was used as a reward. Thermal pain was experienced when the animals' cheeks, after they were sensitized with capsaicin cream, contacted the thermal element placed at 45 °C. We tested subcutaneous injections of buprenorphine (0.01, 0.05, 0.1, 0.2 mg/kg, plus saline). All animals in each group received each dose plus vehicle only in a crossover fashion with a washout period of 4 to 5 d between testing. The total number of licking events was divided by the number of facial-contact events to generate a ratio of reward-licking events to facial-stimulus contact event per dose. Results indicate that buprenorphine dosages of 0.05 ($P < 0.5$) and 0.1 ($P < 0.01$) mg/kg resulted in a statistically significant difference in analgesic response in male mice. However, only 0.05 mg/kg ($P < 0.05$) was effective in female mice. The sensitivity of this assay can directly improve postsurgical pain treatment by successfully finding the most effective analgesic dose tailored specifically to gender while at the same time having a direct effect on the 3Rs by: 1) reducing the number of animals used when compared with incisional pain models and 2) refining the pain assessment paradigm through the application of a less invasive method.

PS20 Evaluation of Analgesic Efficacy of Meloxicam Following Laparotomy in Sprague–Dawley Rats

JL Goldman*, EA Nunamaker, JD Fortman

BRL, University of Illinois at Chicago, Chicago, IL

The management of postoperative pain in rodents is an important component of an animal care and use program. Nonsteroidal antiinflammatory drugs are beneficial alternatives to opioids for postoperative

pain management because they are not controlled substances, have a different mechanism of action, and do not have the same side effects. Meloxicam in particular is an attractive option for postoperative pain management in rodents because of its once a day dosing regimen. In this study, rats given meloxicam were evaluated to determine whether meloxicam could provide adequate postoperative pain relief alone in a rat ovariohysterectomy (OHE) surgical model. Sprague–Dawley rats (n = 10 per group) received 1 mg/kg meloxicam, 2 mg/kg meloxicam, or saline subcutaneously at the time of surgery and for 2 d afterwards. OHE animal groups were compared with naïve and anesthesia-only control groups, and pain was assessed for 8 d following surgery using weight loss, home cage activity, vertical rises, cage-side observations of posture, activity, and coat appearance, and real-time rat facial grimace scoring. There were significant differences for the first 2 d following OHE surgery for all pain indicators, except home cage activity, between the surgery-only and anesthesia-only control groups. However, there was no significant difference in pain indicators between surgery control animals and animals that received OHE and either dose of meloxicam. These results suggest that 1 to 2 mg/kg meloxicam used alone does not provide adequate analgesia for laparotomy procedures in rats.

PS31 Evaluation of Retroorbital Injections as a Technique for the Creation of Humanized Mice

C Bekkevold[*1], B Clapp[2], DW Pascual[2], MK Reinhard[1]

[1]Animal Care Services, [2]Department of Infectious Diseases and Pathology, University of Florida, Gainesville, FL

Humanized mice are a valuable model for studying human immunobiology and hematopoiesis. They are especially necessary when there is no appropriate small animal model of a human disease, such as with many human viral diseases. The engraftment of human peripheral blood mononuclear cells (PBMC) into immunodeficient strains is one of the most prevalent protocols for the generation of humanized mice. Currently, 2 methods for engraftment are described: 1) tail vein injections of adult mice and 2) intracardiac or intrahepatic injections of neonates. Previous studies have described increased engraftments from the neonatal protocol; however, increased pup mortality with this technique is widely observed. The adult mice protocol has significantly decreased mortality but also has poorer engraftment of human cells compared with the neonatal protocol. This presentation gives an overview of an alternative technique by intravenous delivery of stem cells isolated from core blood and PBMC into neonatal mice via a retroorbital injection. Both neonatal and weanling NOD-scid IL2rϒnull were irradiated and subsequently injected with human PBMC cells via the retroorbital and tail vein routes, respectively. Results demonstrated that the retroorbital route had increased survival rates and comparable engraftment to the intracardiac and intrahepatic routes in neonates. This retroorbital protocol is compared with the 2 current techniques with emphasis on engraftment and animal welfare. This technique may serve as an example of refinement in the generation of humanized mice in research.

PS32 A Novel *Streptococcus* Associated with Meningoencephalitis in Naïve Weanling C57BL/6N Mice

G Braden[*1,2], R Ricart Arbona[1,3], S Monette[1,2], M Lepherd[1,2], NS Lipman[1,3]

[1]Tri-Institutional Training Program in Laboratory Animal Medicine and Science, New York, NY; [2]Memorial Sloan-Kettering Cancer Center, The Rockefeller University, and Weill Cornell Medical College, New York, NY; [3]Memorial Sloan-Kettering Cancer Center and Weill Cornell Medical College, New York, NY

Significant mortality associated with runting, abnormal gait, and decreased activity was observed in experimentally naïve C57BL/6N weanlings born to time-pregnant females obtained from a single commercial vendor over a 1-y period. Gram-positive, aerobic, α-hemolytic, coccoid bacteria were consistently isolated from the meninges (n = 18) and occasionally the blood and kidneys (n = 1) of a representative number of affected weanlings. Additionally, the bacteria were isolated from the uterus (n = 1), meninges (n = 1) and oral cavity (n = 3) of 3 dams from the same colony, 2 of which exhibited similar clinical signs. Multifocal fibrinosuppurative meningoencephalitis and ventriculitis with intralesional gram-positive coccoid bacteria, and thymic lymphoid depletion were observed in affected animals. The bacterium was also isolated from the oral cavity of a time-pregnant dam (1 of 23) from the same vendor cultured upon receipt. Sera collected from 6 dams known to have affected offspring and an affected weanling were subject to screening by MFIA and determined to be free of antibody to a comprehensive list of murine viruses and select bacteria, endo- and ectoparasites by direct examination, and pathogenic bacteria by culture. Complete blood counts from a select number of affected animals (n = 7) indicated an increased neutrophil/lymphocyte ratio and neutrophil cytoplasmic basophilia indicative of inflammatory demand. The commercial streptococci-species identification system yielded 2 bacterial profiles: *Aerococcus viridans* 2 or 3. Antibiotic sensitivity demonstrated susceptibility to all common classes of antibiotics. Amplification and sequencing of 16S rRNA fragments by PCR using generic bacterial primer sets yielded ambiguous results with the closest matches being *Streptococcus acidominimus*, *S. sanguinis*, and *S. gallinaceus* (both 96% and 99%, respectively). Growth in 6.5% NaCl, colony morphology, and Gram stain suggest a novel *Streptococcus* species. Additional analyses are ongoing to determine species identity.

PS33 Management of a Mouse Norovirus Outbreak in a Specific Pathogen-Free Barrier Facility

J Wallace[*1,2], K Boyd[1,2], KL Jackson[1], P Chen[1,2], JC Farley[1]

[1]Division of Animal Care, [2]Division of Comparative Medicine, Vanderbilt University, Nashville, TN

In January 2013, antibodies to mouse norovirus (MNV), an excluded pathogen, were detected in sentinels in one of 7 mouse housing rooms located within an SPF rodent barrier facility (Barrier). Animals housed in the Barrier must be rederived into the facility or purchased directly from an approved vendor. Initial steps were taken to isolate the room in preparation for 100% cage testing using serology and fecal PCR for immunocompetent and immunodeficient mice respectively. Thirty-five cages confined to a single investigator on 2 of 5 individually ventilated cage racks within the room tested positive by serology. PCR was negative on the samples submitted for testing. After discussion of management options, the decision was made to manage the outbreak at the individual investigator/rack level rather than relocate the entire room and rederive mouse lines back into the facility. The 2 positive racks were moved to a MNV positive facility. The 3 negative racks were moved to a transitional housing room adjacent to the Barrier anteroom but outside the main corridor. The transitional room was entered/serviced last while additional testing (3 rounds of 100% cage testing) was performed to confirm the animals' MNV negative status. Simultaneously, enhanced sentinel testing was done in the other Barrier rooms to confirm MNV had not spread. Results on both Barrier sentinels and animals in the transitional room were negative, and the animals were subsequently returned to the Barrier. Testing done since the animals' return has been negative. We will describe successful elimination of MNV from a Barrier facility using an individual rack/investigator approach rather than a room level approach. While it may not be a viable strategy for all excluded pathogens, it should be considered in situations involving MNV that would otherwise require rederivation of a significant number of valuable and unique genetically engineered lines.

PS34 Correlation of Infrared Thermography Compared with Rectal Body Temperature Measurement in Rats and Mice

RD Sullivan[*1], C Volpe[2], D Hamilton[1], TD Mandrell[1]

[1]Department of Comparative Meidicine, University of Tennessee Health Science Center, Memphis, TN; [2]Department of Biology, Christian Brothers University, Memphis, TN

Body temperature is one useful physiologic parameter for objectively assessing an animal's health status. Surgically implanted telemetry devices for recording body temperature have a high level of accuracy, but are invasive and subject to complications associated with the surgical procedure. Additionally, rectal temperature measurements subject animals to handling stress or require sedation which may alter their body temperature. Infrared thermography has been used in veterinary medicine as an aid to detect hoof disease and lameness in horses,

infectious diseases in wildlife, and in human medicine to assess skin perfusion and elevated body temperature. The objective of this study was to compare, in rats and mice, body surface temperature obtained using infrared thermography (IRT) with rectal temperatures obtained using a precision calibrated thermometer. A handheld IRT camera was used to measure surface temperature while rectal temperatures were simultaneously recorded in adult CD1 ($n = 10$), recombinant inbred BXD ($n = 20$), nude ($n = 9$) mice, and Sprague–Dawley rats ($n = 9$). Following baseline temperature measurements, all animals were subjected to isoflurane anesthesia without supplemental heat for 60 min to simulate hypothermia while body surface and rectal temperature measurements were obtained. Baseline rectal and IRT body surface temperatures were compared using linear regression analysis. The results showed a statistically significant correlation between IRT and rectal thermometer temperature recordings. IRT accurately detected body surface temperature changes caused by hypothermia which correlated with changes in rectal temperatures recorded simultaneously. Coat color was found to have no effect on the accuracy of IRT, but body surface area to mass ratio may account for a weaker correlation between similar temperatures recorded in rats. IRT is an accurate and noninvasive method for measuring rodent body temperature and can be performed without removing animals from their microenvironment.

PS35 Renal Failure in a Colony of LRRK2 Rats

HA Zimmerman*, F Poulet, M Fell, S Iliff

Safety Assessment and Laboratory Animal Resources, Merck, Kenilworth, NJ

A colony of LEH-Lrrk2tm1sage (LRRK2) rats, a commercially available model used for Parkinson disease research, was noted for an increase in acute dyspnea, weight loss, and spontaneous deaths among knockout (KO) rats approximately 1 y of age. These animals were noted to be in poor body condition (average BCS of 1.5/5) at necropsy, but exhibited few other gross lesions except for darkly pigmented kidneys, an established phenotype for LRRK2 KO animals. Colony management procedures were put in place, including weighing animals weekly and training husbandry staff on prompt recognition of humane endpoints, in order to secure animals for diagnostic workup. Animals were euthanized if their weight loss totaled 20% or more of baseline, while animals exhibiting dyspnea were euthanized immediately. Age- and genotype-matched controls for these animals were provided by the investigator at the conclusion of the study. In all, 32 male and female rats aged 7 mo or older were submitted for diagnostic workup, 19 of which exhibited the clinical signs of weight loss and/or dyspnea. At necropsy, a specified tissue set including mandibular and mesenteric lymph nodes, spleen, kidneys and adrenals, liver, heart and lungs, and brain, was collected from each animal and placed in 10% neutral buffered formalin for histologic processing. On histologic examination, all KO animals exhibited phenotype-related pigmentation of the renal tubular epithelial cells. Aged (11 mo and older) KO and wildetype animals had histopathologic findings consistent with chronic progressive nephropathy, including glomerulopathy with associated proteinaceous casts and/or tubular dilatation. The clinical signs of weight loss and/or dyspnea in these animals were attributed to renal failure consistent with the end-stage kidneys noted histologically. There was no apparent correlation between the phenotype-related pigmentation of the renal tubular epithelial cells in the KO animals and the severity of the chronic nephropathy lesions.

PS36 Refinements in Medical Management of a Bovine Chronic Heart Failure Model

LC Sherwood*[1,2], MA Sobieski[2,3], SC Koenig[2,4], GA Giridharan[2,4], MS Slaughter[2,3]

[1]Research Resources Facilities, [2]Cardiovascular Innovation Institute, [3]Division of Thoracic and Cardiovascular Surgery, [4]Department of Bioengineering, University of Louisville, Louisville, KY

The development and testing of mechanical circulatory support (MCS) devices has historically been performed in animals with normal cardiovascular systems due to the lack of a clinically relevant, stable, and reproducible large animal chronic heart failure (HF) model, thereby limiting the evaluation of device efficacy. High mortality rates have been reported with large animal chronic HF models. In this study, refinements in medical management to improve survival rate (SR) were investigated. Chronic ischemic HF (IHF) was induced in Jersey calves using a microembolization technique. Three groups of animals were used and divided into: 1) control, multiple embolization procedures with conservative therapy ($n = 9$); 2) treatment group 1 (TG1), single embolization procedure with moderately aggressive therapy ($n = 8$); and 3) TG2, single embolization procedure with aggressive medical management ($n = 20$). The groups were not randomized and data was analyzed retrospectively. Mean SR, body condition score, body weight, hemodynamic, echocardiography, and histopathology indices were recorded up to 60 d postembolization. An aggressive medical management regimen of analgesia, diuretics, β-blockade, antiarrhythmics, vasodilators, and inotropes improved SR by 34% over conservative therapy while still producing a clinically relevant and reproducible chronic IHF model.

PS37 Interdigital Furunculosis: Clinical Prevalence and Histopathologic Evidence in a Research Beagle Colony

EA Nunamaker*[1,2], SJ Morgan[2], CL Medina[2], W Buck[2]

[1]Biologic Resources Laboratory, University of Illinois at Chicago, Chicago, IL; [2]Comparative Medicine, AbbVie, North Chicago, IL

Interdigital furunculosis is a common but poorly understood inflammatory dermatological lesion seen in dogs. In order to better understand both the etiology and pathophysiology of this disease process, 242 beagle dogs were evaluated for body weight, body condition score, and the presence of current foot lesions. All identified lesions were scored for severity based on swelling (0 to 3) and redness (0 to 3) of the lesion and the presence of pain or lameness. All of the animals' records were also analyzed for a history of interdigital furunculosis. Statistical analysis revealed that age and duration in the facility were the most significant risk factors associated with the occurrence of interdigital furunculosis. It was further determined that there was a predilection for the primary weight bearing digits, especially on the front feet. Histopathologic evaluation of the feet of dogs pulled from the colony for other study-related reasons revealed a gamut of pathologic lesions, including moderate to severe changes in dogs without clinical furunculosis. These findings suggest that interdigital furunculosis in beagle dogs is a spectrum disorder that is exacerbated by repetitive trauma to the interdigital skin of the primary weight bearing digits.

PS38 Effects of Weekly Blood Collection in Male and Female Cynomolgus Macaques (*Macaca fascicularis*)

CR Adams*, LC Halliday, EA Nunamaker, JD Fortman

Biologic Resources Laboratory, University of Illinois at Chicago, Chicago, IL

This study was designed to evaluate the maximum amount of blood that can be safely collected in healthy, adult male and female cynomolgus macaques for 4 consecutive weeks with minimal impact on animal wellbeing. General guidelines for blood collection volumes in laboratory animals are not species-specific and there is currently limited information evaluating blood collection in macaques. In this study, blood was removed at 7.5%, 10%, 12.5%, 15%, or 17.5% of total blood volume (TBV) for 4 consecutive weeks. Hematologic parameters and body weights were evaluated immediately prior to each blood collection timepoint and for an additional 4 consecutive weeks following the last collection. Male and female macaques tolerated up to 15% TBV with minor clinical effects; whereas macaques in the 17.5% TBV group exhibited an increased incidence of vomition and anorexia in the first 24 h after blood collection. Based on these results, we recommend collecting no more than 15% TBV per week for 4 consecutive weeks in healthy, adult male and female cynomolgus macaques.

PS39 Clinical Use of Sustained-Release Buprenorphine in Rhesus Macaques

K Lencioni*, JF Baer

OLAR, California Institute of Technology, Pasadena, CA

Buprenorphine is widely used as a part of multimodal postoperative analgesia regimens in many species, including nonhuman primates (NHPs). Sustained-release Buprenorphine (SR-Bup) is a recently available formulation that may provide up to 5 d of analgesia with a single subcutaneous dose. This regimen offers a significant improvement in postoperative care, as buprenorphine HCl has traditionally been administered by intramuscular injection every 4 to 12 h to provide adequate analgesia. However, there is no clinical data regarding optimal dosing of SR-Bup for NHPs. We administered SR-Bup to rhesus macaques (*Macaca mulatta*) undergoing vasectomies (expected to experience mild to moderate pain, $n = 3$) as well as animals undergoing a variety of related neurosurgical procedures (expected to cause moderate pain, $n = 4$). We used a range of doses of SR-Bup from 0.12 to 0.2 mg/kg as part of a multimodal analgesia plan. Animals were observed frequently for signs of postoperative pain or discomfort as well as sedation and reduced appetite (2 potentially significant side effects of opioid treatment). The initial animal undergoing a neurosurgical procedure received 0.2 mg/kg. This dose was associated with significant sedation, with concurrent reduced food intake over the first 12 h postoperative. The next 3 animals undergoing neurosurgical procedures received 0.15 to 0.16 mg/kg, which appeared to provide adequate analgesia throughout the postoperative recovery period without significant sedation or reduction in appetite. Animals undergoing vasectomies received 0.12 mg/kg. None of these 3 animals showed signs of pain or discomfort after the surgical procedure. None of the animals in this study required additional doses of buprenorphine. We did observe that increased levels of isoflurane were required to achieve surgical anesthesia compared with previous neurosurgical procedures. Review of the medical records for similar neurosurgical procedures using buprenorphine HCl ($n = 11$) revealed that the average concentration of isoflurane used throughout surgery was significant higher with SR-Bup ($n = 4$) (1.68% compared with 1.23%, $P < 0.0001$).

PS40 Tuberculosis Diagnostics and Antituberculosis Therapy in Suspected Chimpanzees

R Khadka[*1,2], E Schallenberger[1]

[1]Christian-Albrechts University, Kiel, Germany; [2]Tacugama Chimpanzee Sanctuary, Freetown, Sierra Leone

Mycobacterium tuberculosis, a causative agent of chronic tuberculosis disease, is widespread among humans, domesticated and wild animals and is transmitted within and between species by aerosol route. Because of zoonotic potential and ability to spread rapidly, it is of utmost importance to diagnose and prevent the spread of the disease. We will explain the various diagnostic tests and successful treatment for tuberculosis in 10 suspected chimpanzees from our institution. Skin tests were performed intrapalpebrally at the sanctuary in various occasions. Simultaneous serological tests were carried out with a novel, rapid lateral-flow assay for tuberculosis in blood serum or plasma. Gastric lavage samples were collected for Ziehl Neelsen (ZN) staining methods from 8 chimpanzees and tested in human hospital. Nine chimpanzees had their chest radiographs taken to confirm for the presence of TB lesions. All 10 chimpanzees were suspected of infection, with either mammalian old tuberculin or avian and bovine purified protein derivatives skin tests. One positive, 6 negative and 3 inconclusive results were observed in the tests. Gastric lavage samples turned out to be inconclusive in one case. Chest radiographs of 2 confirmed hilar adenopathy, one with consolidation of lungs, and 6 showed no abnormality. A multiple drug combination of isoniazid, rifampicin, ethambutol, and pyrazinamide were used for the treatment. Sanctuary staff members were tested negative with ZN staining method. Antemortem diagnosis of TB is quite challenging. Due to mixed results with different TB diagnostics and high risk of spreading of disease, the chimpanzees were isolated and treated individually with human medicine. Due to their endangered species status, chimpanzees are closely monitored while undergoing treatment for TB. After the completion of antiTB medications, all 10 chimpanzees were confirmed negative with tuberculin skin tests, rapid lateral-flow assay tests, and chest radiographs. We could not confirm TB with culture and molecular detection methods, which are generally impractical and inaccessible in field settings. We successfully treated 10 chimpanzees, which are now back with the other chimpanzees in the group, while also taking into account the costs and ethical issues.

PS41 Training Nonhuman Primates to Check Their Watering System as a Means to Increase Psychologic Wellbeing and Increase Efficiency for Husbandry Staff

KA Giordano[*]

Worldwide Comparative Medicine, Pfizer, Pearl River, NY

the *Guide for the Care and Use of Laboratory Animals* states that "the primary aim of environmental enrichment is to enhance animal wellbeing by providing animals with sensory and motor stimulation, through structures and resources that facilitate the expression of species-typical behaviors and promote psychologic wellbeing through physical exercise, manipulative activities, and cognitive challenges according to species-specific characteristics." The *Guide* also states that "watering devices, such as drinking tubes and automated water delivery systems, should be checked frequently to ensure appropriate maintenance, cleanliness and operation." The Veterinary Science and Technology team at our institution constantly strives to increase efficiencies across comparative medicine groups while also increasing animal psychologic wellbeing. Currently, our practice is to use a pole to check automatic watering systems in primate caging on a daily basis. This method can be tedious for our husbandry staff as the watering system is a very small lever on an automatic watering system device. The use of the pole has also required certain animals to be separated away from the pole during checks due to aggression and/or playfulness with the pole. This creates additional work related to water checks while also causing a potential increase in stress to the animal. Through the use of operant conditioning methods, that is, clicker training, it is possible to train nonhuman primates to check their automatic watering system within the cage creating a daily method of enrichment while also decreasing stress and increasing overall wellbeing. This method also provides increased efficiency for husbandry staff. We will detail a training study conducted at our institution in which sex, age, and housing status will be evaluated as variables in training. We will also detail time saving metrics for husbandry staff using the pole watering check method compared with trained primates.

PS42 Rabbit Hypnosis as a Form of Mild Restraint: A Forgotten Method

CS Pater[*]

CP Consulting, Edgewood, MD

Rabbit hypnosis or "trancing" was a common restraint technique used for minor procedures such as: nail clipping, tooth trimming, eye exams, and subcutaneous injections. In years past the technique was originally used on pet rabbits and then moved into the research environment. Rather than being taught in school it was passed along through on-the-job training. Among the reasons the technique was neglected was because there were anecdotal reports that fear, rather than relaxation, was the cause of the hypnosis and as the people who knew the technique left the field there were no longer people available to teach. The technique has regained popularity again due to workshops, conferences, meetings, and group communication. While there are other methods in use for minor procedures, this version allows the technician to work without anesthesia, without a restrainer, and work alone. New methods have been explored that extend the time animals are "tranced" and require less labor effort. Various pieces of equipment along with the basic technique now allow one person to do different procedures without needing another person to help hold the rabbit in place. Recovery is observed to be instantaneous without harm to the animal. This technique has been shown to work on both large and small rabbits and should be considered a viable alternative for minor procedures.

PS43 Can Regular Access to Floor Pens Affect Caged Rabbit Behavior?

J Cruden[*]

[1]Office of Animal Welfare Ethics and Stratergy, [2]Office of Animal Welfare

Ethics and Stratergy, GlaxoSmithKline, Ware, United Kingdom

The main objective of the program of research, of which this study is part, is to evaluate the housing and environmental enrichment for laboratory rabbits. The aim is to determine whether modifications to basic housing will improve the wellbeing of laboratory rabbits. It was hypothesised that regular access to an enriched floor pen may affect the behavior of individually housed caged rabbits. This paper discusses an initial study comparing the behaviors of 8 individually cage-housed 2.5 kg (12 wk old) Hsdlf:NZW female rabbits. Rabbits were randomly assigned to 2 groups for the duration of the study (8 wk). All rabbits were handled throughout the study period. The first group of rabbits was given access to floor pens 3 times per week for the first 4 wk while the second group had no access. For the second 4 wk the access to pens was switched around. Rabbits were videoed in the home-cage for 6 h per day 3 times per week over an 8-wk period. The amount of times an animal performed a particular behavior or its body was in a particular position were counted and the resulting percentage daily counts were analyzed using ANOVA. Both groups had a significant reduction in the amount of time spent grooming when pen access was given ($P < 0.0001$ and = 0.0045). Group 1 showed a significant decrease in grooming ($P = 0.0045$) and their body positions were less alert when pen access was given ($P = 0.0061$) as was group 2 who showed a significant decrease in alertness ($P \leq 0.0001$). All animal studies were ethically reviewed and carried out in accordance with Animals (Scientific Procedures) Act 1986 and the our institution's Policy on the Care, Welfare and Treatment of Animals.

PS44 Conducting a Good Laboratory Practice-Compliant Program in a University Setting

A Ogden*

Division of Laboratory Animal Medicine, University of California, Los Angeles, Los Angeles, CA

Food and Drug Administration (FDA) Good Laboratory Practice (GLP) regulations are a minuscule portion of our education as Lab Animal Professionals, but few often get the opportunity to truly experience the regulatory burden of the GLP regulations and fewer, experience a GLP-related FDA inspection. The mainstay of drug and medical device testing and traditional business model has been the use of private and expensive Contract Research Organizations (CROs). As public research funding dries up and academic institutions shift their focus to translational research, however, university-run GLP research is rapidly becoming an appealing alternative to the traditional CRO setting. This shift has forced academic professionals to quickly familiarize themselves with the high demands of GLP. Expanded and accelerated education is necessary to avoid potential regulatory exposure that could seriously damage the researcher's and institution's reputation, and incur unnecessary expenditures on repeat studies as a result of botched experiments or sloppy record keeping. Despite seemingly insurmountable challenges, we clearly recognize that the inhouse conduct of GLP studies holds great promise, potential and certain advantages, such as: 1) enhanced quality, reproducibility, and confidentiality of the data, as compared with inconsistent methodologies or outsourced locales, 2) quality and consistency of staff training, 3) cost-effectiveness of the studies billed "at cost" maximize declining research dollars, and 4) fostering of mutual trust and better relationships between researchers and testing facility staff. Developing a GLP-compliant infrastructure—with all appropriate levels of management, documentation, and training—is a monumental task. Nevertheless, even with a suboptimal system, GLP grade is attainable, as long as we continue to increase our quality and regulatory awareness to identify and address the challenges inherent to implementing and sustaining a quality system within an academic setting. Examples of common challenges and areas of GLP noncompliance will be presented and discussed at this session.

PS45 Development of a Successful Web-Based Site for Consolidating Animal Resource Operations

A Treat, MP Swan, N Daniels, D Hickman*

Laboratory Animal Resource Center, Indiana University School of Medicine, Indianapolis, IN

Our institution's Laboratory Animal Resource Center (LARC) provides supportive care to 200 investigators and their laboratory staff. Recently, LARC transitioned from a paper system that managed forms for animal ordering, animal housing and study transfers, drug distribution operations, health reporting, and community space reservations to a low cost online interactive software-based site that is accessible from a link off of our university-based website. This has allowed LARC to centralize investigative operational activities to one location that can be managed by the administrative staff. LARC can grant or deny access to the site based on protocol approval, licensing, and training, and the web-based forms have increased operational efficiency by restricting submission from incomplete and inaccurate forms. This has reduced LARC administrative support time by 20%, a significant ongoing cost savings. Additionally, research staff are able to submit forms from any computer or location at any time of day. Feedback from investigative staff has been positive and LARC continues to add more operations to this web-based program. This topic will present how we designed and implemented these changes and will be of interest to other facility managers and administrative staff.

PS46 Innovation in Training: Animal Use Training, Instruction, and Certification System

DE DeOrnellis*, K Astrofsky

Laboratory Animal Services, Novartis Institution for BioMedical Research, Cambridge, MA

Prior to working with research animals at our instituion's BioMedical Research campus in Cambridge, MA (NIBR Cambridge), all employees must be trained and certified as competent by the Laboratory Animal Services' (LAS) training staff. This training requirement, which was instituted when the Cambridge site initially opened in 2004, is based on policies of the NIBR Cambridge IACUC and is intended to meet the spirit of the Animal Welfare Act, which states that "all scientists, research technicians, animal technicians, and other personnel involved in animal care, treatment, and use are qualified to perform their duties. This responsibility shall be fulfilled in part through the provision of training and instruction to those personnel." In October 2007, a thorough review of the entire NIBR Cambridge LAS's training program and associated in vivo research needs was conducted. After the review was completed, a novel and innovative training program was conceptualized. Implementation of our instituion's Animal Use Training, Instruction, and Certification System (NAUTICS) was completed during 2008, improving the documentation associated with training, significantly expanding the scope and quality of training provided to the entire in vivo research community, and resulting in a wide range of accolades from customers and oversight bodies. NAUTICS was designed to meet the broad scope of the training requirements of the more than 400 employees in 13 research departments who work with the 6 species in 4 different vivaria, using a variety of delivery formats and modules. The implementation of NAUTICS has eliminated several issues related to the previous program. Notably, NAUTICS has streamlined the training process, allowing more individuals to be trained on a broader basis and to a higher standard in a shorter amount of time. The modular approach of NAUTICS has also created a large number of inherent efficiencies. In addition to increased customer satisfaction, NAUTICS has improved animal welfare due to the increased knowledge and skill of the trainees.

PS47 Correlation between Fur Tape Microscopy and Fur Swab PCR for Fur Mite Detection and Surveillance

BM Zamora*, L Thomas, A Hancock, B Koerper, JS Cole, A Battles, M Reinhard

University of Florida, Gainesville, FL

Simultaneous *Myobia* spp. and *Myocoptes* spp. infections in a central mouse breeding facility were successfully detected and controlled with the help of intensive surveillance testing techniques using fur tape microscopy, fur swab PCR and pelt examination. The first 2 positive cases were detected in a retired breeder and a mouse carcass in February 2011. This was followed by sampling and treatment of directly and

potentially infected colonies. Surveillance testing was done until August 2012 before the affected facilities were declared free of fur mites. During this 18-mo period, test specimens were collected from various groups consisting of retired breeders (n = 150), investigators' mouse carcasses kept at 4 °C for at least 24 h (n = 414), investigators' mice in potentially infected colonies (n = 4,368), investigators' mice prior to transfer from the breeding suite to other facilities for experimentation (n = 7,211), and dirty bedding sentinels (n = 2,100). *Myobia* spp. and *Myocoptes* spp. were identified via fur tapes based on the distinguishing morphologic features of the parasites' various developmental stages and confirmed by PCR testing in an external commercial laboratory. The positive results were traced back to 6 investigators' colonies with prevalence rates ranging from 4.35% to 47.62% during the first 3 mo after initial detection. None of the dirty bedding sentinels in the affected colonies tested positive until 3 mo after the first case was diagnosed. Pooled fur swabs for PCR (n = 195) were collected from fur tape-defined positive and negative populations, and a good kappa correlation index of $\kappa = 0.852$ was obtained. Pelts were examined and one of 199 samples yielded a positive result, which correlated with fur tape microscopy. This presentation describes the efficiency of direct microscopic examination of fur tapes in combination with fur swab PCR for diagnosing fur mite infections and for following the incidence over time as a means to evaluate the efficacy of the treatment regimen for eliminating fur mites in the facility.

PS48 Colony Life without Mouse Norovirus

KM Slinkard*, J Kalishman, S Greco

DCM, Washington University in St Louis, St Louis, MO

Mouse norovirus (MNV) provides an experimental model of human noroviruses; however, an outbreak among severely immunocompromised mice would have major economic, health, and research-related repercussions. A colony of severely immunodeficient mice has been maintained free of MNV for more than 7 y using an enhanced care protocol, restricting animal and human traffic, and instituting a more rigorous health surveillance system. The enhanced protocol relies on a meticulous care staff using specialized animal handling procedures, liberal use of a concentrated disinfectant, and preparation of preassembled autoclaved cages. Entering the suites requires special training in procedures found to effectively prevent the introduction of MNV. Incoming animals are acquired from approved vendors or rederived by research staff. Once removed from breeding rooms, animals cannot return. To augment sentinel surveillance, sentinel cages were added representing individual racks and tested for MNV with increased frequency. Advantages of these additional sentinels are earlier detection and localization of suspect positives. Serologic testing for MNV is performed inhouse, with confirmation of positive results performed by a commercial laboratory. The research lab is responsible for fecal PCR testing of rack sentinels and individual cages (if necessary). Over the past 4 y, more than 1,300 blood samples have been tested. All blood samples were MNV negative. One suspicious case occurred, when serology from one room sentinel tested positive. Feces were quickly collected from all breeder cages and rack sentinels in the room for PCR analysis; however, the suspicious result was determined to be a false positive. With increased diligence, the maintenance of an MNV-free colony within the larger barrier facility has been a huge success. Although the size of the breeding colony doubled and experimental colonies are increasing in size, the management and MNV-negative status remains unchanged. The achievements can be attributed to the enhanced care procedures, restricted access, and intensive health surveillance.

PS49 Ultraviolet Tattoos: A Refinement in Ear Tattooing

BH Hess*

Abbvie, Abbott Park, IL

Although the use of ear tattooing in rodents proved to be extremely useful, the main drawback was the lack of visibility when using dark pigmented animals. The green or black ink was not easily distinguishable when it was used on species such as C57 Black mice or Armenian hamsters. To overcome this disadvantage, UV tattoo ink which fluoresces under black light was considered. The fluorescent UV ink differs from glow-in-the dark ink since it contains no phosphorus, which is carcinogenic and should not be used in animals. Eight different colors of human UV tattoo ink were obtained and the best 4 colors, when examined under UV light, were selected for a trail study. After IACUC approvals were obtained, 4 Armenian hamsters received ear tattoos using a microtattoo system and a 25-gauge needle along with a small pocket UV flashlight used for illumination. The animals were monitored daily for 7 d and then weekly for a total of 2 mo. The visibility of the tattoos and the potential to quickly identify the animals with the pocket UV flashlight were tracked for all 4 colors of ink. Based on the initial results, neon orange provided the best visibility for the dark pigmented skin. A follow-up study using 12 animals lasted 4 mo, the animals were still easily identifiable and the tattoos remained vibrant, without any negative effects on the animals for the duration of the study. The small UV flashlight was easy to use for a single technician and the duration of UV exposure near the eyes of the animals was only momentary. By using this refined method with human use UV tattoo ink, it has eliminated the main drawback of ear tattoos. Dark pigmented animals can now be permanently identified using UV ear tattoos replacing the need for ear tags with a much quicker and more reliable method.

PS50 Evaluating the Need for Inhalant Anesthesia for Tail Tattooing in Mice

L Nedved*

Garvan Institute of Medical Research, Darlinghurst, NSW, Australia

Permanent individual identification is critical in avoiding errors in the selection of mice for breeding and to ensure accuracy in monitoring and sample collection during research. Many permanent identification methods are invasive, inconsistent in application, or difficult to read. While an automated tail tattooing system provides definite advantages in terms of consistency and readability, no studies have addressed the issue of whether the tattooing is associated with pain and stress when preformed in conscious animals. This study assessed whether anesthetic induction with isoflurane-altered behavioral and hormonal parameters that may reflect pain or stress caused by an automated tail tattooing system. Two strains of 3- to 4-wk-old mice (*Mus musculus*, strain C57BL/6J and BALB/cJ) were divided into 5 study groups: isoflurane only, isoflurane with tattooing, isoflurane with tattooing and tissue collection, conscious tattooing, and control group (restraint only). Behavior was scored during the procedure (conscious groups 4 and 5) and following the procedure (all groups). Fecal glucorticosteroid metabolite levels were measured in samples that were collected on the day prior to the procedure and the afternoon following the procedure. Behavioral assessment of the mice during conscious tattooing indicated sensory perception, but it is not clear whether the tattooing was perceived as painful. There was no evidence of pain in the 2 min after conscious tattooing and any pain felt during conscious tattooing was likely to be either very mild or absent. There is evidence, however, that tattooing was stressful, both from the behavior of the mice and the fecal glucocorticosteroid metabolite levels. This stress does not seem to be effectively alleviated by isoflurane anesthesia, as both anaesthetized and conscious mice had significantly elevated fecal glucocorticosteroid metabolite levels. This study does not suggest that the use of isoflurane anesthesia provided any significant animal welfare benefits during the tail tattooing of mice 3 to 4 wk of age.

PS51 Readiness Rounds: Ensuring Inspection Readiness and Flawless Animal Care while Engaging Our Animal Care Staff

M Brown*[1], L Cowlishaw[2], T Levkoff[1], M Rodolfo[3]

[1]Comparative Medicine, Pfizer, San Diego, CA; [2]Comparative Medicine, Vision IT, San Diego, CA; [3]Comparative Medicine, Charles River at Pfizer La Jolla, San Diego, CA

Our institution is committed to the ethical treatment and high-quality care of all animals used in research. To fulfill this commitment, La Jolla Comparative Medicine (CM) complies with all laws, regulations and accrediting standards related to the use of animals in research. In addition, we constantly strive to provide flawless animal care, as well as maintain compliance with external, internal, regulatory and AAALAC standards.

In order to facilitate this compliance and maintain a flawless animal care and use program, an "inspection readiness" team made up of CM colleagues in a variety of roles conducts weekly "readiness rounds" of the vivariums. The Readiness team inspects all areas in the vivariums each month on a rotating basis, ensuring that all animal holding and procedure rooms, storage space, cage wash areas, and halls are inspected monthly. The standards used for the assessment include the Animal Welfare Act and Regulations, the *Guide for the Care and Use of Laboratory Animals* (eighth edition), AVMA Guidelines for the Euthanasia of Animals, Corporate Policy no. 901, CM Standard Operating Procedures, La Jolla IACUC Guidelines, Global IACUC Guidelines, and our EH&S Laboratory Safety Inspection Checklist. After each inspection, a "readiness rounds" form is completed, and any findings are assigned to the responsible individuals to be completed by a reasonable due date based on task. Since initiating Readiness Rounds in January 2012, the overall number of weekly inspection findings has steadily decreased and there has been a concomitant reduction in findings on both the IACUC semiannual inspections and quarterly EH&S inspections. Readiness Rounds has also provided an opportunity for our animal care technicians to learn more about the assessment/inspection process with the added benefit of increased colleague engagement and an "own it" culture regarding inspection-readiness. "Readiness rounds" is an effective and efficient process to proactively self-inspect our facilities with limited resources and no additional staff. Through this novel approach, we are meeting CM strategic imperatives to execute flawless animal care and welfare, engage colleagues, and create efficiencies and flexibility.

PS52 Creating and Managing a Performance Assessment System for Animal Care Technicians

K Murray, T Butler*, L King, K Young, JD Thulin

Biomedical Resource Center, Medical College of Wisconsin, Wauwatosa, WI

Managing animal care technicians that rotate into different areas with different teams created inconsistencies for the multiple supervisors of our animal facility, especially when addressing an employee's ongoing work quality. Additionally, employees requested more standardization for the evaluation of errors and clarifications regarding the quality expectations for their specific positions. To resolve these issues and concerns, a formalized and consistent method to document and track errors was developed and error thresholds were created for each technician position level. Every error is investigated by a supervisor, designated as either an animal or procedural error, and then categorized as a minor, major, or critical error based on the impact the error has to our animal program. All errors are documented by the supervisors on a shared spreadsheet, creating a convenient and successful system to monitor an employee's performance. Supervisors meet regularly with their staff to evaluate any documented errors and discuss the progress towards each employee's quality goals established during annual performance reviews. Employees now have a standardized system for performance evaluation to maintain consistency in quality assessment and performance expectations, regardless of the team or area of the animal facility into which they have rotated.

PS53 Implementing a Research and Development Complete Cost Savings Initiative Program

DL Goldsteen*

Translational Sciences, MedImmune, Gaithersburg, MD

Faced with a reduction in overall lab supply budget funding, a cross-departmental team was formed to develop ideas and implement cost savings. In 2012 our Research and Development (R&D) Department set a goal to reduce laboratory spending by 5% over the 2011 baseline. Early forecasts predicted we would not meet that goal. To create the necessary focus, a group of representatives across research, development, laboratory operations, laboratory animal resources, finance, and strategic sourcing formed the Cost Savings Initiative Team, (CSI). Using a variety of Operational Excellence approaches the team developed and implemented plans to save money on lab supplies. Because the potential impact that reducing spending in animal resources can have on the overall budget, our Laboratory Animal Resources program became heavily involved in the process. The CSI Team determined primary sources of waste within their functional areas and developed strategies to decrease or eliminate waste or excess spending. The team members presented data to their individual program areas informing them of budget restrictions, providing them with our suggestions for savings, and gaining feedback for other areas of improvement. A variety of methods were used to advertise and explain the program within R&D and to implement processes to achieve our goals. Many significant changes were made in our spending and work habits, which resulted in the R&D department coming in 12% under budget for lab supplies and the LAR Department coming in 14% under budget for 2012. These savings will continue to accrue in 2013 and beyond. This team approach was instrumental in identifying the problem areas and working together across departments to successfully achieve our goal.

PS54 Implementation of a Novel Organizational Leadership and Management Training Program for Senior-Level Laboratory Animal Technicians

DE DeOrnellis*, K Astrofsky

Laboratory Animal Services, Novartis Institution for BioMedical Research, Cambridge, MA

The skills associated with leadership and management are vital to the success of any organization. We will describe the Leadership and Management Training program implemented in the Laboratory Animal Services (LAS) department at our institution's BioMedical Research campus in Cambridge, MA (NIBR Cambridge), designed to provide a broad set of leadership and management skills for nonmanagement staff (coordinators, team leaders, etc.). In 2008, the LAS department at NIBR Cambridge was reorganized. As part of the new organizational structure implemented at that time, the ratio of management to associate staff was dramatically reduced from 0.333 to 0.125. During the following 4 y, LAS more than doubled the number of employees, from 33 to 72. The reduction in management staff within LAS, a large increase in the number of departmental staff, and a rapid expansion in the scope of programmatic responsibilities, created a leadership gap within LAS. To address this issue, LAS management partnered with an external training company to design a novel training program. The LAS Leadership and Management Training program is unique since it uses concepts and a delivery paradigm historically reserved for senior level managers. Once the external training company was identified, LAS management worked with their instructional designers and trainers to create a curriculum. The curriculum was created to address the following leadership and management skills: communication, motivation, delegation, continuous learning, training and mentoring, self-awareness, and ownership. Key individuals within LAS were identified to participate in the training, based on their responsibilities within the department. A schedule was created for both training and coaching sessions. We will provide additional background for this training program, describe the curriculum in detail, explain delivery logistics, and discuss the challenges and successes encountered. It will be instructive for attendees who are trainers and personnel managers.

PS55 Meaning and Pedagogical Impact of Class Notes Translated into Mandarin Chinese for Scholars from China Enrolled in Research Techniques Training

T Whitcomb*[1], J Hu[2]

[1]Comparative Medicine, [2]Pathology, Penn State College of Medicine, Hershey, PA

A hands-on training program for common laboratory animal research techniques was established at our institution's College of Medicine in August of 2010. Since its inception, 21% of the participants enrolled have been Chinese researchers who have recently arrived in the United States. In an effort to support our new colleagues, who were struggling with communication during class, a collaboration was formed to translate the class notes into Mandarin Chinese. To evaluate the impact of this endeavor, a qualitative study was designed with the goals of identifying the pedagogical value of the translated notes, determining what

personal meaning this gesture had for the recipients of the notes and soliciting suggestions about what interventions might be employed to improve the hands-on training experience for this group of scholars. This IRB-approved study consists of semistructured, in-depth interviews conducted with participants from our program who used the translated documents. Interviews are transcribed and data analysis is performed using open coding, followed by constant comparative method. Preliminary findings indicate that although the translated notes were useful to all students, they may have been associated with an unintended negative message for some. Suggestions for additional interventions include materials that support visual learning (like videos), facilitation of inclusion in community activities, and a mentoring program. Study results are being used to guide continued development and improvement of our hands-on training program.

PS56 FESSACAL: Bridging the Gap between South American Laboratory Animal Science Associations

A Salvarrey-Strati[*1], A Guaraldo[2], A Romera[3]

[1]University of Uruguay, Boston, MA; [2]Animal Biology Department., State University of Campinas, Sao Paulo, Brazil; [3]Lab.herpes Virus, Instituto Virologia-CiCVyA-INTA, Castelar, Argentina

The Federation of South American Associations of Laboratory Animal Sciences (FESSACAL) was created in 1999 at the International Council of Laboratory Animal Science (ICLAS) annual meeting. The South American Continent is made up of 10 countries: Argentina, Bolivia, Brazil, Chile, Colombia, Ecuador, Paraguay, Peru, Venezuela, and Uruguay. The economic and political climates in these countries were not well positioned to support laboratory animal science community. As the research community has grown, local associations have been created and some of these countries were able to develop animal welfare laws—Brazil was the first to have such laws in 2008, and then Uruguay introduced them in 2009. In this context, a group of professionals started working to organize FESSACAL.The first International FESSACAL Meeting took place last December in Sao Paulo, Brazil. In a third event last April in Uruguay, elections were held and the new regulations were approved. The organization has a promising future, but we must continue our efforts to coordinate resources and build a large and diverse internal and external program in order to develop continental and international relationships. Education is an area that requires gradual progress since in most of these countries the veterinary programs do not have postdoctoral training in laboratory animal medicine. Finally, we do not have a similar level of laboratory animal technician certification such as ALAT, LAT, and LATG. Nevertheless, an increasing number of animal facilities obtained the AAALAC accreditation. We have strong associations in 6 countries (Argentina, Bolivia, Brazil, Chile, Venezuela, and Uruguay) and Colombia has a strong IACUC network (red Colombiana de Cicuales) and is firmly working toward the organization of a national LAS association. FESSACAL is working to foster Paraguay's effort to create a similar association. In this way we look forward to work in order to achieve FESSACAL objectives.

PS57 Operant Conditioning with Laboratory Beagles

GM Savastano[*]

Merck Research Labs, Boston, MA

There is increased regulatory emphasis on positive reinforcement training for husbandry, research procedures, and restraint devices. We practice acclimation, association, and positive reinforcement training with our beagle colony with goals to reduce stress for dogs and improve efficiency for personnel. Desensitization and acclimation practices are used to prepare dogs for participation in PK studies. Dogs are desensitized to sounds and vibrations of the electric shaver to prepare skin for catheterization, and acclimated to the sling for up to 1 h. Association training was used to decrease anticipatory barking. Staff enter the room many times throughout the day, and we noticed that barking became louder around lunchtime, with the dogs not knowing which entry was for feeding. The caretaker now rings a dinner bell when they enter with feed, signaling lunchtime. This has reduced the amount of barking and signals the dogs housed in kennels that do not face the door that lunch has arrived. Positive reinforcement techniques were used to train dogs to return to their kennel on command. This behavior has an ergonomic benefit to the staff, who no longer have to lift dogs, and saves over 50 h of labor per dog room per year. Positive reinforcement training techniques were also used to train the dogs to jump onto a platform and present a paw for voluntary blood collection. Blood collection usually requires a second operator to restrain the dog, or requires putting the dog in a sling. With voluntary paw presentation a single operator can rapidly collect the blood sample. Behavior modification via clicker training is an effective and positive way to not only enrich the lives of the animals but also to ease the lives of the animal care, research, and veterinary staff.

PS58 An Aquarium as a Passive Enrichment Item and Its Effect on Locomotor Stereotypy in a Group of Singly Housed Rhesus Macaques (*Macaca mulatta*)

TM Meade[*], C Krall, EK Hutchinson, J Watson

Molecular and Comparative Pathobiology, Johns Hopkins University, Baltimore, MD

Locomotor stereotypies are behaviors often seen in singly housed rhesus macaques (*Macaca mulatta*) and are considered to represent a maladaptive response to captive environments. Social housing is the most effective means for decreasing abnormal behaviors but is sometimes unrealistic as a result of individual incompatibility or research needs. Active and passive enrichment items are therefore commonly used as a means to mitigate these behaviors. Active enrichment items allow physical manipulation and are frequently successful in reducing stereotypies. However, their beneficial effects are usually confined to relatively short periods of active use. Passive enrichment items are less well studied and the results are mixed. This study evaluated an aquarium with live fish as a passive enrichment item. We hypothesized that the frequency of locomotor stereotypy would decrease after its introduction to a group of singly housed rhesus macaques with a known history of stereotypy and self-injurious behavior. The study followed an ABBA design and each block was 2 wk in duration, consistent with the concurrent enrichment and cage wash rotation schedule. Behavior was scored at 15-s intervals for 2 h daily. The average frequency of locomotor stereotypy was calculated per individual for each time block. Statistical comparisons were made between blocks using ANOVA and post hoc tests. Unexpectedly, locomotor stereotypy significantly increased with introduction of the aquarium. Stereotypy frequency decreased over time and no significant difference was noted during the second 2 wk compared with baseline. However, once the aquarium was removed, frequency of stereotypy decreased to below baseline levels. We suspect that the increased rate of abnormal behavior is a manifestation of neophobia resulting from the stress of a novel environmental object. Therefore, in the context of reducing abnormal behavior, enrichment items may not always be beneficial and should be critically evaluated upon their introduction to a new group of animals.

PS59 Investigation of Commonalities among Laboratory Animal Facilities Associated with the Group A Rotavirus Outbreaks in Spring 2013

VS Dole[1], K Boyd[2], K Jackson[2], J Wallace[2], J Asher[3], J Cohen[4], V Gillespie[4], KS Henderson[*1]

[1]Research Animal Diagnostic Services, Charles River, Wilmington, MA; [2]Vanderbilt University School of Medicine, Nashville, TN; [3]Temple University, Philadelphia, PA; [4]Icahn School of Medicine at Mt Sinai, New York, NY

Group A rotavirus (GARV/EDIM/murine rotavirus) remains one of the prevalent viruses in laboratory mice. We reviewed the data from January 2012 till May 2013 for rotavirus positive mouse facilities in the US identified by our Research Animal Diagnostic Services. Mice from 13 independent facilities tested positive for GARV by serology and/or real-time RT PCR assay with the majority appearing in spring 2013. A region of the rotavirus NSP3 gene (284 bp) was sequenced from 5 of these samples and found to be genetically identical. This appears to be a novel mouse rotavirus strain that is only 92% to 94% identical to published murine rotavirus sequences and previously sequenced EDIM isolates

in our laboratory. Local incursion of wild rodents seems unlikely to be the source since these facilities are in geographically distant regions (New York, Tennessee, Pennsylvania, an undisclosed midwest location and an undisclosed mideast coast location), instead we hypothesize a possible common source for the 2013 strain described in this study. We compared the sources of feed, water, bedding, sentinels, study animals, and sterilization/decontamination treatments in 4 of these 5 facilities that participated in the survey. All used corncob bedding and rodent chow diet from the same respective manufacturers; supplemental feed was used by some facilities. The bedding was not irradiated by the manufacturer and no further treatments were performed at the 4 facilities, while irradiated feed was procured by one facility. Other parameters surveyed were not consistent between these facilities. In comparison, 2 of these facilities also housed mice at their locations in barrier rooms supplied with sterilized feed and bedding that remained free of rotavirus. The survey suggests further investigation of procedures for decontamination of husbandry supplies to mitigate introduction of environmentally stable rodent pathogens.

PS60 Development and Implementation of a Training Program for Cardiopulmonary Resuscitation in Nonhuman Primates

RM Kramer*[1,2], S Rasmussen[3], R Tolwani[3]

[1]Tri-Institutional Training Program in Laboratory Animal Medicine and Science, New York, NY; [2]Memorial Sloan-Kettering Cancer Center, Weill Cornell Medical College, and The Rockefeller University, New York, NY; [3]Comparative Bioscience Center, The Rockefeller University, New York, NY

Clinical emergencies can happen anytime. Laboratory animal veterinarians, technicians, and investigators should be appropriately trained and prepared to effectively respond and make decisions in the event of respiratory or cardiopulmonary arrest in nonhuman primates. Effective cardiopulmonary resuscitation (CPR) requires a team approach of at least 3 knowledgeable individuals; therefore, training of staff and investigators to assist veterinarians is necessary to ensure the best clinical outcome. As an added component to our program of animal care, institutional guidelines and a didactic curriculum for CPR were developed using clinical veterinary guidelines adapted for nonhuman primates. Both veterinary technicians and nonhuman primate investigators attended an initial didactic CPR training, including a discussion of postarrest care and prognosis. Investigators with limited to no experience with clinical emergencies developed realistic expectations about postarrest survival rates and humane endpoints. The initial training was followed by a hands-on, mock emergency, where trainees practiced the techniques described in the didactic session. Cognitive aids, such as simple signage, were created, distributed to trainees, and posted in the surgery suite for quick reference. Because CPR is a rare event in a research setting, a hands-on CPR wet lab has been incorporated into our annual nonhuman primate refresher training curriculum. To ensure that veterinary and research staff are prepared to respond in the event of a clinical emergency, formal CPR training should be considered by any facility that may perform CPR.

PS61 Light Spectral Transmittance through Red Laboratory Animal Cages Impacts Circadian Metabolism and Physiology in Nude Rats

RT Dauchy*[1], M Wren[2], EM Dauchy[2], JP Hanifin[3], MR Jablonski[3], B Warfield[3], GC Brainard[3], L Mao[1], SM Hill[1], LM Dupepe[2], TG Ooms[2], DE Blask[1]

[1]Department of Structural and Cellular Biology, [2]Department of Comparative Medicine, Tulane University School of Medicine, New Orleans, LA; [3]Department of Neurology, Thomas Jefferson University, Philadelphia, PA

Light, a potent biologic force essential to life on our planet, is responsible for entraining normal circadian rhythms of physiology and metabolism in all mammals, including laboratory animals. Previous studies from our laboratory demonstrated that spectral transmittance (color) of light passing through standard laboratory rodent caging impacts these responses in rats. With the publication of the new edition of the *Guide*, concern arose regarding the use of red tint, a color commonly used on observation windows and in nighttime safety lighting. Here, in conjunction with our GLAS-supported studies, we examined how enhanced cage transmission of light in the red-appearing portion of the visible spectrum (610 to 670 nm) can affect laboratory rodents' daily rhythmic nocturnal melatonin signal, thereby altering temporal coordination of normal metabolic and physiologic functions. Female nude rats (Hsd:RH-*Foxn1*^{rnu}; $n = 6$/group) were maintained on a lighting regimen 12:12-h light:dark cycle (300 lx; 123.0 μW/cm^2; lights on 0600) in either standard polycarbonate translucent clear (A, controls) or red tinted (B) rodent cages in an AAALAC-accredited facility. After 1 wk, animals were subjected to 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, insulin, leptin, and corticosterone concentrations. Results showed no differences in dietary and water intake, or body growth rates between the groups. Plasma melatonin levels in pg/mL (mean ± 1 SD) were low in the light phase (1.0 ± 0.2) in both groups, but significantly higher during dark phase in A (154.8 ± 3.8), compared with B (105.0 ± 2.4; $P < 0.001$). Arterial plasma diurnal rhythms of TFA, glucose, lactic acid, leptin, insulin, and corticosterone levels were significantly disrupted in B, compared with A ($P < 0.05$). Together with our previous results, the present findings indicate that a wide variety of spectral transmittances through differently colored cages have a profound impact on the circadian regulation of neuroendocrine, metabolic, and physiologic parameters that influence laboratory animal health and wellbeing, and ultimately the outcome of scientific investigations.

PS62 Animal Facility Light Exposure at Night-Induced Disruption of Circadian Dynamics in Fatty Acid Metabolism, Aerobic Glycolysis (Warburg Effect), and Clock Gene Expression in Human Breast Cancer Xenografts in Nude Rats

RT Dauchy*[1], EM Dauchy[1], MA Wren[2], L Mao[1], SD Zeringue[1], S Xiang[1], PC Tirrell[3], SM Hill[1], VP Belancio[1], DE Blask[1]

[1]Department of Structural and Cellular Biology, [2]Department of Comparative Medicine, Tulane University School of Medicine, New Orleans, LA; [3]Department of Medicine, Mary Imogene Bassett Hospital, Cooperstown, NY

Lighting and lighting protocols in animal facilities, as outlined in the *Guide*, influence laboratory animal health and wellbeing and the outcome of scientific investigations. Recent evidence indicates that rotating night shift workers have an increased risk of developing breast cancer that is directly associated with light exposure at night (LEN). Previously, we demonstrated that animal facility dark phase light contamination 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 disrupts temporal coordination of mammalian central and peripheral clock gene mechanisms regulating metabolism. Female nude rats bearing tissue-isolated MCF-7 steroid receptor-negative human breast cancer xenografts were maintained on either a control (C) 12:12-h light:dark cycle (300 lx; 123 μW/cm^2) or experimental (E) 12:12-h light:dark cycle (0.2 lx; 0.08 μW/cm^2; lights on 0600). Tumor tissue clock (BMAL1, CLOCK, PER1, CRY1) and clock-associated (WEE1 and cMYC) gene mRNA and protein expression, arterial plasma melatonin, total fatty acid (TFA), and corticosterone concentrations, and tumor aerobic glycolysis (Warburg Effect) measurements were made every 4 h over a 24-h period ($n = 6$ per time point). Plasma melatonin levels in C were high in the dark phase (108.8 ± 6.5 pg/mL), low (1.0 ± 0.2 pg/mL) in the light phase and low throughout the 24-h period in E. Normal circadian expression of tumor clock gene mRNAs in C was disrupted in E. Diurnal plasma TFA were similar for C and E (high, night; low, day; E > C), but corticosterone levels in C (high, night; low, day) were disrupted in E ($P < 0.05$). Tumor cAMP levels, TFA-uptake, and DNA [3H]thymidine incorporation, and aerobic glycolysis were elevated significantly ($P < 0.001$) by over 25%, 700%, 900%, and 200% respectively, in the light phase over the dark phase in C, and high throughout the 24-h period in E. These findings show in vivo that the integrated circadian rhythms of clock gene expression and metabolism underlying human breast cancer growth can be disrupted by LEN, as sometimes occurs in laboratory animal facilities.

PS63 Spectral Transmittance of Light through Laboratory Animal Cages Alters Circadian Rhythms of Metabolism and Physiology in Male Sprague–Dawley Rats

MA Wren*[1,4], RT Dauchy[2], JP Hanifin[3], MR Jablonski[3], B Warfield[3], GC Brainard[3], DE Blask[2], SM Hill[2], TG Ooms[1], RP Bohm[4]

[1]Department of Comparative Medicine, [2]Structural and Cellular Biology, Tulane University School of Medicine, New Orleans, LA; [3]Department of Neurology, Thomas Jefferson University, Philadelphia, PA; [4]Division of Veterinary Medicine, Tulane National Primate Research Center, Covington, LA

Light synchronizes circadian rhythms via a recently described nonvisual system. In this system, intrinsically photosensitive retinal ganglion cells containing the photopigment melanopsin send signals to the suprachiasmatic nucleus (SCN) via the retinohypothalmic tract. The SCN is the master biologic clock that serves as a pacemaker to regulate circadian rhythms. Previous studies conducted with tinted laboratory cages demonstrated significant disruptions of metabolic and endocrine parameters in pigmented female nude rats. We explored the hypothesis that spectral transmittance (tint) of light through laboratory caging affects the SCN, and therefore circadian rhythms of various endocrine and metabolic plasma constituents in nonpigmented male Sprague–Dawley rats. Rats (Crl:SD; $n = 12$ per group) were housed in a 12:12-h light:dark cycle environment (300 lx; 123.0 µW/cm^2; lights on 0600) in either clear, amber-, blue-, or red-tinted rodent cages; lighting intensity and duration was constant for all groups. Blood was collected at 6 circadian time points (0400, 0800, 1200, 1600, 2000, and 2400) and measured for melatonin, total fatty acids, pH, glucose, lactic acid, corticosterone, insulin, and leptin. Plasma melatonin levels in pg/mL (mean ± SEM) were low in the light phase (10.99 ± 0.69 pg/mL), but significantly higher during the dark phase (198.30 ± 10.18 pg/mL) in all groups as expected; however, animals in amber- and red-tinted cages had 29% and 47%, respectively, greater total daily melatonin levels when compared with the clear cage group due to an increased duration of the nocturnal melatonin signal. No significant differences were found either in dietary and water intake, body growth rates, total fatty acids, pH, or glucose among groups. Significant ($P < 0.05$) disruptions in circadian rhythms were seen in multiple groups for melatonin, lactic acid, corticosterone, insulin, and leptin, such as phase timing, peak amplitude, or peak duration when compared with the clear cage group. These results demonstrate that the use of tinted animal caging altered normal circadian rhythms of plasma measures of metabolism and physiology in laboratory rats. These findings indicate that cage tint could influence the outcomes of scientific investigations.

PS64 Melatonin Inhibition of Aerobic Glycolysis (Warburg Effect) and Fatty Acid Metabolic Signaling in Human HeLa Cervical Cancer

RT Dauchy*[1], EM Dauchy[1], MA Wren[2], L Mao[1], SD Zeringue[1], SM Hill[1], VP Belancio[1], DE Blask[1]

[1]Department of Structural and Cellular Biology, [2]Department of Comparative Medicine, Tulane University School of Medicine, New Orleans, LA

Over 640,000 people in the US alone this year will be diagnosed with either breast, prostate, colorectal, or cervical cancers, which represents nearly 50% of all reported malignancies. Epidemiologic studies have revealed that the risk of these cancers is increased in persons exposed to ocular light at night, as experienced by night-shift workers, a finding associated with suppression of the nighttime production of the circadian neurohormone melatonin. In previous studies, we determined that melatonin, produced by the pineal gland primarily at night, inhibits human tumor growth via suppression of aerobic glycolysis (Warburg effect) and an MT1 melatonin receptor-mediated suppression of tumor cAMP leading to an inhibition of tumor linoleic acid (LA) uptake and its metabolism to the mitogenic signaling molecule 13-hydroxyoctadecadienoic acid (13-HODE), culminating in downregulation of the epidermal growth factor and insulin-like growth factor-1 pathways. Here we explore the effects in vivo of melatonin (500 pM) on perfused, tissue isolated HeLa cervical adenocarcinomas in nude rats. HeLa cervical adenocarcinoma latency-to-onset and growth rates, respectively, 8 d, and and 0.09 ± 0.01 g/d; mean tumor weights were 5.6 ± 0.2 g ($n = 20$). Tissue-isolated human HeLa cervical ($n = 20$) adenocarcinoma xenografts in nude rats perfused in situ for 60 min with donor blood augmented with physiologic nocturnal levels of melatonin (500 pM) resulted in substantial reductions of nearly 30% in tumor aerobic glycolysis (Warburg Effect), a complete inhibition of LA uptake, 13-HODE release, ERK 1/2, MEK, Akt, and GSK3β activities, and over a 90% reduction in tumor cAMP levels and [3H]thymidine incorporation into tumor DNA and DNA content. Addition of the non-selective MT1/MT2 melatonin antagonist S20928, forskolin, 8-Bromo-cyclic-AMP, or pertussis toxin completely reversed the tumor growth inhibitory response. These results are the first to demonstrate in HeLa cervical adenocarcinomas that nocturnal melatonin levels suppress tumor growth via a melatonin receptor-mediated signal transduction mechanism. An understanding of this signaling pathway for the control of LA uptake in cancer could lead to new approaches for therapeutic intervention and/or chemoprevention.

PS65 Comparative Analysis of Peripheral Angiographic Vascular Analysis in Swine Breeds: Impact of Animal Model Selection in Study Design for Peripheral Device Evaluation

A Dardenne*, A Peppas, C Hyon, C Gongora, P Mount, LV Anglin, A Carter, M Morales, A Tellez, G Kaluza, J Grenada

Skirball Center for Cardiovascular Research at the Cardiovascular Research Foundation, Orangeburg, NY

Due to similarities between swine and human peripheral vascular anatomic and response, the swine is recognized as the preferred animal model for interventional cardiology research, intravascular imaging, and device evaluation. Despite these anatomic similarities there are fundamental differences that need to be recognized to understand its limitations as an animal model. Different swine breeds provide different advantages such as size, maturity, and metabolic state. For this reason the peripheral vascular dimensions are critical to determine, age, size and weight of the animals during study design. This study we aimed to compare the vascular dimensions of the peripheral vasculature in the regular domestic swine (Yorkshire), the Yucatan miniswine and the familial hypercholesterolemic swine (FHS). A total of 224 peripheral vessels in 112 animals were included in this study. Animals consisted of 3 different breeds: Yorkshire (21 animals), FHS (53 animals), and Yucatan (38 animals). Software analysis was used to measure the peripheral vasculature of all animals at baseline of their respective studies. Each main peripheral artery (iliac artery, superficial femoral artery, and profunda femoral artery) was divided into 3 subsegments (proximal, distal, and total). Each measurement consisted of a proximal and distal vascular diameter as well as length. Animals were categorized by breed and subsequently categorized by age in months from 3 to 13 mo. Yorkshire animals aged between 3 to 5 mo in comparison to the use of older FHS and Yucatan animals (age range from 5 to 13 mo). As expected, the Yorkshire breed demonstrated a higher vascular diameter in all analyzed vessels as compare to Yucatan and FHS. Therefore, no Yorkshire animals are able to be evaluated in the catheterization laboratory after 5 mo of age. Yucatan swine demonstrated slightly larger vascular diameter and length compared with FHS and this was consistent in all 3 analyzed peripheral vessels and this relationship was maintained up to 1 y of age. Knowledge of swine peripheral vasculature will guide preclinical scientists and veterinary researchers to best determine swine breed and age selection for peripheral device evaluation based on accurate knowledge of arterial size.

PS66 Determination of the Mouse Resonance Frequency Range and the Cardiovascular Effects of Vibration at These Frequencies

Y Li*[1], RN Karyne[2], RP Reynolds[1], J Norton[1], D Schmitt[2]

[1]Division of Laboratory Animal Resources, Duke University Medical Center, Durham, NC; [2]Department of Evolutionary Anthropology, Duke University, Durham, NC

Vibration has been shown to produce stress and cardiovascular changes in laboratory animals. Although vibration is thought to be detrimental in mice, no information is currently available concerning the amplitude or frequency ranges that could potentially have harmful effects

on the animals. The resonance frequency range (RFR) is the range of frequencies whereby an object most readily vibrates and the range over which an animal may sense and respond to vibration. In this study, we hypothesized that short-term exposure to vibration only within the RFR would result in reversible changes to cardiovascular function. We initially determined the whole body RFR of mice, and subsequently studied the effects of vibration at frequencies both within and outside of the RFR on heart rate (HR) and mean arterial pressure (MAP). Mice with a body weight between 25 to 30 g were placed on a vibration table with a small accelerometer mounted on their back and another accelerometer attached to the table. A range of 0 to 1000 Hz vibrations at 20 db amplitude were applied. Over most of the vibration range the mice damped all vibrations, but in one specific range the accelerometer readings from the mouse were higher than that of the table. We then evaluated the cardiovascular response to vibration using radiotelemetry where mice implanted with a transmitter were exposed to vibrations of various frequencies. The HR and MAP were significantly higher than the baseline parameters within the defined RFR range, whereas no significant change in MAP or HR occurred in frequencies below or above the defined RFR. Mice appear to most physiologically sensitive to vibration in the defined RFR. These data are important to establish the harmful ranges of amplitude and frequency of environmental vibration that should be minimized or altogether avoided in the mouse facility and will be presented.

PS67 Impact of Rederivation and Associated Microbiome Changes in a Model of Inflammatory Bowel Disease

ML Hart*[2,1], J Cornelius-Green[1], A Goerndt[1], AC Ericsson[1,3], CL Franklin[2,3]

[1]Veterinary Pathobiology, [2]Comparative Medicine Program, [3]Mutant Mouse Regional Resource Centers, University of Missouri, Columbia, MO

Rederivation of mice is a common practice used to render animals free of unwanted infectious diseases. This also occurs when mutant mouse strains are maintained as cryopreserved germplasm and subsequently recovered for use in research. While rederivation eliminates unwanted pathogens, it may also result in changes in microbiota that impact model phenotypes. To this end, we investigated whether rederivation of a common mouse model of inflammatory bowel disease (IBD) onto different recipient strains of mice would result in changes in microbiota that correlated with changes in disease severity. In addition, we assessed whether the segmented filamentous bacterium (SFB) status of recipients impacts disease severity. C57BL/6 and C3H/HeJBir mice with targeted mutations in the IL10 gene were rederived onto SFB-negative CD1, SFB-negative C57BL/6 (B6) or SFB-positive B6 recipient mothers. To induce inflammatory bowel disease, pups were inoculated with *Helicobacter hepaticus* at 3 and 5 d after weaning. Cecal lesion scores and changes in microbiota were evaluated at 90 d postinoculation using histopathology and Autosomal Ribosomal Intergenic Spacer Analysis (ARISA), respectively. Differences in the microbiota were seen between mice derived onto SFB-negative CD1 or B6 recipients; however, no differences in IBD lesion scores were seen. Differences in the microbiota were also seen between mice derived onto SFB-negative and SFB-positive B6 mice. Moreover mean cecal lesion scores were significantly elevated ($P < 0.05$) in SFB-positive mice. These findings suggest that intestinal microbiota, including SFB that is obtained during rederivation, can alter mouse model phenotypes.

PS68 Conditioned Aversion to Carbon Dioxide, Isoflurane, and Argon in Laboratory Rats: Implications for the Welfare of Animals Undergoing Euthanasia

H Golledge*

Centre for Behavior and Evolution, Newcastle University, Newcastle upon Tyne, United Kingdom

There is ongoing debate about whether inhaled agents used for rodent euthanasia are humane. CO_2 is suggested to cause distress because rodents show aversion to it. Premedication with the anesthetic isoflurane and Ar-induced asphyxia are suggested by some authors to be more humane alternatives, yet it is unclear if they cause less aversion. The aversiveness of these agents was compared using conditioned place aversion (CPA) which measures an agent's ability to cause a Pavlovian association between the experience of exposure to the agent and the place where it was administered. CPA is measured in the absence of the aversive agent (the unconditioned stimulus, UCS), and therefore, likely represents the memory of affective responses to the UCS. The ability of Ar, isoflurane, and CO_2 to induce CPA in Lister-hooded rats (*Rattus norvegicus*) was measured. Animals ($n = 10$ per agent) were exposed for 90 s to isoflurane (5% in a flow of 20% of the chamber vol/min O_2), Ar (> 95% static concentration) or CO_2 (20% of chamber vol/min) in one chamber and to an equivalent air-flow (control) in a second chamber, which was made distinctive with visual and tactile cues. After 6 conditioning exposures each to test agent and control (one exposure per day), animals were allowed to move between the chambers and their chamber preference measured. All agents caused significant increases in time spent in the control chamber following conditioning ($P < 0.01$); Ar caused the strongest aversion (20.6 ± 3.1% (mean ± SE) increase in time spent on the control side compared with preconditioning), CO_2 caused a 13.1 ± 2.1% increase and isoflurane a 10.4 ± 2.2% increase. The relative aversiveness of the most and least aversive agents was compared directly in a second experiment. One chamber was paired with Ar and the other with CO_2 for each rat ($n = 10$). In this case there was a significant ($P < 0.01$) shift in preference towards the CO_2-paired chamber (17.4 ± 3.4%), confirming that Ar caused stronger aversion than CO_2. These data suggest that none of the agents are entirely humane as all cause CPA, and therefore, presumably induce a negative affective state. However, Ar appears to be significantly more aversive than CO_2, and should not be considered a more humane alternative for rat euthanasia.

PS69 Defining Alterations of Tryptophan Metabolism in the Self-Injurious Behavior Macaque to Determine the Cause of Serotonin Imbalance

RL Cohen*, EK Hutchinson, KA Metcalf Pate, D Graham

Molecular and Comparative Pathobiology, Johns Hopkins, Baltimore, MD

In people with major depression disorder, posttraumatic stress disorder, and other psychopathologies, there is a lower concentration of central serotonin. It has been proposed that the major etiological factor of depression is a disturbance in tryptophan metabolism away from the 5-HT pathway, which leads to serotonin, shunting towards the kynurenine pathway. Supporting this hypothesis is previously published work indicating that macaques supplemented with tryptophan have a decreased incidence of self-injurious behavior (SIB). We hypothesized that in macaques with SIB, central tryptophan metabolism would be shifted toward kynurenine production, leading to lower central 5-HT. Further, given that 5-HT does not appreciably cross the blood brain barrier and kynurenine does, we hypothesized that this shift in metabolism centrally would result in the reverse shift peripherally, leading to higher peripheral 5-HT. We analyzed paired cerebral spinal fluid and blood samples from SIB macaques via ELISA and mass spectrometry for tryptophan metabolites to determine whether and where tryptophan metabolism is altered in these animals compared with normal controls. We will present our findings demonstrating that macaques with SIB have lower central and higher peripheral concentrations of serotonin compared with normal macaques, and that these shifts are correlated with the severity of abnormal behavior in these animals. We will also discuss how this disparity in central and peripheral serotonin level relates to the tryptophan metabolite profile. Determining the alterations in the tryptophan metabolic pathway may lead to the identification of targets within the metabolic pathway for treatment of self-injurious macaques, and self-injurious humans.

PS70 Vibrating Needle during Venipuncture Reduces Insertion Force and Yields Lower and Less Variable Average Corticosterone Levels in Rodents

RS Clement*[1], EL Unger[2], SA Cavigelli[2], RM Sheehan[1], RB Bagwell[1], VA Kellogg[1], ML Mulvihill[1]

[1]Actuated Medical, Bellefonte, PA; [2]Department of Biobehavioral

Health, The Pennsylvania State University, University Park, PA

Painful needle puncture can cause discomfort in research subjects and increase stress hormone release which may confound blood chemistry analysis and trigger vasoconstriction, making serial blood sampling more difficult. To address this problem, an investigational handheld device designed to reduce the force of needle insertion was evaluated for its ability to lower overall stress of the blood sampling procedure. The device produces low frequency, axially-directed vibration at submillimeter amplitudes to reduce needle insertion forces. Sprague–Dawley rats were divided into 2 groups: treatment (n = 10) and control (n = 9). On 3 separate days (spaced 1 wk apart) tail vein blood sample collections were conducted; each trial within a day (n = 3) was separated by 1 h. Rats in the treatment group were always punctured with the device turned on, while the control rats were always punctured with the device turned off. Sample tail sections from a separate group of animals were also obtained for supplemental in vitro measurements of insertion force. To assess the level of stress associated with the punctures, plasma concentration of corticosterone in the blood samples was estimated via radioimmuno assay, and behavioral indications of stress (that is, animal movement and vocalization) were scored by 4 independent viewers of video footage of the blood sampling procedures. With the investigational device on, average insertion force was reduced by 68%. Blood samples obtained with the investigational device on yielded lower (> 60%) average corticosterone levels, reaching statistical significance at weeks 2 and 3, and individual variance in corticosterone levels over the study span was also reduced by 71%. Behavioral indications of stress in the rats were also lower when the investigational device was on compared with off. Experiments are currently underway to evaluate whether similar corticosterone and behavioral effects are repeated in mice. Vibrating needles during insertion reduces the force needed to penetrate tissues and may lead to less stressful venipuncture procedures in research subjects—a particularly important methodological advance for investigators interested in studying and assessing behavioral and physiologic stress processes.

PS71 Clopidogrel Treatment of Lentivirus-Related Coagulopathy in Pigtailed Macaques (*Macaca nemestrina*)

CE Hotchkiss*, B Agricola, J Ahrens, KW Vogel, S Hu

Washington National Primate Research Center, University Of Washington, Seattle, WA

Macaques infected with lentiviruses such as SIV or SHIV are prone to the development of pulmonary arteriopathy associated with thrombi in the right ventricle and pulmonary arteries. This condition can be rapidly fatal if a thrombus or embolus occludes the pulmonary arteries; therefore, it would be advantageous to identify this condition early and institute appropriate treatment to prevent thrombus formation. We have evaluated several hematologic and echocardiographic parameters, and have found that the earliest and most consistent predictor of this condition is persistent thrombocytopenia. We hypothesized that thrombocytopenia is due to the consumption of platelets associated with a hypercoagulable condition, implying that inhibition of platelet aggregation and activation might be therapeutic. Clopidogrel inhibits activation of platelets by binding to the ADP receptor, and was selected as a candidate therapeutic agent, even though potential side effects include thrombocytopenia and excessive bleeding. Two pigtailed macaques (*Macaca nemestrina*) infected with a chimeric SHIV virus developed persistent thrombocytopenia consistent with pulmonary arteriopathy/thrombosis. Treatment with clopidogrel (3 mg/kg PO SID) resulted in an increase in platelet count in both animals (from 73 to 324 and from 49 to 177 thousand/mL, respectively). Platelet function tests revealed that treatment increased closure time in response to ADP stimulation, indicating appropriate pharmacologic activity. There have been no adverse effects noted with this treatment. The animals continue to be monitored hematologically and by echocardiography. Clopidogrel shows strong potential for the prevention of pulmonary thrombosis/embolism in lentivirus-infected macaques.

PS72 Randomized, Double-Blind, Placebo-Controlled Study to Assess Safety and Efficacy of Lysozyme in Juvenile Rhesus Macaques (*Macaca mulatta*) with Diarrhea

KR Kelly*[1], CA Cooper[2], JD Murray[2,3], DM Hyde[4]

[1]Primate Medicine, California National Primate Research Center, Davis, CA; [2]Animal Science, [3]Population Health and Reproduction, [4]Anatomy, Physiology, and Cell Biology, University of California, Davis, Davis, CA

Lysozyme is a glycoprotein that is produced in high levels in human breast milk and modulates inflammatory responses through nonspecific antimicrobial mechanisms. Infectious and noninfectious insults to the gastrointestinal tract can result in inappropriate inflammatory responses, and lysozyme has the potential to resolve gastrointestinal inflammatory responses. Transgenic goats have been engineered to produce human lysozyme in their milk (hLZ-goat milk), and hLZ-goat milk has been found to help young pigs recover from experimentally induced *E. coli* diarrhea. As diarrhea is a significant cause for hospitalization in young monkeys, we implemented a randomized, double-blind, placebo-controlled study to assess safety and efficacy of hLZ-goat milk in captive, juvenile rhesus macaques (*Macaca mulatta*) presenting to the hospital at our institution with diarrhea. Monkeys received initial emergency veterinary treatment and then were assigned to one of 3 treatment groups (n = 5): experimental group (hLZ-goat milk), placebo group (goat milk), and standard-of-care group (oral rehydration fluid). Animals received 5 consecutive days of enteric treatment followed by 5 d of health monitoring. Clinical subjective and objective monitoring parameters were assessed at time of presentation and compared with day of discharge. Hospital diarrhea readmission rates were monitored for 30 d after intervention in order to best assess treatment efficacy. Experimental treatments were well-tolerated by all monkeys, and no significant side effects were reported. Enteric administration of hLZ-goat milk resulted in a ≥ 40% decrease in predicted diarrhea reoccurrence and significantly decreased 1-mo readmission rates. Animals administered hLZ-goat milk tended to have improved body condition scores (P = 0.08), and select subjective and objective clinical monitoring parameters were found to return to baseline faster in the hLZ-goat milk treatment group as compared with other groups. As such, lysozyme, as found in hLZ-goat milk, can be considered a safe and effective treatment option for captive, juvenile rhesus macaques with diarrhea.

PS73 Cage Movement as a Method of Creating Positive Social Interactions in Rhesus Macaques Demonstrating Abnormal Behavior

RJ Mistretta*

Advanced Bioscience Laboratories, Rockville, MD

Abnormal behavior is commonly seen in clinical and nonclinical populations of captive rhesus macaques. The behavior is usually presented as a coping mechanism triggered by stressors, internal and external. The most commonly seen abnormal behaviors in captive rhesus macaques include: self-injurious behavior, slapping, hair pulling, hair plucking, grasping, head banging, and pacing. Implementing a behavioral analysis and relocation program has been effective in the reduction and elimination of abnormal behavior by providing changes to the primate's social group composition. Once a primate has been designated as demonstrating abnormal behavior, the current social group undergoes detailed behavioral observations related to the displayed behaviors of the primate and the interactions with other primates within the group. Once the observations are performed, specific changes to the social interactions can be made by moving primates within the social group or to a completely new social group to alleviate the stressors. Using this practice and depending on the specific triggers to the behavior and the degree of the reaction, it has been possible to reduce or eliminate the abnormal behavior by removing stressful social interactions such as visual contact and promoting positive social interactions; including positive visual contact and social pair housing. If the degree of reaction is larger than the reduction of negative social interactions can alleviate or if the social group does not have any acceptable animal movement opportunities, then the primate will be moved into a new socially acceptable group. The new social group can be composed of smaller less dominant primates and possibly primates of a different sex. Movements

within the social group or to another social group have been successful in reducing or eliminating the unwanted behavior in 12 active cases. Three cases had a reduction of frequency in behavior by 50%, 4 by 75% and 5 by 90% or more. Through detailed behavioral observations social stressors can be identified and the appropriate social movements can be made to remove the stressors, reducing or eliminating the abnormal behavior.

PS74 Gastric *Helicobacter* spp. Identified in Captive Sooty Mangabeys (*Cercocebus atys*) with Gastric Adenocarcinoma

M Esmail*[1], AG Swennes[1], Y Feng[1], Z Shen[1], Q Duthoit[3], P Sharma[2], J Cohen[3], MT Whary[1], JG Fox[1]

[1]Division of Comparative Medicine, Massachusetts Institute of Technology, Cambridge, MA; [2]Division of Pathology, [3]Division of Animal Resources, Yerkes National Primate Center, Emory University, Atlanta, GA

Helicobacter pylori is a class I carcinogen that causes human gastric adenocarcinoma (ADCA). Nonhuman primates have been used to model human gastric *H. pylori* infection; however, naturally occurring gastric ADCA is rare. Natural infection with *H. pylori* and *H. suis* (previously classified as *H. heilmannii*) have been documented in cynomolgus and rhesus macaques and humans. In a recent report, spontaneous gastric ADCA was identified in 8 sooty mangabeys (SMs) without evidence of *H. pylori* infection by using human reagent serology, IHC, or Steiner silver stain. In this study, selected diagnostic and necropsy samples collected from this colony of approximately 200 indoor/outdoor SMs were screened for *Helicobacter* spp. infection by several modalities. Nineteen of 24 (79%) SMs were genus-specific 16S rRNA PCR (PCR) *Helicobacter* spp. positive by gastric wash with 2 samples being *H. pylori* positive by species-specific PCR. Because species-specific PCR assays do not exist for non*H. pylori* gastric *Helicobacter* spp., other genus-specific PCR products were sequenced. PCR products from 2 gastric washes shared 99% homology with *H. suis* and 4 were novel *Helicobacter* spp. most closely related (95% homology) to MIT 02-6899, a *Helicobacter* spp. isolated from woodchucks. To associate *Helicobacter* spp. gastric infection with gastric carcinogenesis, SM ADCAs were retrospectively examined. Three of 4 (75%) SMs with ADCA were infected with *H. suis* based on fluorescent in situ hybridization (FISH). In addition, 4 of 12 gastric tissues were FISH positive for *H. felis*, *H. bizzozeroni*, and/or *H. salmonis*, but negative for other type 2 *H. heilmannii* species. To further characterize *Helicobacter* spp. infection, ELISA was performed to determine *Helicobacter* spp. titers using sera of 19 SMs. Using an inhouse antihuman IgG ELISA, most SMs had higher titers to *H. pylori* compared with *H. suis* ($P < 0.01$). This is the first report to demonstrate gastric *Helicobacter* spp. infection in SMs. While PCR analysis of gastric washes has been used for diagnosis of *Helicobacter* spp. in cats and humans, this is the first reported use of this technique in NHPs. Further research is warranted as SMs may be a reservoir for human infections and may model the natural progression of gastric ADCA associated with *H. pylori* and *H. suis* infection.

PS75 Natural Infection of *Burkholderia pseudomallei* in 2 Pigtail Macaques (*Macaca nemestrina*) after Importation into the United States

CH Johnson*

Division of Scientific Resources, Animal Resources Branch, Centers for Disease Control and Prevention, Atlanta, GA

Identification of the select agent *Burkholderia pseudomallei* in macaques imported into the US is rare. Two purpose-bred, 5- to 6-y-old female pigtail macaques (*Macaca nemestrina*) imported from Southeast Asia were received from a commercial vendor in January and March of 2012. After the initial acclimation period of 5 to 7 d, a physical examination of the macaque received in March of 2012, revealed a subcutaneous abscess surrounding the right stifle joint. The wound was treated and resolved over 3 mo. Two months after completing treatment for the wounds surrounding the stifle joints, the animal presented with new-onset clinical manifestations including a left head tilt, full body tremors, and vertical nystagmus. On physical examination, a lack of pupillary light reflex was noted along with complete stiffness and muscle rigidity of the entire body. Radiographs were taken and diagnostic testing, which included hematology, serum chemistry, and urinalysis, was performed. There were no significant findings seen on the hematology report or radiographs. Collection of cerebrospinal fluid (CSF) at the cisterna magna was attempted, but was unsuccessful. Hyperglycemia, glycosuria, and ketonuria were noted on the serum chemistry and urinalysis results. Over the course of 2 d, despite medical treatment, the macaque progressed to inability to ambulate, vertical nystagmus with anisocoria, and hypothermia. The animal arrested while receiving supportive care including sodium chloride fluids (50 mL/kg), buprenorphine (0.1 mL/kg), regular insulin (0.25 U/kg), penicillin (20,000 U/kg) and dexamethasone (1 mg/kg), and was submitted for necropsy. A second case, the macaque received at our facility in January of 2012, was noted to have an abscess surrounding the right metacarpal joint approximately 1 y after being housed in our facility. No other clinical signs of disease were apparent. Based on information obtained from the initial case, the abscess was aspirated, cultured, and removed with tissue samples obtained from the surrounding area for Immunohistochemistry. Blood and CSF were also collected for culture and Indirect hemagglutination assay (IHA) titers. Based on histology, IHA titers, culture and PCR, both animals were diagnosed as infected with *Burkholderia pseudomallei*, the causative agent for melioidosis.

PS76 Evaluation of Sustained-Release Analgesics as an Alternative to Buprenorphine in a Postlaparotomy Mouse Model

S Kang, K Dorsey, LV Kendall*

Laboratory Animal Resources, Colorado State University, Fort Collins, CO

Buprenorphine and carprofen are commonly used analgesics for minimizing pain and distress in laboratory animals which require redosing every 8 to 12 h. Alternative opioid analgesics such as butorphanol and fentanyl require more frequent redosing. A single injection of a sustained-release (SR) formulation would provide prolonged analgesia and facilitate the use of the shorter acting opioids such as butorphanol and fentanyl. The efficacy of sustained-release formulations of buprenorphine (0.6 mg/kg), butorphanol (18 mg/kg), fentanyl (3.5 mg/kg), carprofen (15 mg/kg), and meloxicam (6 mg/kg) were compared with immediate-release formulations of buprenorphine-HCl (0.5 mg/kg BID), carprofen (5 mg/kg SID) and meloxicam (1 mg/kg BID), with the expectation that they would provide comparable pain relief for 72 h after laparotomy. A midline laparotomy was performed followed by an acute crushing injury to the intestines in an experimental mouse model. Pain was assessed postoperatively at 1, 3, 6, 12, 24, 48, and 72 h based on the frequency of behaviors, facial expressions, nest building, and change in body weight, and change in feed and water intake. Over time, there was a significant decrease in some of the parameters when compared with the buprenorphine-HCl treated group. Specifically, there was decreased activity in the SR-buprenorphine, SR-butorphanol, and SR-fentanyl groups; decreased rearing and wound licking in the SR-fentanyl group; and increased mouse grimace score in the SR-fentanyl and SR-meloxicam groups when compared with buprenorphine. There was no significant difference over time of the other behavioral parameters measures or in the other treatment groups when compared with buprenorphine-HCl. All treatment groups completed nest building within 24 h postlaparotomy, and all treatment groups lost weight following surgery. The overall evaluation suggests that sustained release formulations are as effective as buprenorphine-HCl and can be used as alternatives for postoperative pain.

PS77 Rederivation of Guinea Pigs via Hysterectomy

K Pritchett-Corning*, GB Mulder

Research Models and Services, Charles River, Wilmington, MA

For removal of infectious agents, embryo transfer has become the gold standard in mice and rats. In many rodent species, however, rederivation via embryo transfer is difficult or impossible due to lack of defined flora recipients, challenging anatomy, or lack of information on hormone protocols or pseudopregnancy induction. Although fostering can work with most rodent species, finding compatible defined flora recipient dams can also be difficult. With their precocial young, guinea pigs can survive without parental care, obviating the need for

defined flora recipient or foster females. We performed hysterectomy rederivation on full-term Hartley guinea pigs in order to eliminate guinea pig cytomegalovirus (GpCMV), a β-herpesvirus. GpCMV may be transmitted both transplacentally and after birth during passage through the vagina, postnatal grooming by the dam, or via shedding in milk. Transplacental transmission and its serious sequelae are most likely when animals have a primary infection during pregnancy; rates of human transplacental transmission decrease drastically if the mother has antibodies to cytomegalovirus. Critical to the success of this project was the ability to rapidly determine the presence or absence of GpCMV using a combination of serology and PCR assays on salivary gland, uterus, kidney, spleen, and blood of the dams and serology on offspring. Survival of newborn guinea pig pups was improved over that reported in the literature (75% compared with less than 50%) and offspring were virus-free. Husbandry innovations, including feeding a flora-supplemented softened diet and structural enrichment within the cage were also important to the success of this project.

PS78 Comparison of Fecal PCR and Conventional Diagnostic Methods for Detection of Murine Pinworms (*Syphacia* and *Aspiculuris*)

J Morris*[1], VS Dole[2], KS Henderson[2], R Hurley[1], D Cooper[1]

[1]Center for Comparative Medicine, Massachusetts General Hospital, Charlestown, MA; [2]Charles River Laboratories, Wilmington, MA

In an effort to determine the most sensitive means of detecting pinworms in mice at our institution's Hospital Center for Comparative Medicine (MGH CCM) animal facilities, PCR was evaluated against traditional diagnostic methods for *Aspiculuris tetraptera* and *Syphacia obvelata*. Additionally, these methodologies were used to determine the efficacy of 2 fenbendazole (FBZ) pinworm treatment regimens. In experiment 1 we compared fecal PCR, intestinal maceration (IM), and fecal concentration centrifugation (FCC) for detecting *A. tetraptera* and *S. obvelata* in 33 Swiss Webster mice exposed to dirty bedding from known pinworm positive animals over a 12-wk period. After an initial 4-wk exposure, 3 live mice were submitted weekly to the laboratory and individually tested for all 3 assays. In experiment 2 we used the same testing methods to determine the efficacy of MediGel FBZ or standard dietary FBZ treatment to eradicate *S. obvelata* in Swiss Webster and Prkdcscid mice. In addition to FCC and IM, PCR was used to evaluate both feces and anal swabs from each mouse after treatment. Results from experiment 1 indicated that fecal PCR was the most sensitive detection method (16/33 +), followed by IM (13/33 +) and FCC (3/33+). Seven of 16 PCR positive results were confirmed by either IM or FCC. PCR and FCC were both negative 7 of 13 times when *S. obvelata* worms were detected by IM. Results from experiment 2 demonstrated that including anal swabs for *S. obvelata* testing improved the diagnostic sensitivity of PCR. Animals were negative by all diagnostic methods 1 wk after commencement of either treatment and remained so during weekly evaluations for 12 wk. The results from these 2 experiments support that PCR is a highly sensitive method for pinworm screening and treatment verification that can significantly enhance a program's health surveillance/quality assurance program.

PS79 Analgesic Evaluation of Buprenorphine and Tramadol after Voluntary Oral Ingestion in Pair-Housed Rats

BF Taylor*[1], HE Ramirez[1], AH Battles[1], JK Neubert[2]

[1]Animal Care Services, [2]College of Dentistry, Department of Orthodontics, University of Florida, Gainesville, FL

Parenteral administration of analgesics to rodents induces an acute stress response and may lead to morbidity or mortality. Voluntary oral ingestion (VOI) eliminates this stress response making it a more preferable method for administration of analgesics; however, little information is available regarding oral dosing for rats. In addition, currently accepted methods for delivery of substances by VOI require animals to be singly housed. Here we describe the evaluation of different doses of 2 commonly used analgesics after VOI in pair-housed rats using a capsaicin-induced orofacial thermal operant assay. Male and female rats (Crl:SD) were assigned to one of 2 treatment groups (n = 10 males and 10 females per group) and trained to consume 2 g/kg hazelnut chocolate spread from a syringe. Rats then consumed either buprenorphine (0, 0.3, 0.4, 0.5, 0.6 mg/kg) or tramadol (0, 10, 20, 30, 40 mg/kg) mixed with hazelnut chocolate spread. All rats received each dose of their respective drug in a crossover fashion with a washout period of 4 to 5 d between testing. Twenty minutes after drug administration rats were anesthetized with isoflurane and capsaicin was applied to their previously depilated faces. The capsaicin was removed after 5 min with alcohol wipes and rats were allowed to recover for 30 min. They were then tested using an operant orofacial pain assessment device. Rats were rewarded with sweetened condensed milk while the capsaicin-sensitized areas of the face contacted 2 parallel metal thermodes heated to 45 °C. The number of licks and facial contacts were recorded and their ratio calculated. For a positive control, rats were tested using buprenorphine (0.03 mg/kg SC) 30 min prior to testing. Our results indicate that VOI of buprenorphine and tramadol are effective at alleviating capsaicin-induced pain in male rats, but only tramadol was effective in females at tested doses when compared with parenteral buprenorphine.

PS80 Comparison of the Clinical Efficacy of Buprenorphine and Sustained-Release Buprenorphine in Beagle Dogs

EA Nunamaker*[1,2], CL Medina[2]

[1]Biologic Resources Laboratory, University of Illinois at Chicago, Chicago, IL; [2]Comparative Medicine, AbbVie, North Chicago, IL

Buprenorphine is a common opioid analgesic used as part of a multimodal approach to pain management in dogs undergoing ovariohysterectomy. It is a popular opioid analgesic due to its long interdose interval compared with other opioids, and the recently developed sustained-release formulation promises to extend this interval to up to 3 d. As such, the goal of the current study was to compare the clinical efficacy of buprenorphine and sustained-release buprenorphine (buprenorphine SR) in dogs. Twenty healthy adult female beagle dogs underwent routine ovariohysterectomy and received multimodal analgesia consisting of meloxicam and buprenorphine. Ten of the dogs received 0.02 mg/kg buprenorphine subcutaneously twice daily for 3 d and the other 10 received 0.2 mg/kg buprenorphine SR subcutaneously once. Clinical efficacy was assessed at regular intervals by blinded observers using animal sedation scores, behavioral pain scores, temperature, heart rate, and respirations. Nine of 10 dogs in each buprenorphine group had adequate analgesic coverage. Only one dog from each group had breakthrough pain that required rescue analgesia. Dogs that received buprenorphine SR demonstrated a more rapid rebound of body temperature following surgery and had lower average sedation scores than dogs receiving buprenorphine. Pain scores, respiratory rate, heart rate, and side effects were comparable between formulations. Based on the results of this study, buprenorphine and buprenorphine SR are equally efficacious in managing pain associated with an ovariohysterectomy with comparable side effects.

PS81 Breaking Barriers: Promoting Growth with Inhouse Talent

D Byzek*, LJ Hughes, B Davis-Ritchie

Animal Health Care Section, NINDS/NIH, Bethesda, MD

In our progressive society the question remains, "Should we promote from within or hire candidates who appear stronger on paper?" It is a risk assessment each institute or workplace must consider. Both types of candidates bring their own set of expertise into an ever evolving work force. We have come to foster the idea of promoting from within at our Institute after developing an inhouse cross training program. It had been observed that promotions tended to stall once a cage wash technician was promoted to animal care technician (ACT). There was no continued advancement into higher level positions such as veterinary technologist (VT). Some inhouse candidates possessed years of animal experience with certifications under their belt, but were still falling short when compared with external candidates with higher education. As an institute, we wanted to begin investing in our staff to promote from within, thereby increasing employee morale and loyalty. The first initiative was the development of an "Introduction to Veterinary Skills" cross-training program. This program was open to all ACTs interested in learning VT skills. The program was a success and sparked the creation

of several other training endeavors within our institute. A majority of these trainings place the ownership on the individual as they occur during their lunch hour, but offer the potential to develop a multitude of skill sets such as computer proficiency and rodent techniques. A fair amount of consideration must be taken when fostering such an extensive cross-training program. Identifying key personnel willing to invest time and energy in assisting with the program is vital, as well as having the necessary resources available. While still in the pilot stages, excitement fills the air as individuals anticipate these trainings with smiles on their faces. We hope to share our experience as an institution and highlight the advantages and disadvantages of promoting from within compared with hiring externally. We hope to motivate other institutions to foster similar programs and realize that giving current employees more opportunities for advancement can be a win-win situation for everyone.

PS82 Decreasing Repetitive Strain Injury through an Early Symptom Management Program

MA Gregoire*[1], R Boles[1], GB Mulder[2]

[1]Environmental Health and Safety, [2]Research Models and Services, Charles River, Wilmington, MA

Repetitive strain injuries (RSIs) account for over $100 billion in direct and indirect worker compensation costs annually in the United States. RSIs are defined as a range of painful or uncomfortable conditions of the muscles, tendons, nerves, and other soft tissues that are caused by repetitive use of a specific part of the body. Symptoms of RSI include pain, tenderness, throbbing, pins and needles sensation, loss of sensation and loss of strength in affected areas. Highly repetitive rodent husbandry tasks such as cage cleaning and sanitation, animal handling (often with forceps), and manipulation of water bottles can contribute to excessive muscular tension leading to nerve entrapment, pain, numbness, and weakness in the affected extremity. Untreated, employees may progress to loss of function, inability to perform required job tasks, work loss and employer-paid medical expenses. An early symptom management (ESM) program involves identification of poor ergonomic practices responsible for employee symptoms, institution of corrective ergonomic measures, and application of simple techniques to relieve muscle tension and pain that does not require medical oversight. One year after instituting an ESM program in a rodent isolator operation, the Occupational Health and Safety Administration (OSHA) reportable injury rate decreased 47% and employee loss time was decreased by 60%. Direct worker compensation costs (medical expenses + lost work days) were decreased by 77% compared with the prior year. In addition, employee morale increased which led to more active participation in other new occupational health programs. ESMs can be tailored to any work environment and can often be developed using resources that already exist within an organization. Elements of an ESM program and a framework for development of a site-specific plan will be presented that can benefit your organization by generating cost savings, more efficient work practices and contribute to healthier employees.

PS83 An Enterprise Approach to Delivering an Animal Research Strategy

K Humphreys*

GSK, Stevenage, United Kingdom

Regulations governing animal care and use vary throughout the world, the laws reflecting the differing cultures of regions and countries. This presents international organizations with a challenge if they wish all of their research to be carried out to a common standard. Our company addresses this by having an overarching policy on animal care and use which lays out a set of core principles that must be met for all animal studies irrespective of their location or whether they are carried out within company facilities or performed externally on our behalf. In 2012, the Office of Animal Welfare, Ethics and Strategy was launched, responsible for identifying strategy, supporting global initiatives and training, as well as providing governance and advocacy in the animal research and 3Rs space. Being independent of both the therapeutic and operational areas, the group aims to balance the corporate ambition of the business with the needs of the scientist to deliver their programs involving animals. This presentation describes the advantages and efficiencies which have resulted from formation of OAWES and outlines the successes to date along with the challenges we faced during our first year of operation.

PS84 More Than Meets the Eye: A Comprehensive Assessment of Optimal Floor Space for Breeding Mice

R Rajoria*, BS Blank, EK Daugherity, T Southard, B Singh, T Cleland

Cornell University, Ithaca, NY

The eighth edition of the *Guide for the Care and Use of Laboratory Animals* includes new recommendations of additional floor space requirements for female mice with litters. This change signifies that trio-breeding cannot be performed in regular shoebox mouse cages, and a larger cage (≥ 117 in.^{2}) should be used for this purpose. The objective of this study was to assess health and reproductive performance of C57BL/6 mice housed in pair- and trio-breeding scenarios, both in mouse and rat shoebox cages (67 in.^{2} and 132 in.^{2}, respectively). The front of each cage contained a sampling port used for weekly air sampling to assess microenvironment parameters in each group. Trio- and pair- breedings, in both size cages, were set-up (n = 5 for each of the 4 groups), and data was collected until weaning of the third consecutive litter from each female. Reproductive performance analysis included number of pups born, average weekly pup weights, survival to weaning (wean: born ratio), and days between litters. Following weekly cage changes, fresh fecal pellets were collected from each cage to compare corticosterone levels of the 4 groups. Anxiety behavior was measured in weanlings and adults using open-field test and elevated-plus maze. Tissue samples were collected to determine respiratory tract histopathology, to measure Brain-Derived Neurotrophic Factor (BDNF), and to perform brain RNA microarray analysis. No significant differences were noted among the groups in measured parameters of rodent health, reproductive performance, and respiratory tissue inflammation. The data obtained for fecal corticosterone, anxiety, BDNF, and brain RNA microarray analysis will be presented.

PS85 Withdrawn

PS86 Conducting Successful Public Outreach in an Elementary Setting

A Ogden*

UCLA, Division of Lab Animal Medicine, Los Angeles, CA

As lab animal professionals, it is our responsibility to educate the public as well as those we work with. Public outreach in many aspects may not be for everyone, but when the accurate components come together, outreach can be extremely rewarding for all parties involved. As a mom of young children, I have quickly grown to realize how little the public knows and understands the work that we do as a lab animal community. With a little legwork, creativity, and commitment, we made a small attempt to bridge this knowledge gap by exposing our lab animal world to a local elementary school by conducting a classroom-based, hands on, exposure and education program. We worked with the teachers to develop a curriculum that would allow the children to receive a brief introduction to the world of laboratory animal science in a hands-on setting while also meeting the teacher's educational goals for the students. We built 4 learning workstations and divided each classroom evenly into 4 groups of students. Students were allowed about 10 min at each learning station before they were instructed to rotate. Not only did the children and teachers get the opportunity to learn about our work, but we instructors also experienced and grew to appreciate the various learning capacities among the different ages. This opportunity allowed us to introduce the animals, the science, the veterinary medicine, and the possibilities of medical cures. We are confident that we left a vivid, and positive impact on the children.

PS87 A 16-Week Cage Change Cycle for Mice

JL Taylor*, P Noel, B Mickelsen, D Bird

Office of Comparative Medicine, University of Utah, Salt Lake City, UT

The Office of Comparative Medicine at our institution conducted an extensive evaluation of a 16-wk change cycle for mouse cages and accessories. The study was carried out in polysulfone cages docked in an individually ventilated cages (IVC) rack, which used a cage ventilation rate of 30 changes per hour. Two types of bedding, corncob and paper, were used and each cage contained 5 male C57BL/6 mice. The study compared intracage environmental conditions (NH_3, temperature, CO_2, RH, ATP) in cages changed on a 2-wk cycle with those changed after 16 wk of use. Environmental conditions in the room were also monitored. Behavioral parameters, including fecal corticosteroids, were evaluated with both change cycles. Using a unique method, cages on the 16-wk cycle underwent a partial removal (75%) and replacement of bedding every 2 wk with replenishment of food (irradiated) and water. Animals and nests were not disturbed during the process. During the entire 16-wk time period, our data revealed that there was no statistically significant difference in the measured cage parameters between the cages changed every 2 wk and those changed during and after 16 wk of use. Signs of stress and fighting were also much lower in the 16-wk cages compared with those changed on a 2-wk cycle. The results from this study definitively prove that the 16-wk cage changing method is less stressful for the animals and intracage conditions are comparable to those of cages changed at the current standard 2-wk cycle.

PS88 Unrestricted Number of Pups in Trio Breeding of C57BL/6J Mice

JM Cadillac[*1], U Mudunuru[2], TS Simpler[3], FE Lund[3], TD Randall[2]

[1]Animal Resources Program, [2]School of Medicine Division of Clinical Immunology and Rheumatology, [3]Microbiology, University of Alabama - Birmingham, Birmingham, AL

Our institution's IACUC mouse housing density policy currently states that the total number of pups in a trio breeding cage (2 females and one male) must be kept to less than or equal to 9 pups, litters cannot be more than 14 d apart, and there can be no more than 1 litter per female in the cage at one time. A research group with an extensive mouse breeding program (52 strains on the C57BL/6J (B6) background housed on ventilated racks totaling over 1,000 cages) requested an exemption from the current IACUC policy to breed trios leaving all pups born in the cage until weaning age (21 to 25 days after birth). The study objective was to elucidate whether there was a significant impact on breeding performance between the current IACUC mouse housing density policy (group 1) and the investigator's proposed management plan (group 2). Twenty cages of B6 mice with trio matings were managed following the current housing policy (group 1) and 20 cages of B6 mice were managed as requested by the research group (group 2). Data collected included the number of litters born, the number of pups born per litter, the litter weight at weaning, the number of pups weaned per litter (percent survival) and intracage ammonia levels. There were 333 litters born between the 2 groups over a 4.5-mo study period. Both groups had an average litter size of 6.9 pups. The average litter weight at weaning was 52 g in group 1 and 54 g in group 2. The average percent survival was 61% and 66%, respectively. Intracage ammonia levels were zero. There were no statistical differences found between the study groups. The current IACUC mouse housing density policy is under review to address the eighth edition of the *Guide for the Care and Use of Laboratory Animals*.

PS89 Conducting Large-Scale Rodent Infusion: Methods for Success

N West[*], K Rasmussen

Covance, Madison, WI

Infusion studies with rodents are frequently conducted to evaluate the relative safety, metabolic actions, and pharmacologic profile of developing compounds. Increasingly, studies with large numbers of animals and/or long durations are being used in order to evaluate the previously mentioned parameters. Studies of this nature present unique challenges and opportunities, both of which require modified approaches to study design and management. To facilitate successful large, long-term rat infusion studies, consideration should be given to animal housing, animal characteristics, dose administration personnel, roles, and scheduling, as well as infusion apparatus refinement. Modified animal housing that allows free range of movement with minimal stress to the infusion system is a critical initial step. Important animal characteristics to consider include animal weight and age at surgery. An approach to dose administration was developed using 3 specific dosing roles: start dose (person 1), syringe change and flush start (person 2), and end dose (person 3). This approach allowed for more streamlined and efficient dose administration. In addition to the development of specified dosing roles, a scheduling tool was developed to allow for targeted, specific dose start and/or end times, which in turn led to better scheduling for postdose sample collections. Recently, the implementation of an infusion pump control and tracking computer program has allowed for further refinement of the above discussed variables. The development and combination of these method refinements allows for improved study management, efficiency, and overall data quality and study integrity. These modifications should be considered when presented with large long-term rodent infusion studies.

PS90 Evidenced-Based Allergen Exposure Reduction Assessment in a Rodent Facility

J Merk[*1], C King[2], E Georgelos[1], A Kim[2], R Klein[2], JD Macy[1]

[1]Yale Animal Resources Center, [2]Environmental Health and Safety, Yale University, New Haven, CT

Laboratory animal allergen (LAA) exposure is a well-established occupational hazard to both animal care technicians and researchers. To better define location-dependent and/or activity dependent levels of LAA exposure risks, environmental testing was conducted using mouse urinary protein, (MUP) *Mus* m1, as a marker for environmental allergen levels. Sampling was conducted by using an air pump, which was affixed to staff members for a minimum of 90 min for each routine task being performed, including cage changing, euthanasia, and bedding disposal. Samples were also collected in corridors and mouse holding rooms for longer intervals, up to 8 h. Once collected, the samples analyzed by an external laboratory using an immunochemically by ELISA. A total of 49 samples (24 area and 25 personal samples) were taken within 8 separate animal facilities. The results indicate that the highest exposure, cage dumping without an HEPA filtered dump station, resulting in 470 ng/m^3 of MUP (individual protected by powered air purifying respirator), and was followed by cage changing without engineering controls resulting in MUP over 30 ng/m^3. Cage dumping (10 samples) or cage changing (7 samples) with an engineering control (dump station, animal transfer station, biosafety cabinet) reduced ambient exposure to 0.61 to 58 ng/m^3 for cage dumping and 1.4 ng/m^3 to 6.9 ng/m3 for caging changing. Corridor results (10 samples) were all below 0.5 ng/m^3, and similar to a tissue culture room, with one exception. Two background samples within animal rooms containing 6 to 8 ventilated cage racks were < 1.2 ng/m^3. MUP is well-controlled when engineering controls are in use and, when combined with the use of ventilated cages protects against occupational exposure of personnel and prevents migration of MUP to adjacent spaces. These findings will allow an evidenced-based approach to determine the value and appropriateness of practices and procedures used to reduce allergen exposure.

PS91 Mutational Evidence Implicates Increased Oxidative Stress in the Pathogenesis of *Helicobacter hepaticus*-Induced Lower Bowel Inflammation in 129 SvEv Rag2-/-/Il-10-/- gpt Δ Mice

EE Turowski[*1], CL Belanger[2], LJ Trudel[2], GN Wogan[2], NA Parry[1], JG Fox[1]

[1]Division of Comparative Medicine, [2]Department of Biologic Engineering, Massachusetts Institute of Technology, Cambridge, MA

Helicobacter hepaticus is a gram-negative, spiral-shaped, microaerophilic bacterium that causes hepatitis and colitis that progress to neoplasia in susceptible mouse strains. Certain strains of immunocompromised *H. hepaticus*-infected mice are frequently used as models of inflammatory bowel disease, although the exact pathogenic mechanism by which infection leads to colitis and neoplasia remains unknown. We hypothesize that *H. hepaticus* infection causes increases in inflammation and oxidative stress in the lower gastrointestinal tract, resulting in a promutagenic environment that predisposes infected animals to neoplasia. In this study, *H. hepaticus*-infected 129 SvEv Rag2-/-/Il-10-

/- mice possess a 456-bp transgene from *Escherichia coli* that allows quantification of point mutations and small deletions in the genome. As expected, *H. hepaticus* causes significant typhlocolitis with dysplasia at 21 wk postinfection by oral gavage in infected animals compared with controls (n = 7 for each group). Additionally, *H. hepaticus* causes significantly more cecal DNA mutations in male animals at 21 wk postinfection compared with uninfected controls (12.1 ± 8.0 compared with 5.6 ± 6.7 mutations per million bases). These mutations primarily comprise G:C-to-A:T transitions (30.23%) and G:C-to-T:A transversions (20.93%), both of which are consistent with DNA damage resulting from increased oxidative stress. Further studies are underway to determine the mutational spectrum in females in this cohort. In addition, we are exploring the role of cytolethal distending toxin, a genotoxin expressed by *H. hepaticus*, in the development of double-stranded DNA breaks that could also lead to DNA damage. We conclude that *H. hepaticus* infection in these mice leads to lower bowel inflammation while inducing a DNA mutational spectrum that is consistent with increased oxidative stress. These findings support our hypothesis and suggest a role of oxidative DNA damage in the pathogenesis of *H. hepaticus*-induced colitis and lower bowel neoplasia in mice.

PS92 Experimental Infection of Syrian Hamsters with *Helicobacter* spp. of the *H. bilis* Cluster

SE Woods*, C Ek, MT Whary, Z Ge, Z Shen, S Muthupalani, JG Fox

Division of Comparative Medicine, Massachusetts Institute of Technology, Cambridge, MA

Syrian hamsters are used in studies of liver carcinogenesis, including liver fluke-associated cholangiocarcinoma. Aged hamsters naturally infected with novel *Helicobacter* spp. classified in the *H. bilis* cluster develop hepatobiliary lesions and typhlocolitis. To determine if enterohepatic *Helicobacter* spp. are responsible for disease, *Helicobacter*-free Syrian hamsters were experimentally infected with *Helicobacter* spp. after suppression of intestinal bacteria by treatment of dams and pups with tetracycline in the drinking water. After antibiotic withdrawal, female and male weanlings were gavaged 3 times with a cocktail of 4 *H. bilis*-like strains isolated from hamsters (n = 7) or *H. bilis* ATCC 43879 isolated from human feces (n = 7), and compared with control *Helicobacter*-free hamsters (n = 7). *H. bilis* 43879-infected hamsters were necropsied at 33 wk due to lack of detectable colonization by fecal *Helicobacter* spp. PCR; at necropsy, 5 of 7 were weakly PCR positive (10^1 to 10^2 copies/μg cecal DNA). Control and *Helicobacter* spp. cocktail infected hamsters were maintained for approximately 95 wk. *Helicobacter* spp. cocktail infected hamsters (n = 6 colonized; 10^4 to 10^5 copies/μg cecal DNA) had hepatic cysts (n = 6; 1 cystadenoma), adrenal tumors (n = 4), gastrointestinal lymphomas (predominantly involving MALT; n = 3), atrial thrombosis (n = 1), ovarian cysts (n = 1), and splenic hemangiosarcoma (n = 1). Control hamsters had similar lesions, but lacked MALT lymphoma. Compared with younger *H. bilis* 43879-infected hamsters, *Helicobacter* spp. cocktail infected hamsters had more cecal edema, epithelial defects and inflammation (median typhlitis index: 3.5; $P < 0.01$) and colitis ($P < 0.01$), while age-matched control hamsters lacked significant typhlocolitis. Differences in liver lesions between groups were difficult to interpret due to severe hepatic disease present in aged hamsters; all livers were negative for *Helicobacter* spp. Here, we document the first successful long-term experimental infection of Syrian hamsters with *Helicobacter* spp., and suggest that persistent *H.* spp. infection may augment risk for MALT lymphoma and aging-associated typhlocolitis. Future studies will include dual infection of hamsters with *Helicobacter* spp. and liver flukes to more fully elucidate mechanisms of liver carcinogenesis in both hamsters and humans.

PS93 Rodent Pathogen Prevalence as Determined from the Direct Screening of Index Rodents by PCR Compared with Indirect Sentinel Screening by Traditional Methods

KS Henderson*, J Cosentino

Research Animal Diagnostic Services, Charles River, Wilmington, MA

The prevalence of rodent pathogens has traditionally relied on data obtained from bedding sentinels. Pathogens such as viruses that readily transmit to sentinels can contribute a positive result when a couple cages on a rack are positive; conversely, pathogens that do not transfer efficiently to sentinels may not be detected. Both scenarios may contribute to a false determination of prevalence for pathogens in the laboratory rodents. Additionally, initial positive findings for a particular agent are often followed by confirmation testing of the same population which may artificially raise the total reported prevalence. To determine if these variables or others may impact an accurate prevalence, we summarized PCR data collected over a 1y period from mouse (M) and rat (R) samples submitted for screening by PCR panels of agents. These submissions mostly represented quarantined rodents. The direct testing by PCR mitigates the influence of transmission efficiency of the pathogens to sentinels, overrepresentation of positive sentinels by a few positive cages, and the inclusion of follow-up test results from positive populations. Consistent with reports that many agents do not transfer well to bedding sentinels, the PCR-based prevalence (%) was several times (X) greater than previously reported sentinel-based prevalence for many pathogens; β-hemolytic Strep. B (M = 4.9%/20.6X, R = 35.3%/9.4X), CAR bacillus (R = 1.7%/6.4X), *Helicobacter* (M = 15.9%/2.0X, R = 6.6%/2.2X), *K. oxytoca* (M = 2.3%/6.1X, R = 0.85%/2.3X), *K. pneumoniae* (M = 2.2%/21.8X, R = 9.1%/16.5), *M. pulmonis* (M = 0.12%/12.2X, R = 1.2%/7.5X), *P. pneumotropica* (M ≥ 24.9%/80.4X, R ≥ 18.4%/ 3.7X), *S. aureus* (M = 14.03%/2.3X, R = 55.5%/2.36X), adenovirus (M = 0.15%/7.6X, R = 0.3%/5.0X), fur mites (M = 0.98%/8.9X, R = 0.66%/not found by conventional), pinworms (M = 1.5%/4.9X, R = 4.17%/3.5X), *Spironucleus m.* (M = 1.56%/19.5X, R = 2.72%/14.3X). As anticipated for agents that are more readily transferred to bedding sentinels, except for norovirus (M = 29.8%/1.1X), prevalence for parvovirus, group-A rotavirus, coronavirus and theilovirus was 4.8 to 15.6X higher by sentinel monitoring compared with PCR. A PCR-based prevalence based on the direct testing of index rodents may provide an alternative and potentially more accurate prevalence for rodent pathogens.

PS94 Genotoxic *E. coli* Are Prevalent in Research Macaques with Colitis-Associated Cancer

AG Swennes*[1], CM Madden[1], CP Byrd[1], NM Parry[1], EM Buckley[1], K Lodge[2], TW Mitchell[2], L Handt[2], RP Marini[1], JG Fox[1]

[1]Division of Comparative Medicine, Massachusetts Institute of Technology, Cambridge, MA; [2]Merck Research Laboratories, West Point, PA

Idiopathic colitis and intestinal adenocarcinoma are common in research macaques, though their inciting causes are not well understood. Recent studies have associated *E. coli* strains harboring genotoxins and cyclomodulins with human inflammatory bowel disease (IBD) and colitis-associated cancer (CAC). To investigate the prevalence of these *E. coli* strains in macaques, a PCR-based screen was performed on 50 fecal *E. coli* isolates cultured from 48 young to middle-aged rhesus and cynomalgous macaques housed at our institution. Genes encoding intimin (*eae*), shiga-like toxins (stx_1 and stx_2), cycle inhibiting factor (*cif*), cytotoxic necrotizing factors (*cnf*), cytolethal distending toxins (*cdt*), and colibactin (*pks*) were examined. Of these strains, 2 (4%) were *eae*- and *cif*-positive, 6 (12%) were *cnf*-positive, 1 (2%) was *cdt*-positive, and 14 (28%) were *pks*-positive. Intrigued by their prevalence and association with CAC, these genes were investigated in 14 rhesus macaques with intestinal adenocarcinoma (n = 14) and compared with age-matched colony controls (n = 22). This macaque cohort was 26 to 35 y old and predominantly female (81%). A PCR-based screen was performed on fecal DNA, and when CAC-positive and control groups were compared, frequencies of *eae* (9.0% compared with 4.3%, P = 1.000), *cnf* (18% compared with 8.7%, P = 0.5799), and *cdt* (55% compared with 43%, P = 0.7177) were not significantly different. However, CAC-positive macaques were colonized with *pks*-positive *E. coli* at significantly greater frequency than controls (72% compared with 26%, P = 0.0230). While a statistical association was not found between *cdt* prevalence and CAC, its high overall prevalence in the CAC cohort compared with our initial survey population (44% compared with 2%) suggests that *cdt* might also promote CAC given appropriate conditions. In support of these results, *eae*- (n = 1), *cnf*- (2), *cdt*- (3), and *pks*-positive (5) *E. coli* isolates were cultured from 4 adenocarcinoma-positive macaques' frozen resected intestine. These results show that *E. coli* harboring *cdt* and *pks*, whose gene products cause DNA double-stranded breaks, G2/M cell cycle arrest, and apoptosis, are prevalent among macaques with CAC.

The inflamed intestine may provide an appropriate ecological niche for their expansion and their presence may play a role in IBD and CAC.

PS95 Sleep Loss and Sexual Dysfunction: Unexpected Effects in the Offspring

ML Andersen*[1], TA Alvarenga[1], MF Aguiar[1], R Mazaro-Costa[2], S Tufik[1]

[1]Psicobiologia, Universidade Federal De Sao Paulo, Sao Paulo, Brazil; [2]Ciências Fisiológicas, UFG, Goiania, Brazil

Present-day chronic sleep restriction, compounded by other interferences such as sleep disturbances, constitutes a relevant public health issue. In rodents, the lack of sleep affects the action of sexual hormones and interferes with reproductive capability. The present study investigated the effects of sleep deprivation during pregnancy on the reproductive capability of the offspring. Sexual behavior during the adult phase was analyzed in F1 offspring of sleep-restricted (SR) females (first experiment) and males (second experiment; SR or paradoxically sleep-deprived). Our results demonstrate that the lack of sleep during pregnancy may compromise sexual behavior in the offspring when they reach adulthood. F1 male offspring of female rats that were SR throughout pregnancy show significantly reduced sexual motivation, made evident by the increased latency to the first mount and a reduction in the overall number of mounts during the experimental test. Such results may be a consequence of the reduced progesterone concentration that was noted in this group. In contrast, when the parental males were paradoxically sleep deprived (PSD) or SR, the F1 male offspring displayed depressed sexual function accompanied by a reduction in testosterone. There were notable overall differences in the sexual response of F1 females obtained from females and males submitted either PSD or SR. In the F1 offspring of either PSD or SR parental males there was a significant increase in the acceptance of the male during the nonreceptive phase (the estrus phase), in contrast to what was observed in the CTRL group. The F1 females from the SR group also displayed disrupted sexual behavior, characterized by several mounts of the males during all tests. Together, these experiments demonstrate that sleep restriction in progenitors may alter sexual behavior of the F1 offspring in adulthood. Such findings reveal far-reaching consequences of sleep deprivation, and suggest that parental sleep influences the reproductive capability of subsequent generations, a fact of interest for those who plan on parenting.

PS96 Differences in MicroRNA Expression between SIV-Infected and Naïve Sooty Mangabeys (*Cercocebus atys*) and Rhesus Macaques (*Macaca mulatta*)

GR Smith*[1], SE Bosinger[3], SM Jean[1], ZP Johnson[2]

[1]Division of Animal Resources, [2]Division of Developmental and Cognitive Neuroscience, [3]Division of Microbiology and Immunology, Yerkes National Primate Research Center, Atlanta, GA

The AIDS epidemic can be traced back to interspecies transmission of SIV from African nonhuman primates, such as the sooty mangabey (*Cercocebus atys*), to humans. Sooty mangabeys and other SIV natural host species have evolved natural mechanisms that mask clinical signs and consequences despite high viral loads. Alternatively, Asian nonhuman primate species, such as the rhesus macaque (*Macaca mulatta*), succumb to AIDS-like clinical symptoms following infection with SIV, making SIV-infected rhesus macaques a valuable animal model of HIV infection and AIDS. In an effort to better understand the protective mechanisms present in sooty mangabeys, we sequenced all microRNAs (miRNAs) in infected and naïve rhesus macaque and infected and naïve sooty mangabey peripheral blood mononuclear cells. We observed at least a 2-fold difference in expression in 77 miRNA species between infected and naïve sooty mangabeys, 50 miRNA species between infected and naïve rhesus macaques, 157 miRNA species between naïve sooty mangabeys and naïve rhesus macaques, and 158 miRNA species between infected sooty mangabeys and infected rhesus macaques. Of particular interest is the high expression level of miR-9-5p in infected sooty mangabeys compared with infected rhesus macaques. This miRNA is highly expressed in nonprogressing human patients infected with HIV. miR-9-5p is known to inhibit the transcription factor, Blimp-1, which contributes to CD4+ T-cell dysfunction in HIV infections. By exploring the differential miRNA expression patterns in infected and naïve sooty mangabeys and rhesus macaques, we hope to identify molecular pathways necessary for the suppression of clinical signs in these animal models of HIV infection in humans.

PS97 Refinement of Procedures for Repeat-Dose Bolus Intravenous Administration and Blood Sample Collections in Swine

K Rasmussen*, T Quackenboss, M Taschwer, T Ervin, N West

Covance Laboratories, Madison, WI

Swine are rapidly gaining universal regulatory and sponsor acceptance as the nonrodent species of choice in nonclinical evaluations of various compounds. Historically, administering repeat intravenous injections in swine has presented feasibility concerns as vessel integrity in swine can be easily compromised, potentially resulting in data loss and/or study integrity concerns. This study was designed to evaluate an approach to mitigate these concerns while concurrently applying a modified approach to sample collection. Study candidates were acclimated to jackets for 2 wk prior to surgery and maintained in jackets for the duration of the 4-wk study thereafter (total duration jacketed was approximately 8 wk). Thirty animals were surgically implanted with dual lumen catheters placed in the jugular vein and then externalized. One channel of the catheter was used for intravenous administration and the other was used for blood sample collection. Catheter patency was evaluated at least twice weekly, and catheters were locked with a heparin/dextrose solution between access intervals. Bolus doses (2 mL/kg/dose) were successfully administered to 24 animals daily for 28 d. Blood was collected predose and at 6 postdose time points (from 0.083 to 24 h) on dosing days 1 and 28. Blood samples were successfully collected via the catheter (96% success rate; 321 of 336 collections). Through operant conditioning (largely, food rewards), animals readily acclimated to dose administration and sample collections and consequently became compliant participants during these procedures. Fewer technicians were required for dose administration and sample collection, which reflects an important added benefit. Dual-lumen catheter placement for intravenous administration and sample collection represents significant potential for reduction in animal and technician stress, and a technique refinement that allows for greater study success. This novel approach should be considered as a viable improvement/modification for repeat intravenous dose administration and/or repeat sample collections.

PS98 Tamoxifen Administration: Rats Are Not Large Mice

CE Ahner*[1,2], RJ Schehr[1], M McCoy[1,2], EC Bryda[1,3]

[1]Veterinary Pathobiology, [2]Comparative Medicine Program, [3]Rat Resource and Research Center, University of Missouri, Columbia, MO

Tamoxifen administration in mice has been used extensively to induce Cre expression and knockout genes at specific time points in order to generate genetically engineered disease models. With recent advances, it is now possible to make similar genetically engineered rats, yet little is known about appropriate dosage or route of tamoxifen administration in rats. The objective of this study was to determine the best route of administration, dosage range, and potential side effects of tamoxifen administration in rats. Our hypothesis was that rats will require a different dosage than mice due to species differences in tamoxifen metabolism, and that subcutaneous administration will be a superior delivery route to intraperitoneal administration. Adult and neonatal rats of different genetic backgrounds (inbred Fischer 344 and outbred Sprague–Dawley) were placed into treatment groups: intraperitoneal or subcutaneous sham injection, intraperitoneal or subcutaneous injection of sunflower oil only (vehicle) and intraperitoneal or subcutaneous injection of tamoxifen (3.72, 40 or 100 mg tamoxifen/kg body weight). Adults were given 2 injections 24 h apart, and neonates were injected within 24 h of birth with a second injection 24 h later. Weight measurements and clinical assessments were performed daily. Preliminary data showed that adults tolerated all doses of tamoxifen tested whereas F344 neonates had 100% mortality with the 100 mg/kg dose, irrespective of route of administration. Subcutaneous administration at the 2 lowest tamoxifen doses had lower mortality rates (60% and 30%, respectively)

in neonates. Additionally, F344-Tg(hPKD1-ERT2-cre-ERT2)1Bryd adult rats orally gavaged with 40 mg/kg tamoxifen at 0 h and 24 h showed induction of Cre expression based on Western blot analysis. These findings confirm that while tamoxifen can be administered to rats and successfully induce Cre expression, optimal dose and route of injection in rats appears to be different than that routinely used for mice.

PS99 Use of a Spatial Cognitive Bias Assay to Assess the Wellbeing of Rats Provided with Cages of Varying Dimensions

D Hickman*, R Wheeler, MP Swan

Laboratory Animal Resource Center, Indiana University School of Medicine, Indianapolis, IN

The *Guide* states that animals should be allowed to engage in normal postural positions. The required minimum height for rat cages in the US is 7 in., which prevents engagement in a bipedal stance. This study was designed to measure the wellbeing of male Sprague–Dawley rats that were provided with cages of varying dimensions. Wellbeing was assessed by measuring changes in behavior of rats that either started in US standard-size caging and were provided with clear acrylic caging that was twice the floor space and/or twice the height (experimental caging), or started in the experimental caging and then provided with US standard-size caging prior to the behavioral testing. These groups were compared with rats that were housed in US standard-size caging throughout the entire study (control group). A spatial discrimination assay was used to measure emotional affinity by comparing the latency to approach a rewarded goal location between groups. Decreases in latency times have been associated with positive emotional affinity (optimism). Rats that started in experimental caging, but were moved to US standard-size caging demonstrated significantly increased latency times as compared with the control rats ($P = 0.0032$). Rats that started in the US standard caging, but moved to the experimental caging also demonstrated significantly increased latency times as compared with the control rats ($P < 0.0001$), with rats that were provided with twice the height demonstrating significant increases in latency times ($P < 0.0001$). Passive behavioral analysis demonstrated that rats provided with increased vertical space engaged in a bipedal stance approximately 2% of the time. The results of this study suggest that the provision of increased vertical space results in a negative emotional affinity (pessimism) for male Sprague–Dawley rats, despite their ability to engage in normal postural behaviors, and that cage dimension alone is not sufficient to improve the wellbeing of rats.

PS100 Characterization of a Human Amylin Transgenic Mouse Model of Insulin Resistance

J Aitken*[1], M Li[1], C Walker[1], G Cooper[1,2]

[1]School of Biologic Sciences, University of Auckland, Auckland, New Zealand; [2]Centre for Advanced Discovery and Experimental Therapeutics, University of Manchester, Manchester, United Kingdom

Type 2 diabetes mellitus (T2DM) is a growing problem worldwide. It is a multifactorial disease, characterised by insulin resistance and β-cell dysfunction, which with time leads to death through secondary complications. Human amylin (hA), is a 37 amino acid protein that is cosecreted with insulin from the pancreatic islet β-cells upon stimulation with glucose. Human amylin aggregation has been linked to β-cell degeneration in T2DM. We have constructed a mouse model carrying a nonaggregating version of hA, (25,28,29) triprolyl human amylin (tripro hA), which shows insulin resistance and transient hyperglycaemia. Male animals were housed in environmentally controlled conditions with a 12:12-h light:dark cycle and were fed standard rodent chow and water ad libitum. Hemizygous tripro hA transgenic mice and their nontransgenic littermates were used in the study. Body weight, blood glucose, and food intake were measured weekly from weaning onwards. Longitudinal tail serum samples were taken from each mouse at 5 different stages for hormone analysis. Using longitudinal tail bleeds allowed direct comparison of hormonal changes with observed changes in biologic parameters for individual animals at different stages of their life cycle. We used the same serum sample to measure several different hormones which enabled reduction in the number of animals used. Weights of the hemizygous and nontransgenic littermates diverge at around 100 d of age, with the hemizygous animals showing significantly higher body weight by 125 d of age. Blood glucose levels were significantly higher in the hemizygous animals compared with their control littermates after 100 d, but eventually returned to normoglycaemia by around 300 d of age. Weekly food intake rapidly increased in the hemizygous mice beginning just after 100 d of age. Serum insulin, leptin, and tripro hA levels were all increased in the hemizygous animals at 100 d which preceded the observed increases in food intake, weight, and blood glucose. We have identified important biologic parameters associated with the onset of insulin resistance and show that changes in these parameters are driven by the overexpression of tripro hA. We can now target these changes for intervention with drug therapeutics.

PS101 Platelet Activation and Platelet-Monocyte Aggregate Formation Contribute to Platelet Decline during Acute Infection in the SIV-Infected Pigtailed Macaque Model of HIV Infection

KA Metcalf Pate*[1], CE Lyons[1], JL Dorsey[1], EN Shirk[1], SE Queen[1], RJ Adams[1], L Gama[1], CN Morrell[2], JL Mankowski[1]

[1]Department of Molecular and Comparative Pathobiology, Johns Hopkins School of Medicine, Baltimore, MD; [2]Aab Cardiovascular Research Institute, University of Rochester School of Medicine and Dentistry, Rochester, NY

The SIV-infected pigtailed macaque is an important animal model for the study of the pathogenesis of HIV infection. Platelet decline occurs in SIV-infected pigtailed macaques in a biphasic fashion, with a transient decrease in platelet number during acute infection corresponding with the initial peak in plasma viral load and proinflammatory cytokine levels. Platelets are key participants in innate immune responses to pathogens, and we hypothesized that this initial decrease in platelet number may result from activated platelets becoming sequestered in interactions with fellow innate immune cells. Flow cytometry was used to assess platelet activation and quantify platelet-monocyte aggregates longitudinally throughout infection, and 16 SIV-infected pigtailed macaques were compared with preinoculation baselines and to 9 uninfected controls. Platelet production was assessed using mean platelet volume and platelet reticulation, and platelet autoantibodies were measured using ELISA. During acute SIV infection, circulating platelets were activated with increased surface expression of P-selectin, CD40L and MHC Class I. Platelet production was maintained and platelet autoantibodies were not detected during acute infection. Concurrent with a decrease in platelet numbers and an increase in the percentage of activated platelets, platelets were found sequestered in platelet-monocyte aggregates (PMAs), thereby contributing to decline in platelet counts. As the majority of circulating CD16+ monocytes formed complexes with platelets during acute SIV infection and CD16+ monocytes contribute to the pathogenesis of HIV infection by harboring virus and by traversing across endothelium to enter tissues, platelet decline may represent platelet participation in the innate immune response to HIV.

Poster Sessions

P1 Withdrawn

P2 Effective, Reversible Contraception Program for an Owl Monkey (*Aotus* spp.) Breeding/Research Resource

AG Brady*[1], TJ Kuehl[1], B Skinner[2], LE Williams[1], GW Tustin[1], CR Abee[1]

[1]Veterinary Sciences, UT MD Anderson Cancer Center, Bastrop, TX; [2]Animal Resources Branch, Centers for Disease Control, Atlanta, GA

Responding to declining demand for animals while retaining colony arrangements that allow subsequent breeding and social housing for animal welfare is a dilemma for management of primate breeding resources. One solution is a colony contraception program using hormonal intervention. In a colony of approximately 400 owl monkeys, all breeding females (approximately 70 animals) received 4.5 mg IM of medroxyprogesterone acetate. Frequency over 2 y was increased from every 3 mo to every 4 wk as safety and efficacy were assessed.

Pregnancies declined from a mean of 84 ± 0.7 for the previous 4 y to 53 for year 1 and 34 for year 2 of contraception, no adverse effects were noted at any of the frequencies used. After contraception for 2 y, a subgroup of 15 females was removed from the program for a research study. Within 5 wk 2 of the females became pregnant. Within 7 mo, all 15 were pregnant. This effective, reversible contraception program gives colony management added flexibility in responding to changes in demand for owl monkeys.

P3 Genetic Diversity of *Klebsiella pneumoniae* Isolates during an Outbreak in a Nonhuman Primate Research Colony

A Gozalo*[1], WR Elkins[1], L Lambert[2,3], F Stock[4], R Woodward[5]

[1]Comparative Medicine Branch, [2]Laboratory of Malaria Immunology and Vaccinology, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, MD; [3]SoBran, Bethesda, MD; [4]Department of Laboratory Medicine, Clinical Center, National Institutes of Health, Bethesda, MD; [5]Research Animal Medicine Branch, National Institute of Child Health and Human Development, National Institutes of Health, Dickerson, MD

Klebsiella pneumoniae is an important bacterial pathogen that threatens the viability of captive nonhuman primate research colonies. Numerous outbreaks with pathogenic strains have been reported causing peritonitis, pneumonia, and septicemia. Neotropical primates are known for being particularly susceptible. Eleven *K. pneumoniae* isolates, obtained during an outbreak in an owl monkey research colony at the National Institute of Allergy and Infectious Diseases, National Institutes of Health, where DNA fingerprinted by automated repetitive extragenic palindromic-polymerase chain reaction (rep-PCR). The obtained profiles where compared with samples obtained from other nonhuman primate species during the same time period. Pearson correlations between aligned rep-PCR profiles revealed 7 *K. pneumoniae* different types (<95% similarity) were circulating in the owl monkey colony at the time of the outbreak. When compared with relatedness to the squirrel monkey isolate there was 92% and 83% similarity; when compared with capuchin monkey isolates there was 75% and 62% similarity; and when comparing the squirrel monkey isolate to the capuchin monkey isolates there was 62% similarity. These results agree with recent reports that *K. pneumoniae* nosocomial isolates in hospital settings can have high genetic diversity. This potential genetic diversity should be considered when designing strategies for controlling *K. pneumoniae* outbreaks in captive nonhuman primate colonies.

P4 Fecal Impaction in a 5-Month-Old Female Mouse

AC Jones*[1], KR Strait[1,2], CL Courtney[2]

[1]Division of Animal Resources, [2]Department of Pathology and Laboratory Medicine, Emory University School of Medicine, Atlanta, GA

A 5-mo-old female transgenic mouse (hSERT -/-) was reported for fecal impaction. The mouse was housed in a ventilated cage with 4 other female mice, of which 3 were littermates. On physical examination, the mouse was bright, alert, responsive, and in good body condition. The perineal region was moderately distended and the orifice of the vulva was twice the normal size. Also there was no anal orifice. Palpation of the perineum revealed abdominal discomfort and voiding of feces from the vulva. All other mice appeared healthy and were defecating normally. Differential diagnosis included congenital disorder, trauma, infection, or neoplasia. The mouse was euthanized due to poor prognosis. Gross necropsy revealed that the ventral wall of the rectum terminated into the dorsal wall of the vagina, and feces were within the vaginal canal. Microscopically, transition of colonic mucosa to vaginal squamous epithelium was present with focal mucosal erosion and minimal neutrophilic inflammation. The urinary tract was unaffected. Gross and histopathologic findings were consistent with spontaneous rectovaginal fistula with anal atresia. Rectovaginal fistula is a congenital defect characterized by the vulva functioning as the opening for the urogenital and gastrointestinal tracts. Rectovaginal fistula is typically associated with type II atresia ani, in which the rectum terminates in a blind pouch immediately cranial to an imperforate anus. Both disease processes are rare but have been documented in several species including a few strains of genetically engineered mice. Here we report an unusual case in a hSERT -/- mouse.

P5 Necrosuppurative Mastitis in a Sprague–Dawley Rat Following a Prematurely Weaned Litter

AD Pavan*, C Freed

Lab Animal Resources, The Ohio State University, Columbus, OH

A 5-mo-old female Sprague–Dawley rat presented with piloerection, hunched posture, and porphyrin staining at the external nares and left eye. The dam was singly housed in a microisolation cage on a ventilated rack. Corncob bedding was available and the cage was changed weekly, with ad libitum access to reverse osmosis water and standard rodent chow. The dam had given birth 17 d prior to presentation, but the litter had been euthanized at 1 d of age for study purposes. Physical examination identified 2 firm masses closely associated with the mammary gland; one on the left lateral neck and the other cranial to back right hindlimb on the flank. The first pectoral mammary papilla on the left was dark in color. The rat was euthanized and tissues submitted for pathology. Histologic findings support multicentric necrosuppurative mastitis associated with myraiad gram-positive cocci consistent with *Staphylococcus aureus* and *Streptococcus pneumonia* infection. Predisposing factors for the development of mastitis include mammary gland congestion, trauma, and poor sanitary conditions. Mastitis can develop in breeding females of any species but the second edition of Lab Animal Medicine only addresses mastitis in ferrets and agricultural species (cattle, goats, sheep, and swine). Very rarely has spontaneous mastitis been reported in the laboratory rat. One report (1994) identified granulomatous dermatitis due to *S. aureus* in 2 Sprague–Dawley rats and one (1978) identified chronic necrotizing mastitis in F344 rats caused by *Pasteurella pneumotropica*. Laboratory rats used in biomedical research require strict sanitation frequencies that may decrease the likelihood of bacterial invasion following trauma. It is unclear if the damage to the mammary papilla was a primary or secondary finding but, early weaning of offspring leading to mammary gland congestion may have placed this rat at risk and should be considered when managing breeding colonies.

P6 Case Report: Plasmid DNA Embolism in C57/BL6 Mice

AG Chang*, G McKeon, R Luong

Stanford University, Stanford, CA

Five mice injected intraveneously with plasmid DNA in Hanks balanced salt solution (HBSS) resulted in systemic embolism and subsequent death. Histologically, it shares some features with acute tumor lysis syndrome (ATLS). Specifically, the lumina of capillaries, arterioles, and/or arteries of varying calibers within affected organs and tissues contained precipitated DNA that appeared as variably sized aggregates of homogenous moderately basophilic acellular material. ATLS is caused by the rapid destruction of malignant cells with subsequent massive release of cellular contents and breakdown products that overwhelms normal excretory and cell buffering mechanisms producing an acute metabolic crisis and/or disseminated embolism. ATLS is typically seen in association with intensive chemotherapy of large, highly proliferative lymphoid neoplasms; however, the incidence of ATLS is increasing in a variety of tumor types and can be seen in conjunction with corticosteroids, hormones, or cytokines. There have been several studies with systemic administration of plasmid DNA in mice; however, this is the first report of subsequent plasmid DNA embolism postinjection.

P7 Experimental Infection of *Salmonella enterica* in New Zealand White Rabbits

A Panda*[1], I Tatarov[1], A Crusan[1], B Masek[2], J Hardick[2], T Wakefield[2], K Carroll[2], S Yang[2], Y Hsieh[2], R Rothman[2], CA Gaydos[2], LJ DeTolla[1]

[1]Department of Pathology, Program of Comparative Medicine, University of Maryland School of Medicine, Baltimore, MD; [2]Division of Infectious Diseases, Johns Hopkins University School of Medicine, Baltimore, MD

Outbreaks caused by *Salmonella enterica* serotypes have resulted in high morbidity and mortality rates, especially in developing countries. The goal of this study was to develop and characterize an animal model to study *Salmonella* disease pathogenesis and subsequently demonstrate the utility of a novel PCR-based assay for early rapid detection of low level *Salmonella* bacteremia. Ten New Zealand white rabbits (females, 3 to 5 kg) were used in this study. Fecal samples from all rabbits were prescreened and tested negative for presence of *Salmonella* spp. prior to initiation of the study. Two rabbits received 1 × 1012 CFU/mL and 8 rabbits received 1 × 1013 CFU/mL of *Salmonella enterica* serovar Enteritidis by intraperitoneal injection. Blood, peritoneal fluid, and tissue samples (heart, liver, lung, sections of the gastrointestinal tract) were collected at euthanasia. All samples were subjected to microbiologic culture and a 16S PCR assay for detection of bacterial DNA. Blood, peritoneal fluid and tissue samples tested *Salmonella*-positive by culture. All peritoneal fluid samples and 88.2% of the blood samples tested positive for *Salmonella* DNA by PCR. All rabbits displayed clinical signs of disease (fever, weight loss, lethargy). At necropsy, rabbits exhibited gross pathologic findings (friable/discoloration of liver, congested heart, discoloration of spleen, and areas of hemorrhage in the gastrointestinal tract). Histopathologic findings included multifocal inflammatory areas in the lungs, ileum, and cecum coupled with extensive areas of necrosis and hemorrhage in the livers of infected rabbits. An animal model of bacteremia/septicemia induced by *Salmonella* infection in rabbits was developed. 16S PCR was capable of detecting bacterial nucleic acid from blood, tissues, and body fluids of infected rabbits. This assay could prove to be useful in rapid detection and identification of *Salmonella enterica* infection in rabbits. Furthermore, this approach seems promising to be used as a diagnostic tool to detect salmonellosis in humans.

P8 Evaluation of the Effect of Early Age Anesthesia for Genotyping of Mice on Body Weight

A Fletcher*, KL Stewart

Freimann Life Science Center, University of Notre Dame, Notre Dame, IN

The use of tail biopsy is a widely accepted method of sampling tissue for genotype determination of mice. It is often argued that use of anesthesia for this procedure in young mice is either not necessary or may affect the vigor of the mice as they grow. This is a critical issue because for many studies it is required that the mice be genotyped as young as possible. In this study, we evaluated the use of administration of isoflurane anesthesia for tail biopsy in neonatal mice at various time points after birth (1, 3, 5, 7, 9, and 11 d). The 1-d time point was especially important to determine if sufficient DNA could be extracted from the tail biopsy at this early age. Three treatment groups were used; mice biopsied with the use of isoflurane; mice biopsied without the use of isoflurane; and control mice that had underwent neither biopsy nor isoflurane anesthesia. Body weights of the 3 groups were monitored over a 10-wk period. There were no statistically significant differences in mean body weights between the treatment groups. Further, we found that sufficient DNA for genotype analysis was procured from tail biopsies in animals as young as 1 d of age. These findings have significant implications as they contend that the determination of the genotype can be undertaken at a very early age of the animal, with no discernible long-term harm to the animal if isoflurane anesthesia is withheld.

P9 A Bilateral Lower Eyelid Entropion in 1-Year-Old Intact Male Cat

B Reddyjarugu*, R Rajoria, TJ Pavek, BS Blank

Center for Animal Resources and Education, Cornell University, Ithaca, NY

A 1-y-old, intact male domestic short-haired cat (DSH) singly housed in a cubicle presented with severe squinting of the left eye and with no other significant clinical findings. Ophthalmic examination revealed moderate, serous to mucoid ocular discharge, blepharospasm and conjunctivitis with sporadic minimal inward rolling of the eye lid margin (entropion). Intraocular pressure and tear production were within the normal limits. Fluorescein eye stain revealed moderate, focal corneal ulceration of approximately 0.5 cm diameter near the corneal limbus. Feline herpesvirus fluorescent antibody test on air-dried ocular smear was negative but cannot be ruled out as the sensitivity of this test is low. Despite long-term topical antibiotic (5-wk duration) and antiviral (2-wk duration) therapy, minimal resolution of the corneal ulceration was noted. This later progressed to a marked lower eyelid entropion which persisted after topical anesthesia. Hotz-Celsus procedure was performed for entropion on the left eye followed by 2-wk postoperative treatment (topical application of triple antibiotic ophthalmic ointment and use of an Elizabethan collar). The ocular conditions of the left eye were fully resolved within 2 wk postsurgery. Three weeks postsurgery the cat developed spastic lower eyelid entropion in the right eye, which was successfully corrected using a similar surgical procedure and was resolved within 2 wk postsurgery. Developmental or primary entropion is a rare condition in DSH cats in both the private practice and research settings. Thus, we conclude that the cat in our case had spastic entropion secondary to painful ocular disease and the entropion had persisted beyond the inciting cause, possible infectious etiology or noninfectious ulcerative keratitis. This has been referred to in literature as spastic-cicatricial entropion.

P10 Collapse and Dyspnea in a Sugar Glider (*Petaurus breviceps*)

BH Philips*[1], JH Fried[1], J Jensen[1], JB Lok[2], TJ Nolan[2], AK Brice[1]

[1]University Laboratory Animal Resources, [2]Pathobiology, University of Pennsylvania, Philadelphia, PA

A 10-y-old male sugar glider (*Petaurus breviceps*) was found collapsed and dyspneic on the floor of his cage. The animal had a history of experimental *Parastrongyloides trichosuri* infection in 2009 (no longer thought to have a patent infection) and a benign basal cell tumor that was surgically removed with clean margins in spring 2011. Due to his condition, he was euthanized and submitted for postmortem examination. Grossly, there were multiple raised tan nodules throughout the hepatic parenchyma and an enlarged right atrium. Microscopically, there was moderate neutrophilic inflammation surrounding the root of a maxillary molar with intrapulpal bacteria, septic thrombi in the right atrium, pulmonary vessels, and vessels of the head, and nodular hepatic lipidosis. The source of infection is suspected to be the dental disease, with hepatic lipidosis secondary to decreased appetite. Dental disease is a risk factor for the development of septic embolic disease in other species, and is common in captive sugar gliders. It should be considered a differential for any sugar glider with decreased appetite. Diet optimization, regular oral examination, and close weight monitoring may be helpful in addressing dental disease prior to onset of sequelae such as those described here.

P11 Standardized Method to Evaluate Chronic Eye Lesions in Transgenic Mice

CN Mallow*, E Shukan, ND Hitt

AHCS, NIH/NINDS, Bethesda, MD

Use of transgenic mice and rats continues to increase, and with this comes an increase in unanticipated phenotypes. Clinically, challenges include providing consistent evaluations between animal care staff and communicating the severity of the condition to our scientists. We currently have a strain of glial fibrillary acidic protein-thymidine kinase (GFAP-tk) transgenic mice which exhibit periorbital inflammation exacerbated by stress. Periorbital inflammation presents as blepharitis and can progress from mild alopecia and inflammation to lesions extending below the eyes down the cheeks. Lesions are refractory to topical treatments and systemic treatment is prohibited due to study restraints. To address this we developed a grading scale (0 to 5), with the medical endpoint agreed upon as the appearance of lesions below the eyes. To assist in consistent grading of lesions we photographed mice with eyes at different stages of severity and photographs were assigned specific grades to provide guidance for staff training on staging the lesions. We designed a card for recording health observations that is color-coded and taller than the regular cage card so it can be placed behind it; this facilitates identification when viewing the rack. To begin the process, affected animals are identified, eye lesions are graded, and weekly eye evaluations by a technician begin. Information

recorded includes dates of weekly checks, grade for eye lesions of the most severely affected animal in the cage, initials of person performing observations, and a comments section. Because treatment is not allowed, technicians perform weekly checks until the eyes of at least one animal in the cage receive a moderate-severe rating at which time a clinical record is started and a veterinary check is required. The use of a grading scale and study-specific supplementary cage cards has provided specific clinical parameters to guide our technicians in their assessments and has decreased the paperwork required. In addition, the lesion grading scale has allowed the clinical staff to communicate more effectively with the research staff by providing a more consistent and objective scale to evaluate the eye lesions.

P12 Use of Transabdominal Ultrasonography to Determine Fetal Characteristics and Viability of Prion-Infected Muntjac Deer

CD Ledesma Feliciano*, KD Walton, E McNulty, J Hayes-Klug, A Nalls, K Anderson, C Mathiason

Microbiology, Immunology, and Pathology, Colorado State University, Fort Collins, CO

The Reeves muntjac deer (*Muntiacus reevesi*) is a small South Asian cervid species used as prion transmission and pathogenesis model. Vertical transmission has arisen as a specific area of interest for researchers; thus, necessitating insight into maternal reproductive efficacy and fetal viability during infection. This approach will require use of imaging techniques, namely ultrasonography, to collect information on fetal growth and development. Here we describe a transabdominal ultrasonographic technique for assessing fetal growth characteristics and viability in muntjac does. The does had their estrus cycle synchronized with 2 PGF2 α injections given 11 d apart and verified through vaginal cytology that displayed sperm and increased superficial epithelial cells indicating estrus. Evaluations to date have demonstrated pregnancy as early as 35 d after the second PGF2 injection, with embryos measuring between 0.5 to 1 cm in length and displaying fetal heart movement. Fetal mineralization was apparent at approximately 60 d with a fetal crown-rump distance of 3 to 4 cm. At 90 d, the crown-rump distance was 8 to 9 cm. This imaging modality proved to be beneficial in evaluating the fetal development in this cervid species. Current and future goals of this study include reliably detecting pregnancy in muntjac does, continued assessment of fetal development throughout gestation, assessment of fetal variables to estimate gestational time, and identification of any differences in fetal characteristics or viability in various control and experimental protocols of prion infection.

P13 Successful Use of Medicated Diet to Eradicate 2 *Helicobacter* spp. from Mice with Adaptive Immune Deficiencies

CM Garrett*, D Muth, J Watson

Molecular and Comparative Pathobiology, Research Animal Resources, Johns Hopkins, Baltimore, MD

Eradication of *Helicobacter* spp. following the use of a commercial 4-drug diet has been demonstrated in immune competent mice and those with deficiencies in innate immunity. However efficacy has not been described in mice with alterations in the adaptive immune system. We hypothesized that treatment with 8 wk of medicated diet would eradicate *Helicobacter* spp. from young mice lacking functional T cells (*Foxn1*^{nu}) or T and B cells (B6.129S7-*Rag1*^{tm1Mom}/J). We evaluated *Helicobacter* status, weight, and gross pathology for medicated compared with control diet in groups of infected and uninfected animals at 8 and 16 wk after treatment initiation. Mice born inhouse from a colony enzootically infected with *H. hepaticus* and *H. typhlonicus* were tested and confirmed positive by PCR of fecal samples at 4 and 6 wk age prior to study initiation at 7 wk. PCR testing demonstrated that independent of strain and sex, all treated mice tested negative for *Helicobacter* spp. after 4 wk of treatment and remained negative for the duration of the study. All untreated mice remained *Helicobacter* spp. positive. Mice that received medicated diet had enlarged ceca after 8 wk of treatment; however, cecal size returned to normal after a further 8 wk on regular diet. Irrespective of infection status, *Foxn1*^{nu} and B6.129S7-*Rag1*^{tm1Mom}/J mice that received medicated diet gained less weight than did their untreated controls over the 8-wk treatment period. *Foxn1*^{nu}mice but not B6.129S7-*Rag1*^{tm1Mom}/J mice normalized their weight over the 8-wk period following treatment. There were no clinical signs of disease or mortalities observed in any animals during the 16-wk study period. To our knowledge, this is the first report that demonstrates the efficacy of medicated diet for eradication of *Helicobacter* spp. in mice with adaptive immune deficiencies.

P14 *Corynebacterium bovis* Surveillance and Rapid Detection by Sampling Individually Ventilated Caging Rack Exhaust Air Manifolds

C Manuel*[1,2], U Pugazhenthi[2], S Spiegel[1], W Mirach[1], B Waldmann[1], J Leszczynski[1,2]

[1]Office of Laboratory Animal Resources, [2]Department of Pathology, University of Colorado Denver, Aurora, CO

Corynebacterium bovis is an opportunistic infection of nude (*Foxn1*, nu/nu) mice that affects many academic and industry research facilities. Once present, it is highly contagious and extremely difficult to eliminate. Moreover, soiled bedding sentinel programs are not designed for the rapid detection required to monitor eradication efforts. To achieve more rapid *C. bovis* detection, swabs were collected from the horizontal exhaust manifold (HEM) of an individually ventilated caging (IVC) system and evaluated by quantitative PCR. First, rack sanitation methods were assessed for their capability to eliminate *C. bovis* DNA. Forty percent of *C. bovis*-exposed racks, which had housed enzootically infected colonies, tested positive at the HEM following sanitation by rackwash only (n = 5). After autoclaving, all sanitized racks tested negative for *C. bovis* by qPCR (n = 5). HEM sampling for surveillance-based testing requires IVC rack air ventilation; thus, rates of supply and exhaust air movement were recorded. Rack ventilation remained consistent throughout the study with a supply airflow range of 18.6 to 22 ft^3/min and an exhaust airflow range of 31.7 to 33.4 ft^3/min, corresponding to approximately 35 to 40 air changes per hour at the cage level. To determine the rate of *C. bovis* detection and the infected mouse detection threshold of the HEM sampling technique, a cage containing either 1 or 5 experimentally infected, male, nude mice was placed at the first or last cage position on the bottom row of a 70-cage IVC rack. Sterile, dry swabs were used to sample the corresponding HEM beginning 24 h after cage placement. At 24 h, all racks containing a single *C. bovis* positive cage housing either 1 (n = 4) or 5 nude mice (n = 3) were positive. All subsequent samples remained positive during the 4- to 8-d sampling period. Neither the number of mice per cage nor cage proximity to the HEM affected the rate of detection. The cage position closest to the HEM resulted in a significantly higher copy number of *C. bovis* DNA for trials containing 5 mice per cage only ($P < 0.05$). Our findings suggest that HEM sampling is a promising method for routine surveillance and rapid detection of *C. bovis* in nude mouse colonies.

P15 Hepatocellular Carcinoma in a Nile Grass Rat (*Arvicanthis niloticus*)

CC Hofer*[1], BN Bolon[2], D Coble[1]

[1]University Laboratory Animal Services, [2]Veterinary Biosciences, Ohio State University, Columbus, OH

The Nile grass rat (*Arvicanthis niloticus*) is a USDA-covered species that is increasingly used as a research animal model. Nile grass rats are diurnal rodents used to study a variety of research applications ranging from spontaneous metabolic syndrome to retinal diseases. A 23-mo-old male Nile grass rat used in a diet manipulation study was found laterally recumbent and dyspneic. Moderate dehydration and hypothermia were detected upon physical examination. Treatment included thermal supplementation, subcutaneous fluid administration, and oxygen supplementation. Clinical appearance did not improve despite supportive measures and the animal was euthanized and submitted for necropsy. Gross lesions included a large (3.7 × 2.8 × 1.5 cm) multilobulated hepatic mass and bilateral polycystic kidney disease. Significant microscopic findings included a metastatic hepatocellular carcinoma, myocardial degeneration of the left ventricle and septum with a chronic left atrial thrombus, and chronic amyloidosis of the kidneys leading to

protein-losing nephropathy. Thrombi were also noted in the pulmonary microvasculature. A leukocytosis with increased numbers of degenerative neutrophils, nucleated RBCs, and reduced platelets was detected on the complete blood count. Severe hypoglycemia (4 mg/dL), along with increased hepatic and renal values was noted on the serum chemistry profile. Collectively, these findings describe a severely debilitated and diseased animal and adequately explain the clinical presentation. Several other incidental findings represent findings consistent with degenerative changes in an aged grass rat (IVD and sternum cartilaginous degeneration). To the authors' knowledge, this is the first case of hepatocellular carcinoma reported in a Nile grass rat. This case presents several findings that when combined with examination of younger and clinically normal animals will help to develop a reference database for this unique research animal species.

P16 Idiopathic Alopecia in Male Grey Short-Tailed Opossums (*Monodelphis domesticus*)

CC Hofer*[1], JD Harder[2], C Freed[1]

[1]University Laboratory Animal Services, [2]Evolution, Ecology, and Organismal Biology, Ohio State University, Columbus, OH

A small colony (2 female, 18 male) of grey short-tailed opossums (*Monodelphis domesticus*) obtained from a long-standing closed colony were used for a mammology course to demonstrate mating behavior. The animals were individually housed in standard laboratory rat cages with wire tops and wood chip bedding on a 14:10-h light:dark cycle. Temperature and relative humidity were maintained at 75 ± 5 °F and below 25%, respectively. Four of the male opossums (age 15 to 20 mo) presented with a localized, patchy alopecia primarily on the dorsal pelvic region and partially extending laterally over the rear limb. The underlying dermis was not inflamed, crusted, or irritated and the lesions did not appear to be pruritic. No broken hairs, indicative of barbering, were noted. Noninvasive diagnostics (tape test) did not identify ectoparasites. Following course completion, males were euthanized and gross necropsies performed on animals with lesions and nonaffected age matched animals. Gross necropsies identified no significant findings and additional diagnostics were performed; skin scrape, pelt exam, and hair pluck for dermatophyte test medium. All tests were negative. A complete necropsy and CBC was conducted. CBC findings were not clinically significant. Histologic analysis of the skin lesions revealed GMS-positive, round yeast structures, 1 to 3 µm in diameter on the epidermal surface, within the keratin and/or within the follicular infundibulum of 2 skin sections. Based on the histologic examination of the tissues, it is unclear if the yeast represents an incidental finding or the causative agent of the clinical presentation of alopecia. Anecdotally within the originating colony, alopecia has been noted as an age change in both males and females over 1 y of age.

P17 Granular Cell Tumor in a DBA/2J Mouse

C Boehm*, CS Roberts, D Gonzales

LARC, Indiana University, Indianapolis, IN

A 10-mo-old female DBA/2J female breeder mouse presented with a swollen cranium and severe cranial alopecia. No signs of neurologic deficits were noted, and until recently, she had been breeding and delivering pups with no complications. An abscess was suspected, so the animal was euthanized. At necropsy, the calvarium was encased between a mass and the brain. The mass was smooth, white, and shiny, and grossly appeared to resemble neural tissue. The pericardium also had dystrophic mineralization. On histology, the mass was identified as a granular cell tumor. The mass was composed of sheets of round to polygonal cells that encompassed the bone of the calvarium and the underlying meninges to extend to the dorsal surface of the brain. Areas of osteolysis and bone fragmentation of the calvarium were present within the mass. The cells had distinct borders and abundant eosinophilic, granular cytoplasm. The nuclei were slightly eccentric and hyperchromatic with rare mitotic figures. The mass was moderately vascular and contained multifocal to perivascular foci of lymphocytic inflammation. The underlying brain was essentially normal. Although this is the most common primary CNS tumor of the rat, few reports in mice are found, most of which are confined to the genital area. The mass is believed to be meningeal in origin.

P18 Rats Undergoing Category E 6-Hydroxydopamine Administration Surgery Regain Body Weight Lost at Surgery Faster When Provided with a Soft, Bacon-Flavored Recovery Diet Compared with Regular or Wet Feed

CM Weiner*[1], MA Wilwol[1], JL Lecker[2], KM Froberg-Fejko[2], JC Smith[3]

[1]Surgical Services, Taconic Farms, Rensselaer, NY; [2]Bio-Serv, Frenchtown, NJ; [3]Veterinary Bioscience Institute, Winston-Salem, NC

Surgical procedures produce stress in laboratory animals. Proper nutrition postoperatively is critical to their recovery in order to minimize weight and protein loss. This study investigated whether providing nutritional supplementation to rats undergoing a unilateral 6-hydroxydopamine lesion of the nigrostriatal pathway would improve body weight gain after surgery. We hypothesized that animals that received a soft, bacon-flavored, nutritionally complete recovery diet (recovery diet), would regain body weight lost at surgery more quickly than animals provided regular feed or feed softened with water. Forty-eight male NTac:SD rats were used and were divided into 6 weight- and age-matched groups. Groups C through F were modified with the 6-OHDA procedure according to standard institutional protocols. The animals were anesthetized with isoflurane in oxygen, received 30 mL/kg fluid support at the time of surgery and a lidocaine/bupivicaine local block at the incision site. The groups received the following treatments: A) feed and water (no surgery); B) feed, water, and recovery diet (no surgery); C) feed and water; D) feed, water, and wet feed; E) feed, water, and recovery diet, acclimated to the recovery diet 2 d prior to surgery; F) feed, water, and recovery diet. All animals and feed were weighed daily for 7 d after surgery. Groups C through F animals received a rotational evaluation at days 14 and 21. There was a significant decrease in body weight in group C and D animals compared with controls through day 7 ($P < 0.001$). There were no differences in body weight in group E and F animals compared with group B by days 4 and 5, respectively and to group A by days 5 and 7, respectively. There were no differences in spin rates between groups. These data suggest that a refinement in clinical postoperative care by providing nutritional support may decrease recovery time in category E procedures in rats.

P19 Anesthetic Emergency Algorithms for Improved Crises Management

C Lockworth*

MD Anderson Cancer Center, Houston, TX

Veterinary medicine has the same rare but inevitable challenges faced during anesthesia as those found in human medicine. A routine, uneventful anesthetic event may, with little warning, become an emergency. Fortunately, anesthetic emergencies are uncommon; however, their infrequency poses additional challenges to veterinarians and staff. It may be difficult to hone the skills needed to maintain a calm, thoughtful, and deliberate execution of necessary steps in the event of an emergency situation. We found that algorithms exist, but none specifically addressed the needs of our diverse research animal population, and more specifically, the scope of emergencies that may occur in our animals during anesthesia. To support our veterinary staff during these stressful events, we developed a set of anesthetic emergency algorithms aimed specifically for the resolution of anesthetic issues in our small and large animal research population, which includes primates, swine, canines, rabbits, guinea pigs, and rats. Our goals were to provide a simple and succinct set of guidelines for the early detection of problems, resolution of issues without treatment when possible, and treatment when necessary. In addition, quick drug dose reference charts are embedded within algorithms, making treatments more efficient. Crises that are addressed in the algorithms include cardiac arrest, bradycardia, tachycardia, respiratory distress, seizure, and anaphylaxis. As a result, the quality of animal health care has been advanced. Veterinarians and staff have an additional tool at hand for managing crises, providing not only a sense of security to

the individual managing the crisis, but also an overall improvement in animal health due to improved treatment for the animal. Additionally for the future, the algorithms may be used as a training tool for incoming veterinary staff.

P20 Surface Disinfection of Exam Gloves as an Alternative to Using Sterile Surgeon's Gloves for Rodent Survival Surgeries

D Lemoine*[2], C Freed[1], VK Bergdall[1]

[1]University Laboratory Animal Resources, The Ohio State University, Columbus, OH; [2]Ontario Veterinary College, University of Guelph, Guelph, ON, Canada

Adherence to aseptic technique is required for all survival surgical procedures in rodents and generally includes the use of sterile surgical gloves to limit contamination of the surgical site by the surgeon's hands. To explore potential alternatives to sterile surgeon's gloves, this study examined the effectiveness of 70% isopropyl alcohol and a hydrogen peroxide and peracetic acid solution (HP/PA) in disinfecting standard exam gloves, when hands were either unwashed or washed with antibacterial soap ($n = 8$ for each combination of glove type, hand washing, and disinfectant). The fingers of exam gloves were swabbed to evaluate microbial growth after donning, after disinfection, and after an activity designed to simulate manipulations that would occur during surgery. Swabs were incubated in BHI broth 24 h and evaluated for growth. No significant effect of glove type or hand washing condition was found, so data was pooled to evaluate the effect of each disinfectant. Following disinfection, 99.97% of gloves sprayed with HP/PA were negative for growth while only 56.2% of gloves sprayed with alcohol were negative. There was no significant difference between the postactivity contamination rate of gloves disinfected with HP/PA (0.03%) and sterile gloves, while contamination rates on the gloves disinfected with alcohol (25%) were significantly greater than that of sterile surgical gloves ($P < 0.05$). In summary, our data supports HP/PA disinfection of exam gloves as a viable cost-effective alternative to the use of sterile surgical gloves for rodent surgeries and discourages the use of 70% isopropyl alcohol for glove disinfection.

P21 Autoclaving Exam Gloves as an Alternative to Using Surgeon's Gloves for Rodent Survival Surgeries

D Lemoine*[2], C Freed[1], VK Bergdall[1]

[1]University Laboratory Animal Resources, The Ohio State University, Columbus, OH; [2]Ontario Veterinary College, University of Guelph, Guelph, ON, Canada

Adherence to aseptic technique is required for all survival surgical procedures in rodents and generally includes the use of sterile surgical gloves to limit contamination of the surgical site by the surgeon's hands. Many institutions require the use of surgeon's gloves specifically designed for survival surgery. Alternatives to glove recommendations are acceptable provided that they meet the same performance standards. To explore potential alternatives to surgeon's gloves, this study examined the effectiveness of steam sterilization of standard exam gloves, latex and nitrile, based on performance. Exam gloves were sterilized in either an autoclave pouch or wrapped in a surgical towel, with or without surgical instruments, then tested for integrity and performance. A glove performance test was conducted by placing a line of 5 simple interrupted sutures on a suturing model while wearing the gloves to assess whether they withstand manipulation of surgical instruments. Prior to and following this performance test, a stretch test was conducted by manually stretching each finger and the cuff of the glove, with a visual inspection to identify glove defects. Following steam sterilization, 100% of the nitrile exam gloves and 77.2% of sterilized latex exam gloves did not melt and were able to be donned by the surgeon, compared with 100% of the nitrile and latex sterile surgeon's gloves. Overall, 95% of the sterilized nitrile exam gloves, 76.5% of the sterilized latex exam gloves, 87.5% of the nitrile sterile surgeon's gloves and 100% of the latex sterile surgeon's gloves were usable. Pressure tests evaluating whether gloves inflated with oxygen to 15 PSI maintained pressure were completed at study end for a subset of usable gloves but data is still being analyzed. In summary, the use of steam sterilization of nitrile exam gloves is a viable cost-effective alternative to the use of sterile surgical gloves for rodent survival surgeries.

P22 Outbreak of High Mortality in a Northern Leopard Frog (*Lithobates pipiens*) Colony

D Petkov*[1], D Martin[1], D Morck[1], R Hampton[1], P Johnson[3], B LaFonte[3], L Ward[2]

[1]Animal Health Unit, University of Calgary, Calgary, AB, Canada; [2]Infection Prevention and Control Research Lab, Alberta Health Services, Calgary, AB, Canada; [3]Ecology and Evolutionary Biology, University of Colorado, Ramaley, CO

Northern leopard frog (*Lithobates pipiens*) is a semiterrestrial amphibian from the Ranidae family. Habitat loss and environmental contaminants (pesticides, polycyclic aromatic hydrocarbons, and heavy metals) have contributed for the population decline of this species. Northern leopard frog is a valuable research model used in oncology and neuroscience. The 2 modes of frog locomotion (jumping and swimming) also allow for studies on muscle performance. While there are well-defined guidelines for housing, care, and breeding of *Xenopus* spp. in research settings, these standards for Northern leopard frog are vague. In addition, most Northern leopard frogs used in research are wild caught and with unknown health status. Higher overall mortality (67.8%) in a Northern leopard frog colony housed in our indoor laboratory vivarium was observed between March and June of 2011, in comparison to the same period in 2010, when mortality of 1.1% was recorded. We wanted to identify the cause of mortality and implement measures intended to reduce losses and minimize the likelihood of future occurrence. Postmortem examination and parasitic identification were performed along with bacterial isolation, speciation, and antimicrobial sensitivities tests. Several factors could have contributed to the increased mortality such as environmental (higher housing density and organic material in the water), nematode *Rhabdias ranae*, trematodes *Haematoloechus* spp., *Clinostomum* spp., and *Alaria* spp., cestodes *Mesocestoides* spp. and *Acanthocephalans* spp., and multidrug resistant bacteria *Aeromonas hydrophila* and *Achromobacter* spp. Since August of 2011, mortality similar to that seen in 2010 was maintained following reduction of the housing density, frequent water replacement, change of vitamin supplements, and exclusion of the grain husks used for rearing mealworms. This case illustrates the challenges faced when wild-caught animals are housed in a controlled environment of a laboratory vivarium.

P23 Use of Radio-Frequency Identification to Monitor Internal Temperatures of Beagles in Variable Environmental Conditions

D Bengochea*[1], B Kirch[1], LV Kendall[2]

[1]Department of Animal Sciences, [2]Labratory Animal Research, Colorado State University, Fort Collins, CO

A study with 20 beagles was designed to test radio-frequency identification (RFID) chips with temperature sensing technology to monitor body temperatures under different environmental conditions. Twenty beagles ranging from body score 2.5 to 4 were implanted subcutaneously with a RFID technology. Half of the beagles were implanted between the shoulders and the other half were implanted in the left side of the neck to measure the potential of differences due to placement of the chips. The beagles were allowed to acclimatize and readings were taken remotely when they drank from their water bowl. Rectal temperatures were taken at regular intervals to compare to the implant temperatures in addition to environmental temperatures. The dogs were tested at room temperature and with additional heat. Temperatures for the room temperature study average 17.8 °C. The rectal temperatures for the dogs in this study averaged 38.6 °C while the RFID readings averaged 37.08 °C. The RFID consistently read lower with only slightly higher levels of variation. When the dogs were exposed to heat (average temperature of 31.63 °C) the rectal temperatures were again higher than the RFID values (38.59 to 37.82 °C, respectively). The variation of the readings of the rectal temperatures and the RFID temperatures was very similar. The comparison of the placement of the RFID chip demonstrated only

slightly higher temperatures for the chips placed in the neck (0.37 °C difference). However, the variation in readings was 2 times higher for the chips placed in the neck compared with those placed between the shoulders. Additional studies under conditions of exercise will be performed. The results of this study showed that the RFID sensor was capable of emulating or tracking internal temperatures of dogs. When calibrated, the RFID can be a very accurate form of monitoring animals' internal body temperature without handling or invasive activities. There were not significant differences in the location of the subcutaneous chip and the readings they provide. However, calibration of temperatures of chips placed where heat dissipation is prominent may be more problematic due to the level of variation.

P24 Withdrawn

P25 α-B Crystallinopathy in Mice

DK Hirenallur-S.*, JM Snyder, L Maggio-Price, PM Treuting

Comparative Medicine, University of Washington, Seattle, WA

Three 14-mo-old female α-B crystallin/heat shock protein B2 knockout (αBC/HSPB2 KO; background strain C57BL/6J) mice presented with lethargy, tachypnea, a hunched posture, an ungroomed haircoat, and in poor body condition. The mice were submitted for diagnostic workup including necropsy and histopathology. Grossly, all 3 mice had kyphosis and one mouse had hydronephrosis of the right kidney. Microscopically, the base of the tongue and epaxial musculature showed moderate, multifocal myodegeneration with varying fibrillar size, cytoplasmic vacuolation, and perimysseal fibrosis with fatty replacement of muscle fibers. The myodegenerative findings are consistent with previously reported phenotypic characteristics of aged αBC/HSPB2 KO mice, and may have hindered the ability of these mice to eat, accounting for the poor body condition and kyphosis. Additionally, in the cases reported here, mild to moderate cardiomyopathy was noted in all 3 mice. The αBC/HSPB2 protein, a member of the heat shock protein family and molecular chaperone, is primarily expressed in the lens, but can also be found in other tissues including skeletal and cardiac muscle. However, in initial reports describing aged αBC/HSPB2 KO mice, cardiac lesions were expected, but not seen. The discrepancy of cardiomyopathy in our mice compared with previous reports may be due to differences in background strain (C57BL/6J compared with 129S6/SvEvTac X 129S4/SvJae) or gender (female compared with male). Further, there were numerous lymphoid aggregates in several tissues, including the omentum, lung, liver, and ovary, which are common age related changes in mice, especially those on a C57BL/6 background. Our findings reinforce the role that background strain, age and sex may play on the phenotype of genetically modified mice. Histopathology on age- and sex-matched littermate controls would be warranted to determine if the cardiac and age-associated lesions are genotypic or background strain related.

P26 Examinations of Calprotectin and Apoptotic Activity in the Colon of Marmosets with Chronic Diarrhea

E Takahashi*

BSI, RIKEN, Wako, Japan

The common marmoset (*Callithrix jacchus*) wasting syndrome (MWS) is a disease endemic to captive colonies and the pathogenesis is unclear. MWS is one of the most important and least understood problems in laboratory-bred marmosets. Because the most common chronic symptom is diarrhea and pathologic change is colitis, as a first step to understand the mechanisms of chronic diarrhea, we focused on examining the colon of marmosets with chronic diarrhea using biochemical marker assays and histochemical examinations. Chronic diarrhea was defined as persistent high-viscosity diarrhea for more than 2 wk. A control group consisted of marmosets without diarrhea for more than 2 mo. Four animals (one male and 3 females) aged between 46 and 73 mo were included in the marmosets of chronic diarrhea (CD). Six animals (3 males and 3 females) aged between 40 and 66 mo were included in the control. The CD group had bloody diarrhea in the fecal occult blood test. The levels of hemoglobin, hematocrit, and red blood cells were significantly lower in CD than in control. These results suggest subtle blood loss in the intestinal tract of CD. Calprotectin is used as a surrogate marker of intestinal inflammation and induces apoptosis. Fecal calprotectin ELISA test showed higher calprotectin concentrations in CD than in control. The number of white blood cells was higher in CD than in control. These results indicate that inflammatory response occurred. No significant changes were observed in albumin parameters between CD and control groups, suggesting that albumin is not a suitable marker for the identification of CD. To investigate the presence of inflammation, expression of calprotectin, and activation of apoptosis in the colon, we used histopathologic tests including HE staining, immunocytochemistry, and TUNEL assay. The CD showed higher expression of calprotectin in the extravascular neutrophils and apoptosis in the chronic colitis lesions. No internal microbiologic diseases were identified. Our results suggest that fecal calprotectin can be a useful marker for detecting and monitoring colonic inflammation in marmosets with chronic diarrhea, and marmosets with chronic colitis could be a model of inflammatory bowel diseases.

P27 Ossifying Cardiac Myxomas in 2 Sheep

E Liechty*, Y Takemoto, J Jalife, I Bergin

University of Michigan, Ann Arbor, MI

Two unrelated, approximately 1.5-y-old, castrated male, Suffolk-cross sheep presented independently with cardiac masses of the right atria. The sheep were enrolled in a study of atrial fibrillation and were implanted with right atrial pacemaker leads. On routine echocardiogram, a small, nonmobile, hyperechoic mass was noted in the right atrium of each sheep. Progressive enlargement of the masses was noted on subsequent echocardiograms over the course of 4 mo. No clinical signs associated with atrial outflow obstruction were observed in either sheep. Differentials for the masses included myxoma, myxosarcoma, or mural thrombus. At study endpoint, the right atrial masses were harvested for evaluation. Grossly, they were smooth, slightly firm, white, and located on the right atrial septal wall. Histologically, the masses were composed of proliferating neoplastic spindle-shaped cells within a myxomatous stroma. The masses also contained numerous adipocytes and well-differentiated spicules of trabecular bone. This histologic appearance was consistent with an ossifying myxoma. Myxomas are benign tumors believed to arise from mesenchymal stem cells. The occurrence of intracardiac myxomas in 2 unrelated sheep in the same cardiac implant study is unexpected. Cardiac myxomas are rare in domestic animals. In sheep, only pulmonary myxomas have previously been reported. In humans, intracardiac myxomas are not uncommon and often occur adjacent to the fossa ovale in the atrial septum, as in this report. The pathogenesis of this lesion is unknown.

P28 Outbreak of *Pneumocystis carinii* Pneumonia in HIV-1 Transgenic Nude Rats

EN Ateh*, H Davis, J Davenport, J Bryant

Institute of Human Virology, University of Maryland, Baltimore, MD

The HIV-1 transgenic rat bears a replication deficient version of HIV as the integrated transgene on the F344 background. Specifically, a 3.1 kb deletion was made in the pNL4-3 HIV proviral construct spanning HIV-1 gag and pol from bp 1443 to 4551. After observing in our lab overt signs and symptoms of HIV -1 related conditions such as neurologic deficits, kidney disease, cardiac disorders and mild to severe skin disease in the HIV-1 tg rat we hypothesize that expression of HIV-1 viral protein in the HIV-1 Tg rat is responsible for the manifestation of some clinical conditions similar to those seen in HIV-1 infected patients. To further study the effects of these HIV-1 proteins in an immunocompromised background, we created the HIV-1 nude transgenic rat by crossing the nude rat (mutation: New Zealand nude, rnuN) with the HIV-1 transgenic rat and backcrossing the offsprings for 10 generations. *Pneumocystis carinii*, an opportunistic pulmonary pathogen poses a clinical health challenge in individuals with immune deficiency, including those patients with HIV-associated AIDS. Shortly after the development of the HIV-1 Tg nude rat, we observed an outbreak of pneumocystosis within our rat colony. We will be using the immunodeficient HIV-1 tg nude rat to study, among other things, the pulmonary effects of *Pneumocystis* colonization. The HIV-1 Tg nude rats

presented clinically with wasting, loss of weight, and most pronounced dyspnea. The disease was provoked in asymptomatically naturally infected rats by further immunosuppression using intramuscular injection of dexamethasone. On necropsy, the lungs were pneumonic with dark raised patches. Histologically, the lung showed a severe interstitial pneumonia characterized by increased emphysematous material and scattered alveolar macrophages filled with foamy material. *Pneumocystis carinii* was detected using a special silver methenamine stain which demonstrated the pneumocystis organisms as large multifocal clusters within the lungs and confirmed by PCR. The similarity of the disease to human infection will make this an excellent model for studying pneumocystis pneumonia of humans with or without HIV-1 infection.

P29 An Improved Mouse Restraining Device for Tail Vein Injection

F Liu*[1], P Lee[1], Y Chen[2]

[1]Institute of Molecular Biology, Academia Sinica, Taipei, Taiwan; [2]Biomedical Technology and Device Research Laboratories, Industrial Technology Research Institute, Hisnchu, Taiwan

Creating a comfortable confinement for animals and reducing time and manpower are integral to the success of research projects that involved large amounts of repetitive intravenous injection. An improved mouse restraining device has been employed by our staff for both routine works and training courses. The device is a rigid, tapered, cone-shaped tube that has a longitudinal slit from one end to the other end. The device also includes a tab, which is attached at the small end of the tube and provides a surface for placing the tail of animal. The animal may be loaded in the tube backwards, with its tail in the slit, and backed in toward the tab. The tapered shape of the tube also allows it to adjust to animals with various sizes from young adults to obese ones. The device can be made either by using disposable 50-mL sample tubes or industrial injection molding based on the same idea. Both the handmade and the molding-produced devices have been tested using hundreds of mice and have allowed us to collect excellent and reliable results with less time and stress to the animals in comparison with available commercial products. The positive feedback from the attendees of training courses also make us believe that this improved design is applicable for conveniently and efficiently restraining animals with welfare benefits.

P30 Targeting to Overshadow Fears and Anxiety in Sinclair Miniature Swine

G Rivard*, D Brocksmith, I Stewart, GF Bouchard

Sinclair BioResources, Auxvasse, MO

Targeting refers to social nosing which is a natural behavior of miniature swine. We use targeting to replace an unacceptable behavior such as a fear response with an acceptable one that involves a touch in response to the same stimulus. The handler first finds highly prized reinforcers, often a food reward, and gradually teaches the animal that is afraid to interact with people to want to interact with people. In this case the animal is taught to "touch" a target-stick for food-reward first. It is walked a short way following the target stick and asked to "touch" the target, then reward. Once the animal can predict a reward is coming when interacting with people, we can condition a miniature swine to cooperate for SOPs such as loading a cart. Gradually over several sessions, the animal will "touch" closer and closer to the cart. Next the animal gets in, "touches" the target, and immediately exits. Eventually the animal stays in the cart with a target-touch-reward interaction coming at about every 3 s. If the animal stays calm and responds to the cue-response-reward interaction (CRR) consistently, you can add in distractions such as opening the door or people running around the cart or making distracting noises. Every week, caretakers participate in a performance on-cue (POC) tournament with a miniature swine they have trained. Pigs follow targeting cues to navigate through a maze of obstacles leading to a cart loaded with food reward. Fears and anxiety are eliminated because the animal is taught another behavior (touch) that is more enjoyable or pleasant to exhibit in the presence of the stimulus (human interactions, obstacles, and cart) that elicits the abnormal behavior, that is, a fear response.

P31 Barbering in a Colony of Nile Grass Rats (*Arvicanthis niloticus*)

H Bacon*, CC Hofer, D Coble

University Laboratory Animal Services, Ohio State University, Columbus, OH

Barbering is an abnormal grooming behavior of laboratory rodents, common in mice but also reported in rats and guinea pigs. The hair and whiskers are often plucked, pulled, or chewed by a dominant cage mate resulting in a shaved appearance. In mice and rats, the underlying epidermis appears clinically normal without signs of inflammation or irritation. Many studies have theorized that it is a social behavior; however, animals housed singly have also been shown to demonstrate barbering. Barbering was observed in a colony of Nile grass rats (*Arvicanthis niloticus*), a USDA-covered species, used in a behavioral research study. Animals were separated by gender and group-housed following weaning in conventional rat cages with wire tops and aspen bedding. PVC pipe huts and hard nylon chew toys were added to the cage for enrichment. At approximately 3 to 4 months of age, hair loss was noted in several cages of group-housed female grass rats. The appearance and pattern of the hair loss was suggestive of barbering. Pelage tape tests and swabs for fur mite PCR were collected and samples were negative. Barbering behavior continued until the animals were separated and singly housed because of IACUC-approved protocol requirements. Within 10 d after isolation, regrowth of hair was evident in all animals and continued until the completion of the study, 68 d later, by which time the hair coat had nearly returned to normal. In the same colony, a litter was barbered by the dam until weaning, after which the hair rapidly regrew. Just prior to 3 mo of age, barbering was identified around the eyes of 2 of the 3 males pups which were group housed. To the authors' knowledge, this represents the first detailed description of barbering in the Nile grass rat and compares and contrasts the behavior in this species to other more common rodent species.

P32 False Brush Borders in the Cecum of NSG Mice

M Lepherd[4,2], I Redelsperger*[1,2], NS Lipman[2,3]

[1]Tri Institutional Training Program in Laboratory Animal Medicine and Science, New York, NY; [2]Center for Comparative Medicine and Pathology, Memorial Sloan-Kettering Cancer Center, New York, NY; [3]Center for Comparative Medicine and Pathology, [4]Laboratory of Comparative Pathology, Weill Cornell Medical Center, New York, NY

Two months after arrival, 35 out of 40, 3-mo-old, experimentally naïve, female NOD SCID-IL2 γ-receptor knockout (NSG) mice were noted to be unthrifty, hunched, lethargic, and mildly dehydrated. The mice were housed in a room known to harbor *Corynebacterium bovis* in sterile individually ventilated cages. All caging, bedding, nestlets were autoclaved, feed was irradiated and cages changed in a class 2 biosafety cabinet. Water was reverse osmosis provided via automatic watering. Two animals were submitted for necropsy. The only notable gross findings were unkempt hair coats and poor body condition. Cultures of the oral cavity, blood, spleen, and kidneys yielded oral *C. bovis*, but did not detect other bacterial pathogens. Histology confirmed the presence of *C. bovis* dermatitis. Although a definitive cause of unthriftiness was not determined, microscopic examination of the large intestine revealed that the cecal and, to a lesser extent, colonic mucosal surface, was colonized by a carpet of 1 × 4 um gram-negative, argyrophilic rod-shaped bacteria resulting in the formation of a false brush border. To further characterize these bacteria, 10 additional mice from the same shipment were evaluated. Aerobic and anaerobic culture of the small intestine and cecal mucosa of 3 mice failed to yield known bacterial pathogens or a gram-negative bacteria resembling that seen histologically. Based on the histologic appearance of the bacteria, differential diagnoses included enteric *Helicobacter* spp. and intestinal spirochetosis due to *Brachyspira* spp. colonization, as has been described in primates. However, *Helicobacter* spp. PCR was negative in 2 of 2 mice tested and *Brachyspira* spp. immunohistochemistry was negative in 6 of 6 mice tested. A discussion of the presence of false brush borders in immunocompromised mice at our facility and attempts at further bacterial identification will be presented.

P33 Diphenhydramine Hydrochloride, Pramoxine Hydrochloride, and Calamine Lotion Used as Treatment for Ulcerative Dermatitis in Zucker Rats

J Tubbs*[1], TL Blankenship[2]

[1]DLAR, Duke University Medical Center, Durham, NC; [2]Comparative Medicine Branch, National Institute of Environmental Health Sciences, Research Triangle Park, NC

Ulcerative dermatitis (UD) is a condition that commonly affects certain strains of mice, such as C57BL/6, BALB/c, and DBA/2. The skin disease is typically not seen in rats. The progressive condition is thought to be a multifactorial disease that is influenced by factors such as genotype, housing environment, percentage of fat in the diet, and ad libitum feeding. Male Zucker rats (Crl:ZUC-Leprfa), 6 to 12 wk of age developed ulcerative dermatitis on the right side of the thorax, and were treated with a combination of calamine lotion, pramoxine hydrochloride, and diphenhydramine (CCB). The cause of the unilateral location of the lesions remains unknown, but this was the common presentation in those animals that developed lesions. Primary differentials included dermatitis secondary to experimental lab diet, overgrooming, and external parasites. Treatment included initially, shaving around the affected area followed by cleaning of the lesion with dilute chlorhexidine. Then, CCB was applied once daily to the lesion. Lesions completely resolved within 18 d of treatment, and dermatitis did not recur after treatment was discontinued. The clinical response demonstrates that the use of CCB is a valuable alternative for the treatment of UD in rats.

P34 Efficacy of a Novel Foam E-Collar to Promote Comfort and Inhibit Self-Mutilation during Postsurgical Recovery in the Watanabe Rabbit

KN Bird, AL Trimble, CI Stavinoha, R Work, JL Greaver*

Laboratory Medicine, UT Medical School, Houston, TX

In long-term survival surgeries, postsurgical healing can be challenging in fastidious Watanabe rabbits. Surgical recovery and comfort to the rabbit is essential for its wellbeing. Often the rabbit is able to manipulate the removal of the bandage from the surgical site, delaying recovery and jeopardizing his own health. The traditional cone shaped E-collar can prohibit normal feeding and halt species-typical behavior. We developed a collar that eliminates additional bandaging. This foam collar allows increase ambulation, reduces stress when eating and drinking, decreases dermatopathies around the neck, and allows movement in the normal housing area. We have refined the E-collar that works well for smaller patients. The collar consists of foam that is thin, durable, light weight, and easy to customize to the patient's neck. This foam material is safe, economical, and can be cleaned. The rabbits were able to wear the collar as long as needed without developing local dermatopathies. Animals acclimate readily to this collar enabling the patient comfort, while inhibiting mutilation. Use of this device has reduced postsurgical complications and has decreased recovery time, allowing the animal's normal behavior to be expressed.

P35 Struvite Urolithiasis in Long–Evans Rats

J Pang*, TM Borjeson, SE Erdman, JG Fox

Division of Comparative Medicine, Massachusetts Institute of Technology, Cambridge, MA

Spontaneous urolithiasis in rats has infrequently been reported, although it has been experimentally induced by inoculation of the urinary bladder with urease-producing bacteria such as *Proteus mirabilis*, or by dietary manipulation—for example, a vitamin A-deficient diet. A cohort of sixty-eight 8-wk-old male Long–Evans rats was imported to our institution from a commercial vendor. Within 1 wk after arrival, 2 rats were found dead and gross necropsy revealed multiple calculi in the bladder and renal pelvises of both kidneys. Physical examinations were performed on the remaining rats and 2 were identified with hematuria. Diagnostic imaging revealed the presence of multiple, round, variably sized mineralized opacities within the region of the urinary bladder of both rats. Urinalysis showed an alkaline pH with the presence of red and white blood cells and an abundance of bacteria with *Proteus mirabilis* being the predominant organism. Triple phosphate (struvite) crystals were found in the urine sediment and urolithiasis was confirmed at necropsy. The uroliths from these 4 cases were analyzed and determined to be of struvite composition. The remaining rats were clinically normal for an additional 8 wk after arrival and no significant abnormalities were found on radiography, despite several demonstrating moderate triple phosphate crystalluria by urinalysis, suggesting early struvite urolith formation. *Proteus mirabilis* was also cultured from the bladders of several of these animals. The original colony at the vendor was examined and other cases of urolithiasis were identified with similar findings. While no definitive cause has been found to explain why these juvenile rats developed urinary calculi, we postulate that *Proteus mirabilis*, an opportunistic agent, together with a combination of genetics and diet could have contributed to the high percentage of urolithaisis in this colony. To our knowledge, this is the first case report of naturally occurring struvite urolithiasis in male Long–Evans rats, and emphasizes the importance of close clinical monitoring and diagnostic evaluation of rats from any colony, regardless of source.

P36 *Trichosomoides crassicauda* in a Closed Colony of Sprague–Dawley Rats in Trinidad and Tobago

J Johnson*, G Brown

School of Veterinary Medicine, The University of the West Indies, St Augustine, Trinidad and Tobago

Trichosomoides crassicauda occurs in the urinary tract of wild rats, and rarely, in laboratory rats. The worms are usually found free in the lumen or embedded in the mucosa of the bladder, causing mild uroepithelial hyperplasia 4. Transmission is via ingestion of eggs, passed in urine, and probably occurs from dam to pups before weaning. Barthold reported that there are very few worms (approximately 3) present in the bladder at any time. This poster aims to report on the finding of this nematode in a closed colony of Sprague–Dawley rats in the Republic of Trinidad and Tobago. This colony has been closed, housed on wire-bottom cages and inbred via parent: offspring pairs for 20 y. From preliminary reports, the prevalence of the disease is greater than 80% in the colony animals. Based on the results of random postmortems done on these animals (approximately 40 rats), there is evidence that changes in environment and housing affect the number of worms found in the bladder. A larger number of worms (up to 5 worms) have been seen in rooms with higher temperatures and humidity and with shredded paper as bedding. While rooms with lower temperatures and humidity and housed on wire-bottom cages have an average of 2 worms in the bladder.

P37 Detection and Eradication of a Pinworm Infestation in a Mixed Species Nonhuman Primate Research Facility

JM Bruenau*[1], R Errahimi[1], A Fisher[1], C Janssen[1], Z ToniAnn[2]

[1]DLAR, University of Pittsburgh, Pittsburgh, PA; [2]University of Pittsburgh, Pittsburgh, PA

During the necropsy of a cynomolgus macaque, an infestation of metazoan parasites was found. Many adult worms were found in the cecum and colon of the animal. These were later identified as pinworms of the genus *Enterobius*, but the exact species is yet to be identified. Since pinworms of the genus *Entrobius* cannot be effectively diagnosed with fecal flotation methods in a live host, the entire colony from which this monkey originated was then tested using the perianal cellophane tape technique. Initially 16 out of 55 animals in the colony tested positive in a facility comprised of cynomolgus macaques (*Macaca fascicularis*), rhesus macaques (*Macaca mulatta*), and green monkeys (*Chlorocebus sabaeus*). No animals ever showed any typical clinical symptoms of a pinworm infection. Pyrantel pamoate was determined to be the best option for treatment, due to its minimal systematic absorption and therefore minimal experimental impact to ongoing immunology, virology, and infectious disease research. All animals in the colony were dosed once by mouth, then 2 d after treatment cages were changed and holding rooms were scrubbed to reduce reinfection. This process was repeated at 2-wk intervals for 3 rounds of treatment. After 3 rounds of treatment, testing showed that 2 groups remained positive. Alternate regimens

were used for additional treatment. One group was given albendazole by mouth for 3 d, followed by cage changes and room scrubbing 2 d after treatment; this was repeated at 2-wk intervals for 3 rounds. Due to anticipated research complications, albendazole could not be used for the second group; therefore, a more aggressive pyrantel regimen was used, consisting of treatment for 3 d, repeated at 2-wk intervals. After these approaches, follow-up perianal cellophane tape tests have revealed no further positivity. Additional colony testing will continue. Pinworms are hard to control, especially in a nonhuman primate facility due to the eggs being easily spread on fomites and the difficulty of breaking the life cycle with sanitization. In this case, pyrantle pamoate and albendazole were paired with aggressive cage changes and room sanitization with eventual success.

P38 Abdominal Mass in an Aged Male RPGR Knockout Mouse

JH Fried[*1,3], J Marx[1,3], JM Wilson[2], MP Limberis[2], AK Brice[1,3]

[1]Pathobiology, [2]Pathology and Laboratory Medicine, Perelman School of Medicine, [3]University Laboratory Animal Resources, University of Pennsylvania, Philadelphia, PA

A 2-y-old male retinitis pigmentosa GTPase regulator (RPGR) knockout mouse on a mixed 129/Sv and C57BL/6J background was reported to the veterinary staff for dehydration. The expected knockout phenotype of these mice is limited to retinal changes. On physical exam, the mouse was dehydrated with muscle atrophy and unilateral conjunctivitis. A large, soft, freely-movable mass was palpated in the ventral abdomen. Although supportive care, including subcutaneous saline and provision of moist food and gel, was administered for several days, the mouse's clinical condition did not improve. The lab elected euthanasia with postmortem examination. The eyes were collected for protocol-related work. On gross examination, the large abdominal mass palpated clinically was identified as a markedly enlarged right seminal vesicle with a thick white discharge on cut surface. This material and the mucosal surface were cultured; there was no growth. Other significant gross findings included an enlarged, pale liver and a 5-mm diameter round firm red nodule in the mesentery between the duodenum and the liver. Microscopically, the enlarged seminal vesicle was markedly dilated and filled with abundant brightly eosinophilic proteinaceous fluid, with no inflammatory cells or bacteria. This is consistent with age-related acinar dilatation. The liver was also marked by age-related changes including diffuse hepatocellular vacuolation, abundant eosinophilic intranuclear cytoplasmic invaginations, and moderate multifocal random lymphoplasmacytic hepatitis. Based on gross and microscopic features, the red nodule was diagnosed as a hepatic choristoma with neoplastic transformation to hepatocellular carcinoma. Hepatic choristoma is a rare condition in which liver tissue is found in an abnormal location. It has a higher incidence of neoplastic transformation than the regular liver. Hepatocellular carcinomas arising from choristomas have been reported in the dog and cat. To the authors' knowledge, there are no previous reports in the literature of hepatocellular carcinoma arising from a choristoma in the mouse.

P39 Hemoplasma and Small Ruminant Lentiviral Infections in Research Sheep: An Epizootiologic and Hematologic Investigation

JA Hampel[*], I Bergin, MC Dyson

Unit for Laboratory Animal Medicine, University of Michigan Medical School, Ann Arbor, MI

We diagnosed a clinical case of concurrent hemoplasma and ovine progressive pneumonia virus (OPPV) infection in a research wether. The animal presented with severe chronic, cyclic hemolytic anemia and was euthanized prior to study endpoint. Many research protocols using sheep at our institution rely on hematologic parameters to monitor experimental effects. Based on this clinical case, we investigated the epizootiology of hemoplasma and OPPV and their potential effects on common hematologic parameters in sheep. Blood was collected from 67 research sheep and was analyzed for hemoplasma DNA (*Mycoplasma ovis* and *Candidatus Mycoplasma haemovis*) by PCR, small ruminant lentiviral antibodies by ELISA, and *Mycobacterium paratuberculosis* (Johne disease) antibodies by ELISA. Routine hematologic and clinical chemistry parameters were analyzed as well. We discovered a 22% prevalence of hemoplasma infection, 7% prevalence of small ruminant lentivirus infection, and a 2% prevalence of *M. paratuberculosis* infection. Hematology and serum clinical chemistry parameters were compared between hemoplasma-naïve and -infected animals. Red blood cell distribution width (RDW) and blood urea nitrogen were significantly elevated in hemoplasma-infected animals. Reference intervals for hematologic and clinical chemistry parameters were calculated from the tested animals and compared between the hemoplasma-naïve population and the total population. Differences in reference intervals in several parameters were observed. Hemoplasma and small ruminant lentiviral infections are not uncommon in research sheep. In this study, hematology and clinical chemistry parameters were minimally affected by infection with hemoplasma. These findings may be influenced by hemoplasma infection load—utilization of quantitative PCR may allow more accurate assessment of hemoplasma-influenced effects. Small sample sizes, sample population selection, and variability in clinical sampling techniques can also influence interpretation. Further investigation in a controlled population with a known hemoplasma load will be helpful in defining the effects of hemoplasma infection.

P40 Rhesus Macaque Model of Infant Prematurity: Lessons and Refinements

K Prongay[*]

Oregon National Primate Research Center, Beaverton, OR

Intraamniotic infection is associated with preterm birth in humans. A rhesus macaque model of intrauterine *Ureaplasma parvum* (105 CFU/mL) causes lung injury and premature delivery. These infants have underdeveloped respiratory and digestive tracts, and require extended ventilatory, nutritional, and thermal support. Meeting critical care needs is challenging. Equipment and supplies are oversized, and procedures and techniques must be adapted from the human literature. Three infants less than 145 d gestational age have been delivered via caesarian section. Respiratory support included nasal oxygen, continuous positive airway pressure, and positive pressure ventilation. Nutritional support included parenteral nutrition, nasal gastric, and oral administration of formula. Temperature-controlled incubators and heated procedure tables were used for thermal support. Peripheral vascular access was achieved using a 24-gauge intravenous catheter or a peripheral umbilical vein catheter. Central access was achieved using a 1.9-French peripherally inserted central catheter line. All infants required continuous positive airway pressure following delivery, and one required intubation, surfactant, and ongoing positive pressure ventilation. The remaining 2, following initial resuscitation, maintained adequate SpO_2 saturation on room air. Maintenance in a temperature-controlled incubator was required for all infants, and removal from the incubator for diagnostic imaging or blood sampling was associated with temperature decreases. Conducting procedures under a prewarmed procedure station improved thermoregulation. Postdelivery placement of an umbilical vein catheter was easily achieved, although we were unable to achieve central placement of this line. The superficial saphenous vein was the most reliable site for peripherally inserted central catheter line placement. Oral feeding was associated with enterocolitis in 2 infants. Aggressive supportive care, including ventilator, nutritional, and thermal support is required for rhesus infants less than 145 d gestational age. Our experience suggests 135 d gestational age is the viability threshold. Provision of parenteral nutrition is essential for infants who develop enterocolitis.

P41 See PS77 for Abstract

P42 Blood Collection Techniques in Gambian Pouched Rats (*Cricetomys gambianus*)

K Scott[*1], M Sellers[1], E Rogers[1], C Cullin[1], DN Lee[2], AG Ophir[2], TA Jackson[1]

[1]Center for Veterinary Health Sciences, [2]Department of Zoology, Oklahoma State University, Stillwater, OK

Gambian pouched rats (*Cricetomys gambianus*) are large, rodents native

to Africa with body shapes similar to Norway rats and cheek pouches similar to hamsters. In 2003, importation of Gambian pouched rats into the United States was linked to a multistate outbreak of monkeypox in people. Our institution recently received a large shipment of wild-caught Gambian pouched rats for use in behavioral and reproductive physiology studies. Initial examination and quarantine requirements called for the submission of both serum (approximately 0.5 mL) and saliva from each rat to the Centers for Disease Control (CDC) for monkeypox testing. However, blood collection procedures from Gambian pouched rats have not previously been described. To identify appropriate venipuncture sites, the rats were anesthetized with isoflurane via an induction chamber and maintained on isoflurane via face masks sized for cats. Unlike primates, *C. gambianus*' femoral triangles were not easily palpated due to their short, highly muscular legs, and attempts to collect blood from the femoral veins were rarely successful. Lateral tail veins could be visualized, but yielded less than 0.25 mL of blood with multiple collection attempts. These volumes were insufficient for monkeypox testing. Further examination and palpation of the rats' tails revealed a perceived similarity to the tails of cattle. Based on that similarity, we attempted to collect blood from a ventral tail vein. That attempt was successful, and subsequent collections from ventral tail veins haven become routine. When a Gambian pouched rat required euthanasia and necropsy for health reasons unrelated to the anesthesia and blood collection, dissection identified a large vein on the ventral aspect of the tail. In Gambian pouched rats, the ventral tail vein is a suitable location for collecting large volumes (1 to 2 mL) of blood and for making intravenous injections.

P43 Neonatal Care to Increase Survival Rate of Valuable Mouse Strains

K McDonald*, J Litchfield

Comparative Animal Research, Amgen, Cambridge, MA

It is known that some knockout and transgenic animals can have phenotypes that impact neonatal survival. If a particular knockout/transgenic is valuable to research, there may be ways to intervene and increase the survival rate. We explored various methods to increase the chance of neonatal survival in these special needs animals. We set up timed matings so that approximate date of birth would be known and near-term pregnant dams could be monitored closely. Once litters were born, carefully monitoring of the pups for size differences, presence of a milk spot and hydration status indicated when intervention was needed. We developed a mouse milk substitute that was administered via feeding needle and hand fed pups up to 3 times a day. Subcutaneously administered 2% dextrose also helped provide additional fluid and calories. As the pups aged, we gave additional food and water supplements on the cage floor if warranted. Through this intense care, we were able to get some fragile neonates to weaning and enable their use in valuable research studies. This kind of intense care can allow for the study of valuable mouse models that otherwise would not be possible due to neonatal mortality.

P44 Electrocardiogram Abnormalities in the Naïve Laboratory Beagle

K Cohen*

Covance Research Products, Cumberland, VA

Electrocardiogram (ECG) serves as a useful tool for screening dogs prior to study initiation to establish baseline data and to remove animals with abnormalities that could interfere with interpretation of study findings. This screening is often done at production facilities prior to animal purchase to facilitate selection of animal candidates. ECG recordings were conducted on 663 beagle dogs (399 males and 264 females) during a 19-mo timeframe. Two strains of beagles were evaluated (452 LRE strain and 211 HRA strain). The mean age was 5 mo with a range of 5 to 12 mo. The ECG recordings were evaluated and abnormalities reported. Of all recordings analyzed, 12.4% were found to have an abnormality. The most frequent arrhythmia reported was sinus tachycardia (73%). Dogs with sinus tachycardia were generally not excluded from selection as this arrhythmia can be considered a normal variant in this species. Other less frequent abnormalities included tall T waves (11%), low voltage QRS complex (3.7%), P-mitrale (3.7%), occasional second degree AV block (3.7%), S-T elevation (2.4%), first degree AV block (1.2%), and atrial premature ventricular complex (1.2%). Overall, these data suggest that ECG abnormalities in the young laboratory beagle model are minimal.

P45 Outbreak of Pathogenic *E. coli* in an Outdoor-Housed Nonhuman Primate Colony

K Kolappaswamy*[1], K Turner[2], W Porter[1], H Klein[1]

[1]Harlan Laboratories, Indianapolis, IN; [2]Primate Products, Miami, FL

An outbreak of diarrhea associated with enteroinvasive *E. coli* (EIEC) and enterohemorrhagic *E. coli* (EHEC) occurred in rhesus and cynomolgus macaques in an outdoor nonhuman primate (NHP) facility. Over a period of 2 mo, the attack rate or incidence risk was 14% among the rhesus macaques and 62% in the cynomolgus macaque colony. They experienced clinical signs of soft to watery diarrhea, anorexia, and malaise. By a random sampling of 24% of fecal samples of diarrheal cases, 4 cases of EIEC in rhesus macaques and 2 cases of EHEC in cynomolgus macaques were confirmed. The diagnosis was made based on microbiologic culture and PCR. The NHPs were moved indoors for individual intensive care and treatment. All the animals diagnosed with the pathogenic *E. coli* recovered from the infection. The primary source of infection could not be determined. This is the first time EIEC and EHEC are reported in NHPs causing acute diarrhea. This outbreak provides important evidence of natural susceptibility of rhesus macaques to EIEC and cynomolgus macaques to EHEC.

P46 Ulcerative Skin Lesions in a Colony of Rainbow Trout Experimentally Infected with *Ichthyophthirius multifiliis*

KH Allen-Worthington*[1], AJ Carty[1], BH Philips[1], O Sunyer[2], AK Brice[1]

[1]ULAR, [2]Pathobiology, University of Pennsylvania, Philadelphia, PA

Two, approximately 1-y-old, female rainbow trout (*Oncorhynchus mykiss*) were reported for red skin lesions on the proximal aspect of the tail. Fish were housed in the same tank and experimentally infected with *Ichthyophthirius multifiliis* as part of an IACUC-approved research protocol. The observed lesions were not consistent with the typical mild disease and white foci on the gills, fins, and skin due to *I. multifiliis* infection. There had been a 3-wk history involving 6 of 35 infected fish developing red epidermal lesions with a rapidly declining clinical progression culminating in death. Grossly, there were large, poorly demarcated red erosions and ulcerations of the tail with marked ulceration and loss of the distal portions of the pectoral, pelvic, and tail fins. Additionally, one fish was noted to have a pale, white to grey cottony growth overlying the tail lesions. Diagnostic analyses included microbiologic culture, histopathology, and wet-mount slides. Tests revealed a bacterial ulcerative dermatitis due to *Burkholderia* (*Pseudomonas*) cepacia along with several other opportunistic agents including bacterial, fungal, and algal species. Many species of *Burkholderia* are known to be resistant to antimicrobials and can represent a zoonotic health risk which serves to remind us of the importance of constant vigilance with regard to water quality.

P47 Enucleation for Treatment of Rodent Ocular Disease

L Wilding*, M Uchihashi, I Bergin, MH Nowland

University of Michigan, Ann Arbor, MI

Corneal lesions are a common clinical presentation for laboratory mice and rats. This is likely due to their disproportionately large corneas and their exposure to exogenous irritants such as bedding dust and ammonia levels. Corneal lesions can also result from inadequate eye lubrication during inhalation anesthesia, as well as complications of retroorbital blood collection. Currently, our standard of care for rodent corneal disease includes treatment of any detectable primary lesion in conjunction with topical NSAID drops and systemic NSAIDS in severe cases. When intensive medical management is not successful, animals are euthanized. This can lead to premature loss of valuable genetically

modified animals or animals that are on long-term studies. We investigated enucleation as a treatment option for mice and rats with corneal disease that does not respond to medical management. The procedure is performed under isoflurane anesthesia, and involves removal of the globe, extensive hemostasis, and packing of the orbital space with an absorbable gelatin sponge to reduce dead-space and provide continued hemostasis. The lid margins are closed by tarsorrhaphy and a drop of tissue glue. Analgesia is provided with a preoperative injection of carprofen (5 mg/kg SQ), and carprofen-impregnated gel diet as needed postoperatively. At present, we have had a 100% success rate with this procedure (n = 10) in animals followed for up to 60 d postprocedure. The only complication has been dehiscence of the tarsorrhaphy site, which we quickly eliminated by trimming the lid-margins to provide fresh tissue for closure. Histologic examination of the enucleation site 1 mo after surgery revealed focal pyogranulomatous inflammation associated with the remaining gelatin sponge. There was granulation tissue present in the eyelid at the site of tarsorrhaphy; however, the inflammation did not extend into adjacent glands or soft tissue. Enucleation in rodents is a straight-forward procedure that represents a significant refinement to our current standard of care for rodents, does not cause inflammation of remaining associated ocular structures, and has reduced the number of animals euthanized prior to study endpoint due to severe ocular lesions.

P48 Early Intervention Using Nail Trimming to Treat Idiopathic Ulcerative Dermatitis Results in Improved Healing Rates of Mice Compared with Other Standard Practices

LC Richardson*, C Manuel, T Mufford, J Leszczynski

Office of Lab Animal Resources, University of Colorado Denver, Aurora, CO

In November of 2007, the veterinary technicians at our institution began routinely performing hind foot nail trims on mice with lesions due to idiopathic ulcerative dermatitis (IUD). The previous standard of care at our institution was similar to what is generally accepted in the Laboratory Animal Medicine field and included topical or injectable steroids, other ointments such as triple antibiotic or panalog, and/or washing of the skin with dilute betadine. Water with acepromazine and/or amoxicillin trihydrate–clavulanate potassium were used as a last resort in order to provide a tranquilizing effect and also decrease secondary infections in an attempt to lower the impact of inflammation and the pruritus associated with such lesions. In 2009, a retrospective analysis was performed and found that trimming the nails decreases healing time and eliminated the need for steroids, tranquilizers, and systemic antibiotics in most cases of IUD. Since the original presentation of this technique in 2009, animal husbandry staff have been trained on performing initial nail trims immediately upon finding mice with IUD lesions. Analysis done on cases in 2012 compared with those in 2009 shows that trimming the nails earlier in the disease process decreases healing time and the need for drug therapy compared with the practice in 2009 of waiting approximately 24 h for a member of the veterinary staff to perform the task. Therefore, it is recommended that all husbandry staff be trained to perform hindlimb nail trims upon discovery of IUD lesions in order to decrease duration and severity of lesions.

P49 Jugular Blood Collection Technique in the Syrian Hamster

L Severson*, CS Frisk

Department of Comparative Medicine, Mayo Clinic, Rochester, MN

Collecting repeated blood samples in significant volumes from Syrian hamsters can be difficult. A hamster protocol was submitted proposing collection of up to 0.7 mL of blood per 100 g of body weight every 2 wk preferably using a noninvasive technique. Laboratory technicians sought assistance from our training department to demonstrate survival techniques that would meet the blood collection requirement of the protocol. Withdrawing a relatively large volume of blood using a repeatable and reliable technique presented a challenge for the hamster model. Puncture of the lateral saphenous vein would not meet the volume requirement and retroorbital collection technique poses the risk of postprocedural complications. Literature searches did not reveal a jugular vein technique specifically described for the hamster, only methods adapted from the mouse and rat. Skilled in jugular blood collection in the rat, adaptation of a rat technique to the hamster appeared reasonable. The hamster is placed in dorsal recumbency and the manubrium of the sternum is elevated by placing a syringe case under the anterior thorax. The hamster is secured in this position with adhesive tape. Blood collection from the jugular vein is accomplished by holding the needle and syringe parallel to the sternum in a caudal to cranial direction at a 30° angle to the body. The needle is introduced through the skin caudal to the clavicle and just lateral to the manubrium. With gentle negative pressure applied to the plunger of the syringe, the needle is "walked" over the clavicle entering the jugular vein. Gentle aspiration is continued until an adequate volume of blood has been collected. Critical components to the method are proper restraint provided by inhalation anesthesia, animal positioning, and proper placement of the needle relative to the clavicle.

P50 "Red-Leg" in Wild-Caught Native Australian Spotted Marsh Frogs (*Limnodynastes tasmaniensis*)

L Paluch*, A Britton

The University of Melbourne, Melbourne, VIC, Australia

Three male and 6 female wild-caught, native Australian spotted marsh frogs (*Limnodynastes tasmaniensis*) of unknown age were cohoused in a large enclosure in an effort to induce breeding as part of a captive-breeding conservation project. One spawn was produced but did not survive and no subsequent spawn was generated. A sprinkler system was set up to simulate rain and flooding in an attempt to stimulate breeding. Whilst most of the females were gravid and pairs were observed in amplexus, no spawning occurred. In the hopes of triggering a stronger breeding response, 2 males and 4 gravid females were transferred to another enclosure with shallow water levels and some plastic waterweed. Within 3 d of transfer one of the males and 2 females were found dead and the 3 remaining frogs were dying. A postmortem was conducted on the 3 dead frogs and the major findings were that all 3 frogs presented with anasarca and erythema of the feet and legs. The male frog was most severely affected with erythema extending along the ventrum and petechiae present on the distal limbs. The 3 remaining frogs presented with similar signs and were euthanized by immersion in buffered tricaine methane sulfonate solution (MS222). Skin swabs were collected from each of the euthanized animals for *Batrachochytrium dendrobatidis* (chytrid) testing and bacterial culture. We suspected the cause of illness to be 'red-leg' caused by *Aeromonas hydrophila*. Other differential diagnoses included 'red-leg' due to other gram-negative bacteria or chytridiomycosis. Microscopy of the samples revealed numerous gram-negative bacilli, and a moderate growth of *Pseudomonas fluorescens*, *Pseudomonas putida*, and *Acinetobacter* spp. was cultured from the feet and legs. The frogs returned a negative chytrid test on PCR. We presume that the stress of being transferred to a new environment contributed to the frogs succumbing to an opportunistic *Pseudomonas* infection. The frogs that had not been moved from the original enclosure remained healthy. This case highlights the challenges of working with wild-caught animals in a laboratory setting.

P51 OIE Collaborating Center on Laboratory Animal Science and Welfare: A New Role for ILAR

L Anestidou*

Institute for Laboratory Animal Research (ILAR), Washington, DC

Sixty years after its founding, the Institute for Laboratory Animal Research (ILAR) has been adopted by the World Organization for Animal Health (OIE) as a Collaborating Center for Laboratory Animal Science and Welfare. OIE Collaborating Centers are centers of excellence in a specific designated sphere of competence relating to the management of general questions of animal health issues and they are obligated to provide their expertise internationally (http://www.oie.int/our-scientific-expertise/collaborating-centres/introduction/). The new Collaborating Center focuses on scientific and welfare-related issues pertaining to the care and use of laboratory animals in support and advance of the OIE's "international mandate to be a leader on animal

welfare standard setting and…to elaborate recommendations and guidelines covering animal welfare practices." Among the 4 OIE Collaborating Centers with a focus on animal welfare, this is the first to concentrate exclusively on laboratory animals. We will present the scope of the Center, discuss future projects, and elaborate on opportunities for collaboration and training that aim to improve the welfare of research animals and advance scientific outcomes.

P52 Desensitization in the African Green Model

L Devine*

Veterinary Medicine and Surgical Branch, USAMRICD, Gunpowder, MD

A cooperative environment is paramount when working with nonhuman primates (NHP). Desensitization to specific stimulus helps to allow technicians to more safely perform procedures without the use of anesthetics, and with minimal restraint, greatly reducing stress in the NHP. Creating a cooperative and safe environment can be challenging if you are not properly trained to recognize and reinforce wanted behavior, and is compounded by the limited amount of data regarding behavioral training in the African Green monkey. Study protocol dictated the placement of a vascular access port (VAP) to allow for frequent blood collection at various time-points throughout the study, with the intention of training 9 NHPs to voluntarily present their VAP through the front of their home cage with minimal intervention on the technicians' behalf. These NHPs were especially challenging to train because the perches in their home cage are on the left hand side of the cage and their catheters were placed on their left hand side of their abdomen. Normal posture while resting on their perch exposes the right side of their abdomen to the front of the cage. In order for us to get them to present the VAP, we would have to train the NHPs to show little to no resistance to the squeeze bar by using different techniques and through positive reinforcement for desired behavior. We then would train the NHPs to come off their perch, station in the front of the cage, then turn to face their perch. Although presentation challenges existed, we were able to overcome them through physical cut out modifications to the left hand side of the cages and by using a variety of reinforcers we were able to manipulate the majority of NHPs to present using a targeting style method in a minimally stressful and safe environment. Those NHPs that would not fully present at the time of study showed remarkable progress cooperating with staff for procedural work.

P53 Microfilarial Counts in the Mongolian Gerbil Using Saphenous Vein Compared with Retroorbital Bleeding Techniques

LM Kelly*[1], LC Alworth[3], EJ Burkman[2], TL Cooper[3], MD Riggs[2], P Supakorndej[2], AR Moorhead[2]

[1]Office of Animal Care and Use, [2]Department of Infectious Diseases, [3]University Research Animal Resources, University of Georgia, Athens, GA

The NIH's "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 by comparing microfilaria levels in blood samples collected by retroorbital puncture to those collected by saphenous vein puncture. 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 were blinded with respect to method and gerbil. Both technicians' counts of each pair of slides were averaged to provide the count for each collection site. 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 relatively high levels (over 50 mf/20 mL). However, at lower 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, and does not require general anesthesia. Adequate volumes of blood can be collected to create slides for counting microfilaria. 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 levels. While it has not been determined whether one site is 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/20 mL.

P54 Medical Management of Caudal Occipital Malformation Syndrome in a Naturally Occurring Canine Model of Glycogen Storage Disease Type Ia

MB Struck*[1], AJ Specht[2], DA Weinstein[3], TJ Conlon[3]

[1]Office of Research, [2]College of Veterinary Medicine, [3]College of Medicine, University of Florida, Gainesville, FL

A group-housed, 5-mo-old crossbred (beagle-Maltese) glycogen storage disease (GSD) type Ia-affected research dog presented with a mild left sided head tilt and muscle asymmetry of the neck and shoulders. Initial exam revealed resistance to full range of motion of the neck without significant abnormal neurologic or physical exam findings. Blood chemistry, CBC, and urinalysis showed no significant clinical findings. The dog's condition remained stable for 3 mon except for an intermittent pain response when roughhousing with cohorts, which resolved with rest and oral tramadol at 3 mg/kg TID. Three months after onset, the dog experienced an increase in head tilt severity. The dog was referred to the Veterinary Medical Center at our institution for neurologic consult and diagnostic imaging. Neurologic findings included mild generalized ataxia, mild forelimb hypermetria, and mild resistance to full range of motion of the neck. Computed tomography and magnetic resonance imaging allowed a diagnosis of caudal occipital malformation syndrome (COMS or Chiari-like malformation), syringomyelia, and cervical scoliosis. One treatment option is surgical correction of the abnormality. Due to lack of major neurologic impairments and the comfort level and stability of the patient, a conservative, medical treatment plan with monitoring for neurologic signs was elected. Omeprazole 0.6 mg/kg and gabapentin 10 mg/kg were prescribed. Typically, prednisone is part of medical treatment for COMS but is contraindicated in GSDIa. As is routine for dogs in this colony, the patient is monitored 24 h/d, 7 d/wk by research technicians and shows no progression of the head tilt. COMS is usually seen in small-breed dogs like Cavalier King Charles spaniels, miniature poodles and Maltese. Surgery as a curative option could be considered if progression towards increased neurologic impairment or pain is observed. This is the first known report of management of COMS in the research setting.

P55 Is Sheep Lumbar Spine a Suitable Alternative for Human Spinal Research? Morphometric Study Based on Digitized CT Images

M Mageed*[1,2], D Berner[1], H Jülke[4], C Hohaus[3], W Brehm[1], K Gerlach[1]

[1]Faculty of Veterinary Medicine, University of Leipzig, Large Animal Clinic for Surgery, Leipzig, Germany; [2]Department of Surgery and Anesthesia, Faculty of Veterinary Medicine, University of Khartoum, Khartoum, Sudan; [3]Department of Neurosurgery, BG Hospital Bergmannstrost, Halle, Germany; [4]Microsurgery and Animal Models Core, Translational Centre for Regenerative Medicine, University of Leipzig, Leipzig, Germany

The human cadaveric specimens are the ideal model for new implant testing. However, it is difficult to obtain them fresh and in large quantities to avoid the heterogeneity. Therefore, animal models have been used as alternatives for human spine. Sheep are well accepted as appropriate models in orthopedic research due to similarities with humans in morphologic and biomechanical features. We aimed to assess the feasibility of using sheep as an animal model for the human spine. To achieve this aim, computed tomographic scanning was carried out in 5 healthy Merino sheep (2 y old, 62 ± 5.3 kg) under general anesthesia.

Transverse images were acquired with 1- and 5-mm slice thickness from the cranial level of L1 through L6, and images were reconstructed in sagittal plane. The volume of vertebral body and total of 11 parameters on CT images of each vertebra were measured and 3 spinal indices were calculated and compared with human published literatures. The parameter was defined comparable if the ratio sheep/human of each individual vertebra showed variation less than 20%. This study showed that endplate and spinal canal indices of the sheep were comparable to human, where the pedicle index was not. The comparison between sheep and human revealed that sheep has smaller, narrower, and longer vertebral bodies and thinner intervertebral disc, where human spinal canal was wider and deeper than sheep. Sheep pedicles were narrower, higher and have greater angulations toward lateral than human. Sheep have smaller volume as much as 48.6% than human. In summary, sheep lumbar spine has good similarity to human in terms of vertebral endplate and spinal canal. Sheep pedicles can be used as model if anatomic differences are taken into account.

P56 Measurements of Thoracolumbar Spinal Cord in Sheep: Computed Tomographic Study

M Mageed*[1,2], J Ionita[1], E Ludewig[3], W Brehm[2], K Gerlach[1]

[1]Large Animal Clinic for Surgery, Faculty of Veterinary Medicine, University of Leipzig, Leipzig, Germany; [2]Department of Surgery and Anesthesia, Faculty of Veterinary Medicine, University of Khartoum, Leipzig, Germany; [3]Department of Small Animal Medicine, Faculty of Veterinary Medicine, University of Leipzig, Leipzig, Germany

Sheep are widely used as a model for experimental spinal cord injury to investigate pathophysiologic mechanisms of injury, recovery after trauma and to examine the potential benefits of therapeutic agents. Quantitative measures of the spinal cord provide the basis for understanding and interpreting clinical implications. Moreover, the dimensions of the spinal cord are important in cordotomy and other spinal operations. This study aimed to obtain basic morphometric data of the sheep spinal cord. To achieve this aim, computed assisted myelography scanning was carried out in 5 healthy blackhead sheep (2.0 ± 0.4 y, 80.6 ± 28.7 kg) under general anesthesia. Transverse images were acquired with 2-mm slice thickness from T1 to L6. Sagittal and transverse diameters and cross-sectional area of spinal cord at the level of middle third of vertebral body were measured. To determine the anatomic relationship between the spinal cord and osseous structures of vertebral canal, the pedicle-dural sac distance and available space for dural sac were calculated. Available space for dural sac is determined by subtracting the sagittal diameter of the dural sac from the sagittal diameter of the vertebral canal. The transverse diameter was largest at T1, and decreased progressively to T5. However, the sagittal diameter did not change distinctly with the vertebral level. The cranial thoracic and lumbar enlargements were determined by the transverse diameters of the spinal cord. T1 had the largest cross-sectional area, at 84.9 mm^2. In lumbar spine, the pedicle-dural sac distance was larger as much as 15.8% than thoracic vertebrae. That means lumbar spine is safer for preclinical test of new spinal implants than thoracic spine. The generated data can serve as a reference values for the sheep thoracolumbar spinal cord and may be helpful in using sheep spine as a model for human spinal research.

P57 Ulcerative Dermatitis on the Muzzle and Ventral Neck in a Colony of *Prkar1a* Knockout Mice on a FVB/n Background

MK Hogan*[1], DR Pringle[2], C Freed[1]

[1]University Laboratory Animal Resources, [2]Internal Medicine-Division of Endocrinology, The Ohio State University, Columbus, OH

Ulcerative dermatitis (UD), a common condition of laboratory mice on a C57BL/6 background strain, is considered to have a multifactorial etiology. An increased incidence of UD was noted in a colony of mice serving as models of follicular thyroid cancer carrying a *cre*-mediated, thyroid specific ablation of the floxed *Prkar1a* gene on a predominantly FVB background. Unlike the classic midscapular and dorsal lesion presentation, mice 6 mo to 1 y of age, presented with bilateral erythemic, alopecic, raw foci on the muzzle and/or raw moist edematous lesions to the ventral portion of the neck, all displaying extreme pruritus. Incidence within the colony reached 30%. A presumed clinical diagnosis of UD was made on the basis of clinical signs and treatment with topical antibiotics and systemic nonsteroidal antiinflammatory (NSAID) drugs was initiated. Bacterial cultures noted *E. coli* and *Klebsiella*, but lesions did not heal despite long-term treatment with systemic antibiotics. Mice were euthanized and tissues submitted for necropsy. Histologic findings indicated substantial UD in conjunction with systemic changes consistent with an appropriate immunologic response to long standing systemic inflammatory condition. The lesions continued to sporadically erupt within the colony, but have decreased in incidence based on selective breeding by the researcher, supporting a genetic component. An update to the early removal criteria of the protocol now addresses the adverse effects of this phenotype. Subsequently, IACUC approval was granted for long-term systemic treatment with NSAIDs to provide relief of pruritus and minimize self-mutilation until mice neared scheduled endpoints, upwards of 1 y with either metastatic carcinoma or benign adenoma development. UD lesions of the muzzle and neck region are rarely reported. Despite a FVB/n background, we presume that the unique presentation of UD is related to the genetics of this mouse and specifically the absence of the *Prkar1a* gene.

P58 Use of Dried Blood Spot Samples in Serological Testing of Rodents

M Myles*, CL Besch-Williford, R Livingston, B Bauer, L Riley

IDEXX RADIL, Columbia, MO

Serological evaluation for the detection of antibodies to infectious agents is a critical component of a comprehensive rodent health monitoring program and has benefited significantly from recent technological advancements. One such advancement is the use of dried blood spot (DBS) sampling technology. DBS has been used as a sampling technique for newborn disease screening, molecular testing for infectious disease diagnosis, and therapeutic drug monitoring. Further, pharmaceutical companies have recently adopted DBS sampling methods for preclinical pharmacokinetic and toxicokinetic testing in rodents in order to enhance animal welfare, reduce animal numbers, improve study precision, and reduce costs. To investigate the utility of DBS sampling technology for serological health monitoring in mice and rats, our laboratory compared DBS to serum for detection of antibodies to prevalent infectious agents. To this end, 6 groups of 20 ICR mice each were inoculated with epizootic diarrhea of infant mice virus, mouse hepatitis virus, murine norovirus, mouse parvovirus, Theiler murine encephalomyelitis virus, or sham and serial DBS and serum collected at 2, 4, 6, and 12 wk postinoculation. In addition, 3 groups of 8 Sprague–Dawley rats each were inoculated with Kilham rat virus, rat theilovirus, or sham and serial DBS and serum collected at 2, 4, 6, and 12 wk postinoculation. Further, to evaluate the clinical utility of DBS, 500 mouse paired DBS and serum samples were collected from collaborating institutions. DBS and serum samples were evaluated by MFI2 and data were compared for magnitude of MFI signal, signal to noise ratio, correlation, and stability. The magnitude of the MFI signal from all positive DBS samples was equal to or greater than the corresponding serum sample. The signal to noise ratio from the DBS samples was equal to or greater than the corresponding serum samples. The diagnostic correlation of DBS and serum was 100% and there was no degradation of MFI signal after 7 d when DBS samples were stored at room temperature and protected from humidity. DBS samples need no further processing, are stable at room temperature, and require a small sample volume; thus, providing an excellent alternative to traditional serum for rodent serological health monitoring.

P59 Novel Surgical Repair of Rectal Prolapse in Mice

M Uchihashi*, L Wilding, MH Nowland

Unit for Laboratory Animal Medicine, University of Michigan, Ann Arbor, MI

Rectal prolapse is a common problem in laboratory mice. It can occur spontaneously, or may be a complication associated with particular genetic background, such as those with collagen defect, or experimental design, such as colitis models or animals experimentally infected with *Helicobacter* or *Citrobacter* species. At our institution, the current stan-

dard of care for mouse rectal prolapse is to monitor affected animals and euthanize if and/or when they meet the criteria in our end-stage illness guidelines. This conservative approach often leads to euthanasia of valuable animals prior to the study endpoint. At present, there are no published reports describing corrective procedures for rectal prolapse in mice, and treatment is generally limited to empirical therapy consisting of the application of topical ointments or medications. Recently, we have investigated surgical correction as a treatment for rectal prolapse in mice by employing a procedure commonly used for large animals. The procedure lasts approximately 5 min, and involves gentle reduction of the prolapsed tissue and placement of purse-string suture in the perineum under general anesthesia. Analgesia is provided with carprofen (5 mg/kg SQ) and topical lidocaine. We currently have animals with no sign of recurrence 3 mo after the procedure. The most common complication observed across all of the strains is trauma to the surgical site induced by a cage mate. We have eliminated this complication by postponing recombination with cage mates until 7 d postoperatively. In addition, we have seen self-mutilation of the surgical site and postoperative fecal impaction from lack of defecation in mice on a C57BL/6 background. Using a multimodal analgesic regimen, we are currently investigating the possibility that these complications are associated with increased sensitivity to pain in this particular strain of mice. Surgical repair for rectal prolapse is a straightforward, rapid procedure that represents a significant refinement of the current standard of care for mice at our institution. Additionally, it offers the potential to reduce the number of mice that are euthanized prior to completion of a study, and should be considered as a treatment strategy for rectal prolapse in mice.

P60 Maintaining the Intraoperative Temperature during Swine Surgery: An Analysis of Heating Devices

M Bradley*, D Coble

The Ohio State University, Columbus, OH

The aim of this study was to compare 3 thermal supplementation devices during experimental swine surgeries. Currently, there are no studies examining the efficacy of different heating devices during swine surgery. Swine are suitable animal models for surgical research and teaching because of their size and physiologic and anatomic similarities to man. The cardiovascular and CNS effects of anesthesia can result in intraoperative hypothermia, thus thermal supplementation is critical during surgery. The devices compared in this study were a convective heating blanket, a circulating warm water blanket, and a heated table. Esophageal temperatures were recorded every 15 min from the onset of anesthesia, during both survival and nonsurvival surgeries. Significant results were observed following data analysis for the animals in the nonsurvival data set. None of the selected devices maintained normothermia after the onset of anesthesia; however, thermal supplementation minimized the degree of temperature loss. The convective heating blanket maintained higher body temperatures compared with the control after 90 min of surgery. Furthermore, the convective heating blanket maintained higher body temperatures when compared with the heated table after 150 min of surgery. Finally, both the convective heating blanket and circulating water blanket maintained higher body temperatures when compared with the heated table at 180 min. Interestingly, no device showed a difference from control before 90 min of surgical time. The heated table showed no difference compared with the control at any time point, out to 180 min. The most effective thermal supplementation device in this study was the convective heating blanket.

P61 Bedding-Related Ocular Foreign Body Accumulation and Dermatitis in Nude Rats

L Williams, M Campagna*, A Knipe, M Rich

Divsion of Laboratory Animal Medicine, UCLA, Los Angeles, CA

Over 3 mo, 9 Crl:NIH-Foxn1rnu rats housed on a gray, shredded, recycled, autoclaved cellulose bedding were reported for squinting and blepharitis. Three of these rats were also reported as having generalized miliary dermatitis. On examination the eyes had various amounts of, clay-like, red to dark brown, fibrous material in the lower fornix and medial canthus. This material expanded the fornix causing the eyelids to separate from the cornea and occasionally occluded the entire globe. The material was gently removed with forceps and saline-soaked cotton-tipped applicators and collected for further analysis. The eyes were then flushed with saline and the cornea examined for integrity. Blood was also collected from each rat for CBC evaluation. Skin biopsies and culture samples were collected on 2 of the dermatitis cases. Microscopic examination of the ocular material showed concretions of porphyrin and fibrous material consistent with the shredded paper bedding. The histopathology of the skin biopsies was consistent with allergic or contact dermatitis. Several common opportunistic bacteria were cultured. The CBC abnormalities were stress leukograms and variable eosinophilia. A day after sample collection the rats were transferred to a white, square cut, autoclaved, pure cellulose bedding. The rats were rechecked after 1 wk and no new material was found. The dermatitis cases were treated with 5 mg/kg diphenhydramine and 15 mg/kg trimethopirim–sulfamethoxazole. These cases did not resolve, but did not worsen following bedding change. In conclusion, these rats, due to their lack of eyelashes and hair, are predisposed to the accumulation of bedding material within the ocular fornix, as well as potential allergic or contact dermatitis related to certain bedding materials. These findings indicate the need to evaluate all aspects of potential bedding material, especially for compromised and hairless strains.

P62 Tracheal Lesions Following Multiple Procedures Involving Gas Anesthesia in Cynomolgus Macaques

AE Kamholz[1], LE McPherson[2], LW Dochterman[2], MA Koch*[1]

[1]Animal Welfare and Comparative Medicine, [2]Pathology, Covance, Madison, WI

Clinical procedures requiring the use of gas anesthesia are necessary in many animal research studies. For a variety of procedures, gas anesthesia requiring intubation is considered best practice. In one of our primate studies, intubation was performed in cynomolgus macaques during weeks 0, 4, 8, and 13 of the test article dosing phase and additionally during weeks 4, 8, and 13 of the recovery phase. The procedures requiring intubation were performed on all study animals. On histopathologic examination, tracheal lesions were observed in control and treated animal groups. These lesions were considered to have likely been induced by overinflation of the endotracheal tube cuff. Standard intrathoracic sections of the trachea were collected on all the study animals at necropsy. Microscopically, individual animal variation was observed from an acute tissue response of erosion and inflammation to a healing response with hyperplasia of mucosal epithelium. The individual animal differences may have been related to variation in pathology sectioning, variation in techniques of the technicians performing the intubation, or individual animal response. To help prevent these lesions in the future when intubation is required, we instituted the following multistep approach: 1) conducted refresher training for all gas anesthesia certified personnel with particular attention spent on proper cuff inflation techniques, 2) purchased low-cost pressure monitor devices to continuously monitor cuff pressure to maintain it at 20 to 30 cm/H_2O, and 3) our anesthesia lead clinical veterinarian performs routine consistency checks on all anesthesia trained personnel to ensure proper procedures are followed uniformly and consistently. With these changes, the appropriate techniques can easily be used to provide consistent and safe procedures, and minimize the potential for variations in cuff pressure which could lead to confounding study interpretations or life-threatening problems in our study animals.

P63 Addressing the 3Rs: Serial Laparoscopic Liver Biopsies in Dogs and Monkeys

M Taschwer*, R Haas, JR Nelson, A Wathen, V Dinkel, J Stuhler, AE Kamholz, J Lindsay, F Thalacker

Covance, Madsion, WI

The liver plays a key role in metabolism and excretion of endogenous compounds and xenobiotics and is an area of strong experimental focus. The liver may also be a target for efficacy (for example, antiviral therapeutics) or toxicity. Animal models are critical for studying xenobiotic metabolism, biomarkers, and pharmacologic/toxicological effects on

the liver; however, the ability to obtain adequate liver tissue samples for analyses in a survival model has been lacking. A surrogate matrix (for example, blood, plasma, bile) is often used as an indirect measure for liver as its location within the body cavity makes it generally inaccessible for nonterminal sampling. In general, terminal sampling procedures have been needed when larger samples are required and survival procedures have typically been limited to a single collection per animal; the prospect of additional collections being dependent on whether minor (laparoscopic) or major techniques were used. The limitation of a single collection per animal increases the number of animals required to obtain the same data. We have developed laparoscopic surgical techniques for serial liver biopsies of approximately 100 mg to >1 g with samples being taken as close as 18 h to over a period of weeks in monkeys and dogs. All techniques allow for multiple phases and animal use has been decreased from 50% to 88%. In all cases, animals recovered completely following each procedure with no signs of pain or distress. Also, there have been no significant clinical observations, clinical pathology, changes in body weight, or surgical complications with animals being returned to group housing within 24 h of sampling. Overall this work has resulted in the ability to obtain serial liver biopsies in multiple species, providing a significant advantage over current techniques and supporting the 3Rs philosophy through substantial reductions in animal use without compromising animal welfare.

P64 Postsurgical Tibial Vein Thrombosis in a Rhesus Macaque (*Macaca mulatta*)

M Dunbar*, J Na, D Myers, P Lester

Unit for Laboratory Animal Medicine, University of Michigan, Ann Arbor, MI

A 4-y-old, intact male rhesus macaque (*Macaca mulatta*) underwent surgery for a cranial head post implant (dura not penetrated). During surgery, a catheter was placed in the right femoral artery via cutaneous cut down. Initial arterial blood pressure indicated systemic hypotension (mean < 60 mm Hg), which was treated with intravenous crystalloid, colloid, and phenylephrine boluses through a contralateral saphenous vein catheter. The remainder of the surgery was uneventful. Physical examination postsurgery revealed dehydration, lethargy, and generalized edema of the right distal hindlimb with nonweight bearing lameness. Upon evaluation of the right hindlimb, a weak femoral pulse and a firm and warm gastrocnemius muscle was palpated. No joint effusion or crepitus was appreciated and deep pain was present. Biochemical abnormalities included: hypoglycemia, hypocalcemia, hypoproteinemia, hypoalbuminemia, and a moderate elevation of alkaline phosphatase and alanine aminotransferase. Differential diagnoses included: cellulitis, peripheral neuropathy, vasculitis, thrombosis, and hepatic disease. Empirical treatment included: crystalloid fluids, carprofen, enoxaparin, cefazolin, famotidine, metoclopramide, calcium, and orogastric tube feeding. An ultrasound exam revealed a noncompressible echogenic structure in the lumen of the right posterior tibial vein, confirming a diagnosis of deep vein thrombosis. Treatment with enoxaparin and carprofen continued for 28 d and was discontinued after limb swelling resolved. Deep vein thrombosis is a blood clot that forms in a large interior vein, often in the legs, as a result of abnormal blood flow, hypercoagulability, or injury to a vessel wall. Enoxaparin, a low-molecular weight heparin, is commonly used in humans to prevent and treat acute venous thrombosis. We hypothesize that low blood pressure in conjunction with vascular compromise during arterial line placement and limb immobility during the 4-h surgical procedure produced a hypercoagulable state, resulting in tibial vein thrombosis. To our knowledge, this is the first reported case of tibial vein thrombosis in a nonhuman primate associated with placement of an arterial catheter and successfully treated with enoxaparin.

P65 Withdrawn

P66 Two Novel Mite Species Identified in a Research Zebra Finch Colony

M Siddalls*[1], JW Mertins[3], K Lertpiriyapong[1], J Pang[1], T Currier[2], MM Patterson[1]

[1]Division of Comparative Medicine, [2]Fee Laboratory, Massachusetts Institute of Technology, Cambridge, MA; [3]Pathobiology Laboratory, United States Department of Agriculture - Animal and Plant Health Inspection Service, Ames, IA

Zebra finches (*Taeniopygia guttata*) have been maintained at our institution for over 10 y, with inhouse breeding as well as occasional shipments from a commercial vendor. Newly arrived birds undergo a quarantine period during which pooled fecal cultures, testing for endoparasites, and typically a complete necropsy of one animal are performed. The resident colony of approximately 350 birds was considered to be healthy until numerous "feather mites" were observed crawling on a bird. Subsequently a survey found mites on other birds, both in the animal facility and in the neuroscience laboratory where overnight housing was allowed per an IACUC-approved protocol. Because no published reports of feather mites living on zebra finches were available, affected feather samples were sent to a US Department of Agriculture entomologist for mite identification. Detailed examinations revealed the most abundant mite to be a novel, undescribed species in the genus *Neocheyletiella*. Whereas known *Neocheyletiella* mites have been previously characterized as skin parasites of various birds worldwide, the species on the zebra finches is morphologically and biologicly unique, for example, it dwells gregariously in silken nests on the body feathers. Infrequent specimens of a true feather mite and another new species, *Megninialges* spp., were also present. Once the mites had been thoroughly assessed, multiple treatments of the birds, nests, and cages were undertaken using a pyrethrin-based spray at biweekly intervals; sampling to demonstrate successful eradication is ongoing. Due to the insidious nature of this infestation, future finch shipments will be evaluated for ectoparasites, but also treated prophylactically. Routine colony surveillance will be revised to include feather examinations. This case highlights a general dearth of information in the scientific literature about ectoparasites and other clinical issues in zebra finches, even though these are the most popular songbirds used in biomedical research.

P67 Possible Predictors of Inguinal Hernias in Male Rhesus Macaques (*Macaca mulatta*)

N Bacarella*, Z Myles, R DePaz, N Martinez

Advanced BioScience Laboratories, Rockville, MD

Inguinal hernias are a fairly common affliction in rhesus macaques, though no statistics on disease prevalence or incidence currently exist. In this retrospective study, we aimed to examine and identify some of the possible predictors of inguinal hernias in male rhesus macaques. Inguinal hernias can be pathogenic and may cause clinical concerns. The most severe of which occur when bowel or other abdominal contents become entrapped within the hernia ring. Out of a population between 800 and 1100 monkeys over the last 3 y (400 to 550 of them were males), 32 animals presented with an inguinal hernia either bilaterally or unilaterally, ranging from minor to severe upon physical exam. An analytical approach was taken to gather historical data from the animal database to examine the various parameters that might contribute to the incidence of inguinal hernias. Age, body condition and body weights were assessed. The ages ranged from 3 to 15, with 9 as the mean age. The range of body condition scores was 2 to 5 out of 5 with 3.3 out of 5 as the average score. The body weight range was 5.9 to 16.3 kg, with 10.6 kg as the average weight. In the total population in the colony, the mean body weight, body condition, and age was 5.5 kg, 2.89, and 6, respectively. It appears that the incidence of inguinal hernias increases with age, increased body condition, and a higher body weight. This suggests that inguinal hernias could be associated with overweight and middle aged or older male monkeys. No statistics were performed on the data, as our main goal was to identify the possible predictors of inguinal hernias. There are many directions for future research in this area. These include genetic history; that is, heritability of inguinal hernia susceptibility, peritoneum strength/weakness, type of social housing (pair or group housed); whether or not pair-housed monkeys have a higher incidence of hernias than singly housed monkeys or vice versa, and self-injurious behavior. Additionally, and likely most important future direction of research is confirming if age, weight, and body condition are true predictors of inguinal hernias and determining why this is. Identifying the true predictors of inguinal hernias may ultimately lessen the incidence of this condition in laboratory populations in the future.

P68 Severe Respiratory Depression Following Buprenorphine Administration in *Callithrix jacchus*

PS Allen*, MH Nowland, E Liechty, I Bergin

Unit for Laboratory Animal Medicine, University of Michigan, Ann Arbor, MI

Three out of 6 marmosets (*Callithrix jacchus*) that underwent a craniotomy via isoflurane anesthesia as part of an IACUC-approved protocol died acutely in the postoperative period. All marmosets were in an experimental control group. Gross necropsy revealed marked hepatomegaly, histologically diagnosed as acute centrilobular hepatocellular degeneration, a nonspecific indicator of acute circulatory shock. No cardiac, pulmonary, or cerebral lesions were present, though several incidental lesions common to marmosets were identified. Evaluation of the surgical records revealed variations in the administration of buprenorphine. The expired marmosets received a subcutaneous dose (0.03 mg/kg) of buprenorphine pre- or intraoperatively and required artificial ventilation after buprenorphine administration. Two of the 3 surviving marmosets received the same buprenorphine dose postoperatively and only one of those marmosets required artificial ventilation for a portion of the surgery. Buprenorphine dosing for the third surviving marmoset could not be determined from the surgical record. With the correlation between buprenorphine administration, severe respiratory depression, cardiovascular collapse, and death, buprenorphine was removed from the analgesic protocol. Nine marmosets then successfully completed the research protocol without anesthetic or recovery complications. Buprenorphine has been associated with respiratory depression at high doses in some humans and this depression is relatively resistant to reversal by naloxone. The buprenorphine dose used in this case was within the commonly used therapeutic range for nonhuman primates but the cause for the suspected sensitivity to buprenorphine in the current report is unknown. Based on this case series, it is recommended that buprenorphine only be used in the postoperative period and at low doses (0.005 to 0.01 mg/kg) in the common marmoset. Species-specific pharmacologic information is frequently lacking in veterinary medicine, making dose selection very challenging. This case series illustrates the need for heightened caution when extrapolating doses from other species, even those that are closely related.

P69 Field Application of Tuberculin Skin Test and Rapid Lateral-Flow Assay for Tuberculosis for the Diagnosis of Tuberculosis in Semiwild Chimpanzees (*Pan troglodytes verus*)

R Khadka*[1,2], E Schallenberger[1]

[1]Christian-Albrechts University, Kiel, Germany; [2]Tacugama Chimpanzee Sanctuary, Freetown, Sierra Leone

Tuberculosis is one of the most crucial diseases of human and nonhuman primates worldwide, because of its high incidence, zoonotic potential, ability to spread rapidly, and high mortality rates. Due to its zoonotic nature, it is very important to diagnose and prevent the spread of tuberculosis between species. In this study, we aimed to establish a basic tuberculosis diagnostic method and preventive measure particularly for remote captive settings where sophisticated tests cannot be carried out. A basic diagnosis method of tuberculin skin test and rapid lateral-flow assay for tuberculosis have been applied to screen and diagnosis tuberculosis in chimpanzees. Data were collected from 105 chimpanzees (48 males and 57 females) from April 2011 to October 2012 at our institution in Sierra Leone during the normal health checkup and quarantine period of chimpanzees. Tuberculin skin test was done intrapalpebrally with avian and bovine - purified protein derivatives (PPD) antigen. Rapid lateral-flow assay for tuberculosis assay, done simultaneously with skin test in plasma or serum, detects antibodies against both *M. tuberculosis* and *M. bovis* in nonhuman primates that uses a cocktail of recombinant TB-specific antigens. Eighty of 105 and 95 of 105 chimpanzees from 6 different social groups (A to F) and quarantine (Q) were tested with tuberculin and rapid lateral-flow assay for tuberculosis tests, respectively. None of the chimpanzees showed any reaction to tuberculin and rapid lateral-flow assay tests. A digital image for both skin and rapid lateral-flow assay for tuberculosis tests and score recording of skin test (24, 48, and 72 h) had been captured. All the sanctuary staff members ($n = 20$) were negative for acid fast bacilli tests at human hospital. Interpretation of our test results do not rule out the presence or absence of tuberculosis infection at this moment. For the early and reliable detection of tuberculosis, a combination of skin test and rapid lateral-flow assay gives the best testing algorithm in nonhuman primates especially for remote sanctuaries. From the zoonotic point of view, we tested the sanctuary staff members to make sure that everyone is free of the disease. For future, any case, of suspicious or positive chimpanzees with skin or rapid lateral-flow assay test results should be retested and further diagnosis should be done with more reliable tests.

P70 Acute Toxicity Following Intraperitoneal Injection of Azoxymethane in A/J Mice

RC Curtis*[1], B Podell[2], LV Kendall[1]

[1]Laboratory Animal Resources, [2]Department of Microbiology, Immunology and Pathology, Colorado State University, Fort Collins, CO

Azoxymethane (AOM) is a colon-specific carcinogen that is widely used in research to study the pathology and genetics of colorectal cancer in rodents. Previous work shows that while the A/J strain of mice is highly susceptible to the tumorigenic effects of azoxymethane, an acute dose-dependent and age-related toxicity can occur resulting in acute liver failure. Azoxymethane is used as a model of fulminant liver failure at these higher doses. Current research aimed at uncovering certain dietary effects on tumor development, used a 10 mg/kg dose intraperitoneal once weekly for 6 wk. This regimen is reportedly highly tumorigenic without toxicity. Following the first injection in this study, mortality occurred in 9 of 60 mice (15%) over the course of 2 to 4 days postinoculation. All mice necropsied had gross findings of a dark red to black liver with pale borders. Histopathology of the liver revealed a severe, diffuse necrosis with loss of sinusoids causing severe hemorrhage. The necrosis was centrilobular and midzonal largely sparing those hepatocytes in the periportal region. A mild to moderate tubular necrosis was evident in the kidneys of these mice as well. This report describes a relatively high incidence of acute toxicity at a dose (10 mg/kg IP) and age (3 mo) previously shown as nontoxic in A/J mice.

P71 Adverse Events Using Repeat Boluses of Ketamine–Xylazine–Acepromazine for Extension of an Uninterrupted Surgical Plane of Anesthesia in C57BL/6J Mice

SM Jaber*[1,2], FC Hankenson[1,2], J Marx[1,2]

[1]University Laboratory Animal Resources, [2]Department of Pathobiology, School of Veterinary Medicine, University of Pennsylvania, Philadelphia, PA

A surgical plane of anesthesia is often necessary for experimental procedures in mice. During procedures of prolonged length, and for which inhalant agents are contraindicated, repeated boluses of injectable agents tend to be the industry standard used to extend the surgical plane of anesthesia due to the technical difficulty of administration of constant rate infusions in mice. Ideally, there should be no interruption or lightening of anesthetic depth during the procedure. Here, we compared the safety and efficacy of repeat dosing regimens of injectable anesthetic boluses given prior to the lightening of anesthetic depth in 8- to 14-wk-old male C57BL/6J mice. The combination of anesthetic agents used for initial induction was ketamine, xylazine, and acepromazine (KXA) at 80:8:1 mg/kg, respectively. All animals were randomly assigned to experimental groups based on the dose of the repeat bolus. The repeat dose groups ($n = 4$) were 25%, 50%, or 100% of the initial dose of K or 50% of the initial dose of both K/X combined. Mice were administered their repeat bolus 30 min after the initial dose and while still under a surgical plane of anesthesia. Time to loss and return of righting reflex were recorded for each mouse. Mice were monitored for presence of pedal withdrawal reflex, heart rate, respiratory rate, and rectal temperature every 5 min. Initial failure rate of the KXA anesthetic cocktail was 13% with 1 of 30 mice dying from the initial dose and 3 of 30 not reaching a surgical plane of anesthesia. Mortality was unexpectedly high overall (13 of 26 or 50%), with similar mortality between groups regardless of redosing. The average time of continuation of surgical plane after the repeat dose of anesthetic was 21 min for 25% K group, 25 min for the

50% K group, 62 min for the 100% K group, and 62 min for the 50% KX group. In conclusion, if extension of a surgical plane of anesthesia in mice is needed, repeat bolus dosing should not occur prior to the end of the initial plane of surgical anesthesia in order to limit unexpected mortality. Additional work is needed to determine the optimal regimen for prolonged anesthesia using injectable agents in mice.

P72 Lateral True Hermaphroditism with Confirmed Y Chromosome in a Transgenic Founder Mouse (*Mus musculus*)

SC Roshwalb*[1], T Whitcomb[1], TK Cooper[1,2]

[1]Comparative Medicine, [2]Pathology, Penn State University College of Medicine, Hershey, PA

A 6-mo-old FVB/N-TgTn(sb11-tetO/Luc)1 mouse presented with abnormal genitalia, unilateral inguinal swelling, and a 2-mo history of nonproductive breeding. Although identified as a female by the commercial generator, this mouse was used as a male breeder based on positive SRY PCR results, and had been housed with a wildetype female. External examination included imperforate vagina, an enlarged phallus/clitoris, and developed nipples. Internally, there was a small ovary attached to a short uterine horn unilaterally, with a small testicle with epididymis and spermatic cord on the contralateral side. Histologically, tissues were normal except for a complete absence of normal gametogenesis in either gonad. The mouse had phenotypically female characteristics in the mammary gland, submandibular salivary gland, adrenal glands, and kidneys. The presence of the Y-chromosome was confirmed by positive PCR reactions for additional distant Y-chromosome specific genes, Sly and Ssty2. This is an unusual cause of reproductive failure in a genetically valuable founder mouse generated by microinjection technique from a commercial vendor.

P73 Novel Technique for Sampling of CSF in Mouse

S Li*, J Scola

Vertex Pharmaceuticals, Cambridge, MA

It is widely accepted that the CSF concentration of a drug candidate can be indicative of its unbound brain concentration. However, CSF sampling in the mouse is a difficult procedure and yields very little sample volume. We have adopted a unique cisterna magna puncture technique that allows reliable CSF sampling in an anesthetized mouse. This technique requires expert surgical skills and an advanced understanding of mouse anatomy and physiology. Careful dissection of the posterior neck is conducted under a microscope while the anesthetized mouse is restrained in a stereotaxic frame. A 27-gauge needle attached to PE-20 tubing and 10-µL Hamilton glass syringe is advanced through the overlying arachnoid membrane into the cisterna magna. Five to 10 µL of nonblood contaminated CSF can then be withdrawn and stored in glass vials for analysis. As a part of the method validation process, the practicability of sampling CSF was tested in a study with a novel CNS-penetrant compound. The compound was administered by oral gavage at 30 mg/kg. Two hours after administration, 10 µL of plasma was obtained via submandibular bleed and CSF samples obtained using the described above cisternal puncture method. Inspection of mean CSF plasma concentration ratios indicated that the concentrations of the compound in CSF were similar to the unbound drug concentration in the plasma. In conclusion, the cisternal puncture is a suitable method to collect CSF samples in anesthetized mice. This method could be very helpful in pharmacokinetic/pharmacodynamic assessment of centrally acting drugs.

P74 Postoperative Echocardiographic Evaluation of Biologic Mitral Valve Implantation in Sheep

S De Vleeschauwer*, H De Praetere, B Meuris, P Hrijgers, M Herregods

KULeuven, Leuven, Belgium

The ovine model is the animal of choice for bioprosthetic heart valve testing. Although echocardiography is best suited for interim valve evaluation, literature on sheep echocardiography is scarce. Within an anticalcification study of biologic mitral valves, 19 sheep (female, median age and weight of 206 d, 26.5 kg, respectively, Swifter (n = 16), Swifter × Charolais (n = 3)) underwent echocardiography 6 d after surgery. After premedication with 15 mg/kg ketamine IM, echocardiography was performed under isoflurane mask-anesthesia with animals in right lateral decubitus on a table containing a cutout at thorax level. Four images were obtained in the third to fifth intercostal space: right parasternal long axis 4 and 5 chamber view, right parasternal long axis with left ventricular outflow view, and right parasternal short axis view through the mitral valve. We measured aortic annulus and velocity time integral over the aortic valve to determine cardiac output. The mitral valve was evaluated with color Doppler imaging for leakage. Pulsed wave spectral Doppler was used for the evaluation of valve stenosis: velocities, pressure gradients, velocity time integral, deceleration time, and pressure half time. Other structures that could clearly be visualized were both ventricles and atria, wall thicknesses, tricuspid valve. Measurements are reported as median (IQR). Median aortic annulus was 2.01 (1.95 to 2.12) cm, aortic velocity time integral was 11.88 (9.64 to 12.72) cm with a median cardiac output of 4.02 (3.22 to 4.83) L/min. Color Doppler imaging showed a mild valvular leak in one sheep, none of the sheep showed paravalvular leakage. Peak and mean velocities over the mitral valve were 0.91 (0.73 to 1.09) m/s and 0.70 (0.54 to 0.85) m/s, respectively. Peak and mean pressure gradients over the valve were 3.36 (2.15 to 5.21) mm Hg and 2.05 (1.15 to 3.05) mm Hg, respectively. Velocity time integral, deceleration times, and pressure half times of the mitral valve were 17.92 (12.97 to 20.85) cm, 150 (126 to 191) ms and 46.6 (36.6 to 58.3) ms, respectively. This study shows that echocardiography in sheep is feasible under mild anesthesia, that most cardiac structures can be visualized and that echocardiography is an excellent tool for interim evaluation of implanted mitral valves.

P75 Facial and Oral Nerve Blocks with an Opioid Adjunct for Dental Procedures in the Nonhuman Primate

SJ Cital*

Primate Medicine, California National Primate Research Center, Davis, CA

Profound cardiovascular, thermoregulatory, and respiratory effects of inhalant anesthesia have led the veterinary community to investigate adjunctive modalities to decrease the use and effect of volatile anesthetics. The use of various facial and oral nerve blocks, which is the insertion of a local anesthetic near or onto a nerve for pain control, in combination with inhalant anesthesia, has potential for great anesthetic gas sparing effects. Research has shown that mixing an opioid, such as buprenorphine or morphine, with a local anesthetic can extend local analgesia from an average of 2 to 4 h to up to 28 h postoperatively in humans. Other beneficial effects include decreased recovery time and better local analgesia. With the lasting effect of the nerve block, there is also a reduced need for patient compliance, risk of human injury, medications administered and stress to the animal. A dental syringe or tuberculin syringe with a 27-gauge needle is used for administration of local anesthetics. A case series using 6 rhesus macaques (*Macaca mulatta*) showed animals that received a nerve block with the opioid adjunct prior to maxillary cuspid tooth extraction, maintained under general anesthesia, had an isoflurane minimum alveolar concentration of 0.5% to 0.9%, which is reduced from 1.2% to 1.3% for nonblocked patients for cuspid tooth extractions. Postoperatively the blocked animals with the opioid started eating immediately or a few hours after full recovery from gas anesthesia. Nonblocked patients took several hours or up to a day to resume acceptable caloric intake. Thus finding using opioid laced facial and oral nerve blocks in nonhuman primates directly correlates to improved anesthetic and analgesic coverage both intraoperatively and postoperatively. The addition of an opioid may add to the pain-relieving effects and longevity of the local block.

P76 A Novel Organism, *Staphylococcus capitus-ureo*, Isolated from a Mouse (*Mus musculus*) with Severe Disseminated Botryomycosis

TR Bobo*, SC Roshwalb, T Whitcomb, TK Cooper

Comparative Medicine, Penn State Hershey College of Medicine, Hershey, PA

A 4-mo-old female breeder B6.129S-*Cybb*^{tm1Din}/J mouse presented with large soft tissue swellings of the left hindlimb and left caudal mammary gland. Several mice from this principle investigator's colony had presented previously with similar lesions, and culture results identified *Staphylococcus xylosus* as the causative agent. Gross necropsy findings included multiple abscesses, which oftentimes were caseating, within both left and right lungs, left mammary gland, and left popliteal lymph node and tarsus. Histologically, multiple pyogranulomas centered on small colonies of large gram-positive cocci were present. Culture of the lung was positive for *S. capitus-ureo*, a coagulase negative organism commonly isolated from humans. *S. xylosus*, a known commensal of rodents and humans, was isolated from the mammary gland. Both organisms were resistant to the enrofloxacin-medicated feed used in this colony. Recurrent bacterial and fungal infections, including botryomycosis, are common in this strain of mouse because of the targeted mutation of the β-polypeptide of cytochrome b245, a subunit of NADPH oxidase, preventing superoxide mediated destruction of phagocytized microorganisms. To our knowledge, this is the first report of *S. capitus-ureo* identified in a mouse with botryomycosis.

P77 Flouroscopy-Aided Foreign Body Retrieval from a Guinea Pig (*Cavia porcellus*)

TT Chatkupt*

Department of Comparative Medicine, Oregon Health and Science University, Portland, OR

A 5.5-mo-old male guinea pig had previously undergone cochlear implant surgery. While undergoing auditory brainstem response testing as part of an experimental protocol, one of the platinum needle electrodes implanted in the temporal region broke off. An attempt by the researchers to recover the needle via manual palpation and exploratory surgery assisted by surgical scope were unsuccessful. The guinea pig subsequently underwent fluoroscopy at the soonest available date, 6 d later. A cardiac mobile system with digital imaging was used for fluoroscopy and digital X-rays of the guinea pig while it remained conscious and resting in a clear polysufone cage with bedding and filter top in place. The needle electrode was located to the left side of the head, approximately overlying the molars, just caudal to the zygomatic arch. Once the needle electrode was located, the guinea pig was anesthetized, placed on monitoring, and prepared for surgery. A 1-cm incision was made over the belly of the masseter muscle. After some exploration, the needle electrode was identified in the central aspect of the masseter muscle, between the superficial and middle portions, and removed. There was no fibrosis or evidence of inflammation at the site. The incision was closed in 2 layers. Recovery was uneventful. This unique case serves as a reminder that penetrating foreign bodies, while relatively uncommon in laboratory animal medicine, can migrate and thus pose a potential threat if they puncture the heart, intestines, or other major organs. It also illustrates the usefulness of fluoroscopy and other radiographic modalities in laboratory animal practice. Finally, it is a reminder of the tractable nature of guinea pigs, and how many procedures may be performed on them with minimal restraint or anesthesia.

P78 Refinement of a Hematopoietic Stem Cell Engraftment Model for the Novel Use of Gene Therapies

A Allaire*

DSAR - Boston Hub, Genzyme, Framingham, MA

Gene therapy using hematopoietic stem cells shows positive results in treatment of various genetic blood disorders, immune system diseases, and metabolic disorders. In hopes of translating successes observed in early phase 1 and 2 clinical trials where lentiviral-transduced hematopoietic stem cells were used to treat diseases such as severe compromised immunodeficiency and adrenoleukodystrophy, we are developing a hematopoietic stem cell transplant model to evaluate stem cell mobilization and harvest and to evaluate pretreatment regimens for recipient mice. CD45.1+ mice were used as donors and CD45.2+ C57Bl/6 were used as recipients. Due a single allele difference between strains, we were able to determine the level of engraftment with flow cytometry. Various pretreatment regimens, including sublethal irradiation, chemotherapeutics and mobilizing agents, and varying combinations were administered to recipients to reduce bone marrow cells immediately prior to transplantation to permit engraftment of donor cells. Bone marrow donor cells were harvested directly from femurs and tibias of donor mice or mobilizing agents were administered to release hematopoietic stem cells from bone marrow into peripheral circulation for collection. After intravenous injections of donor cells into recipients, blood samples were collected for 45 to 120 d to measure levels of chimerism and host cell populations using flow cytometry. Chimerism levels over 60% were achieved with pretreatment use of mobilizing agents to circulate hematopoietic stem cells in recipient mice in conjunction with mobilized donor hematopoietic stem cells. This percentage is consistent with what was noted with irradiation, chemotherapeutics, and direct bone marrow collection, but requires less invasive methodology and is less detrimental to the overall health of the animals. The use of mobilized donor cells to produce better engraftment of transplant is being evaluated for use in patients, translating to fewer painful procedures, shorter hospitalization periods, and the potential for a longer lasting effect of the transplant. Future studies are planned to determine the ability of enriched mobilized cells to achieve long-term repopulation of recipient animals to further model development.

P79 Inactivating AP1 Transcription Factor Function in Suprabasal Epidermis Produces a Loricrin Keratoderma Phenotype Associated with Enhanced Th1 Chemokine Production

C Young*, E Rorke, J Babus, R Eckert

University of Maryland, Baltimore, Baltimore, MD

The activator protein one (AP1) transcription factors are key controllers of keratinocyte proliferation, differentiation, apoptosis and transformation. To study AP1 factor function in epidermis, we expressed TAM67, a dominant-negative form of c-jun that inhibits the function of all AP1 factors, in the suprabasal epidermis. Suprabasal AP1 blockade results in a progressive symmetric erythrokeratoderma-like phenotype characterized by hyperproliferation, hyperkeratosis, parakeratosis, constriction of the tail and digits, and nuclear localization of loricrin. We hypothesize that TAM67 expression alters epidermal chemokine production and that this is, in part, responsible for the TAM67 impact on the epidermal phenotype. Mice were treated for zero to 21 d with doxycycline and the phenotype assayed. This analysis reveals a sequential activation of events beginning with nuclear loricrin accumulation at 24 to 48 h, increased basal keratinocyte proliferation at 48 to 72 h, and enhanced epidermal production of Th1 lymphocyte chemoattractants, CXCL9, CXCL10 and CXCL11 as early as 12 h. Increased mRNA expression for each of these chemokines was confirmed by qRT-PCR. CD3+ activated T cells are found to be elevated in the epidermis of 8 d doxycycline treated mice by nearly 5-fold. These findings describe a hierarchy of early and late events in disease development and suggest that increased expression of Th1 chemokines may be a contributing factor.

P80 Pdlim7 (LMP) Knockout Mice Display Decreased Trabecular Bone Density in the Spine and Femur with Microcomputed Tomography

C Oliver*[1], M Viggeswarapu[2], MF Gary[3], M Teklemarian[2], S Sangadala[1,2], L Titus[1,2], SD Boden[1,2]

[1]Orthopaedic Research, VAMC, Decatur, GA; [2]Orthopaedics, [3]Neurosurgery, Emory University School of Medicine, Decatur, GA

Our group has described rat and human LMP-1 as an osteoinductive protein whose overexpression induces bone formation in rats and rabbits and enhances BMP-2 efficacy in vitro. To understand the systemic role of LMP-1 during development and maturation, global Pdlim7 (mouse equivalent of LMP) knockout (KO) mice were developed using gene trapping and breeding techniques. Mice were euthanized at 18 or 26 wk for analysis. Cardiac puncture was performed to obtain serum for a chemistry panel. Weight and height were recorded. Spines and femurs were harvested from wild, heterozygous and KO mice for analysis by microcomputed tomography to assess bone structure. The KO mice at 18 and 26 wk weighed less (20%) and were shorter in length (6%), $P = 0.0003$ and $P = 0.001$, respectively, compared with wildetype. uCT analysis of femurs revealed that 18- and 26-wk female KO mice

displayed a decrease in trabecular bone (BV/TV) of 25% ($P = 0.04$) and 45% ($P = 0.001$), respectively, compared with wildetype. uCT analysis of lumbar spines revealed that 26-wk female KO mice displayed a decrease in vertebral body BV/TV ratio by 25% ($P = 0.001$) compared with wildetype. There was no statistically significant difference in uCT analysis of femurs or spines in male 18 or 26 week KO mice compared with wildetype. The 26-wk female KO mice had normal serum chemistries compared with heterozygous and wildetypes. We present here the development of the first LMP KO mouse. We conclude that LMP is an important determinant of mouse weight, length, and female bone density. We speculate that these may be due to altered responsiveness to BMPs, an important regulator of bone formation during development. Further research is needed to elucidate other phenotypic differences and to determine the mechanism causing the reduced body size and gender specific bone loss of LMP KO mice.

P81 Biodistribution Analyses of a Near Infrared Labeled Bispecific Monoclonal Antibody by Optical Imaging

NC Peterson, GG Wilson*, Q Huang, N Dimasi, K Sachsenmeier, DL Goldsteen

Medimmune, Gaithersburg, MD

Biodistribution analyses of pharmaceutical compounds in preclinical animal models typically require the use of radioactive tracers or postmortem, bioanalytical analyses of processed tissues. Using NIR fluorescence tagged proteins and an optical imaging system, an alternative approach for performing biodistribution analyses for biologics-based therapeutics was investigated. A bispecific antibody (Medi6348) which recognizes insulin-like growth factor 1 receptor and epidermal growth factor receptor was labeled with a near-infrared (NIR) fluorophore and intravenously injected into athymic mice. Fluorescent in vivo and ex vivo images of major organs revealed that Medi6348 was retained in the liver and kidneys within first 5.5 h of injection when compared with similarly analyzed NIR-labeled control antibody (R347). Results of biodistribution analyses of a control bispecific antibody (R347bs) were similar to that of Medi6348, suggesting that early retention of these bispecific antibodies in the liver and kidney may be determined by factors other than their binding specificity. These results support the continued development of in vivo optical imaging approaches for the assessment of NIR fluorescent labeled antibody biodistribution analyses.

P82 Generation of Engineered Nucleases Targeting Guinea Pig Recombination Activating Gene

H Holcombe*[1], ML Maeder[2], D Reyon[2], JG Fox[1], JK Joung[2]

[1]Division of Comparative Medicine, Massachusetts Institute of Technology, Cambridge, MA; [2]Center for Computational and Integrative Biology, Massachusetts General Hospital, Charlestown, MA

Guinea pigs are often used as models for infectious disease research, most notably tuberculosis, as well as in vaccine development. In many respects, their immune system is more similar to that of humans than is the mouse immune system. A major drawback to using guinea pigs as animal models is the relative lack of reagents compared with those available for mice. In particular, there are no published methods for genetic manipulation of the guinea pig genome. A number of recent advances have made the generation of genetically modified guinea pigs feasible. Published protocols are now available for superovulating female guinea pigs, synchronizing estrus, and, most recently, transferring embryos. While generation of guinea pig embryonic stem cells has not been reported, progress in engineered nucleases used to knockout genes in a variety of species, including mice, rats, rabbits, and pigs, suggests that this technology may be useful for modifying the guinea pig genome as well. To address this, we have designed 8 zinc finger nucleases (ZFNs) targeting 2 distinct regions of the guinea pig recombination activating gene (RAG). Four pairs have been tested in a guinea pig fibroblast cell line. Additionally, we have generated one pair of transcription activator-like effector nucleases (TALENs) for comparison. Fibroblasts were transfected with DNA encoding each of the nucleases as well as with DNA encoding GFP. GFP-positive cells were sorted and collected for DNA extraction, and the targeted region in RAG was amplified by PCR for mutation analysis. Nonhomologous end-joining (NHEJ)-mediated mutations were identified by a previously described T7E1 assay and by sequencing. We found that engineered nucleases were highly efficient at inducing mutations in RAG, with frequencies similar to those reported for ZFNs targeting genes in human and other cell lines. Two ZFN pairs induced mutations at a frequency of 9.5% to 24.2%. The TALEN pair targeting the same region of DNA resulted in mutations at a frequency of approximately 13%. The remaining 2 pairs of ZFNs failed to induce a detectable number of mutations. We conclude that engineered nucleases can be used to delete genes in guinea pig cells and may be useful for generating novel guinea pig knockouts.

P83 HIV-1 Transgenic Nude Rat: A Novel Disease Progressed Animal Model for HIV-1/AIDS

JM Davenport*[1], S Navas-Reyes[1], M Guo[2], H Davis[1], W Royal[2], J Bryant[1]

[1]Institute of Human Virology, [2]Maryland Center for Multiple Sclerosis Treatment and Research, University of Maryland School of Medicine, Baltimore, MD

Numerous animal models investigate the HIV-1 virus, host cell response, and immune system. Each model has its strengths; none is perfect in translation of HIV-1 pathogenesis, clinical manifestations, or end stage AIDS-related pathologies. In 2001, our lab reported the available HIV-1 transgenic rat (HIV-1Tg). HIV-1Tg appears clinically healthy until 9 mo of age, often not demonstrating clinical disease until 14 mo of age. We decided to create a more reliable animal model for HIV-1 virus, which mimics T-cell deficiency seen in AIDS patients. We report a new animal model, the HIV-1 transgenic nude rat (HIV-1TgNu+). We developed the first HIV-1TgNu+ rat through random pair mating for recombinant inbred advanced intercross lines of the HIV-1Tg rat to the athymic nude rat. The successful breeding design provided a consistent, reproducible model to study T-cell immunodeficiency, nonAIDS cancers and opportunistic organisms in the HIV-1 setting. We phenotyped the transgenic offspring based on congenital cataracts. Transgenic offspring were further identified by PCR amplification of the nef gene in tail DNA preparations. Confirmation of the transgene's integration was performed by Western blots. Several phenotypes can be bred to mimic HIV-1/AIDS-related pathologies. The earliest visible phenotype occurs within 2 wk of age when cataracts are noted. The HIV-1TgNu+ rat can be selectively bred for skin disease based on fur pattern. The HIV-1TGNu+ rat has similar characteristics comparable to the existing HIV-1 Tg rat model with the additional benefit of exhibiting multiple pathologies that relate to AIDS adding clarity on the expression of HIV-1 genes and proteins.

P84 Cardiac Gene Therapy in Sheep Model of Heart Failure: Analysis of Safety, Physiologic Consequences, and Complications

K Mihalko*[1], MG Katz[2], A Fargnoli[2], R Williams[2], J Dvorak[2], AJ Carty[3], CR Bridges[2]

[1]Comparative Medicine, [2]Sanger Heart and Vascular Institute, Carolinas Healthcare System, Charlotte, NC; [3]University Laboratory Animal Resources, University of Pennsylvania, Philadelphia, PA

Current management of heart failure (HF) is limited. In an effort to develop more effective treatments, emerging gene therapy approaches are promising. One of the most attractive HF genes is the sarcoplasmic reticulum calcium ATPase (SERCA2a). Previous studies have demonstrated that restoration of SERCA2a improves cardiac contractility and reverses HF. However, safety, preoperative management, physiologic consequences, and complications in large animals have not been addressed. Ischemic HF was induced by surgical ligation of circumflex artery branches in 25 sheep. Blood samples were taken for complete blood chemistry tests. Thirteen sheep underwent cardiac surgical delivery of adeno-associated virus encoding SERCA2a 4 wk after myocardial infarction, while 12 sheep served as untreated controls. Hemodynamic studies using MRI were performed at baseline, 3 and 12 wk after gene transfer. Postmortem samples were analyzed extensively for biodistribution. Echocardiograms and electrocardiograms were obtained to evaluate function. Surgical delivery of SERCA2a resulted in robust gene transfer in all heart regions with no detectable collateral

gene expression, a distinguishing feature of surgical-mediated delivery. Pre- and postoperative echos revealed no change in function. Four of 25 animals died. All others survived and resumed normal activity. The procedure had associated complications involving the respiratory system (pneumonia, $n = 1$; pleural effusion, $n = 2$), impaired renal function with elevation of creatinine and urea nitrogen ($n = 2$), abnormal liver function tests with elevated alanine aminotransferase or aspartate aminotransferase ($n = 2$), and mild to moderate metabolic acidosis ($n = 4$). After 48 to 72 h, all abnormal blood gases, CBC, renal, and liver function levels returned to normal. The terminal results indicate preservation of cardiac function and rescue from HF. The present study demonstrates that surgical cardiac gene delivery in a large animal model appears to be safe with no toxic effects on major organ functions which support the potential role of HF gene treatment.

P85 Low Cost, High Throughput, Noninvasive Fluorescence Imaging Technique to Phenotype GFP-LC3 Transgenic Mice

NL Patel[*1], S Stern[2], C Robinson[3], C McLeland[2], JD Kalen[1]

[1]Small Animal Imaging Program/Laboratory Animal Sciences Program, [2]Nanotechnology Characterization Laboratory/Advanced Technology Program, [3]Laboratory Animal Sciences Program, SAIC-Frederick, Frederick National Laboratory for Cancer Research, Frederick, MD

Fluorescence protein based reporters are widely used to confirm the presence of a transgene. Green fluorescence protein (GFP) tagged light chain-3 (LC3) is a standard in vivo marker for autophagy. Current uses of the GFP-LC3 transgenic autophagy model include cancer, neurodegeneration, inflammation, and developmental research. Although several in vitro methods to monitor autophagy are well established, they are unable to evaluate autophagy during development, disease progression or in response to pharmacologic agents in living, heterogeneous tissues. Reverse transcription polymerase chain reaction (RT-PCR) is currently used to screen the presence of GFP-LC3 transgene. However, it is invasive, labor intensive, and costly. Here, we report a noninvasive, high throughput and cost effective in vivo fluorescence imaging scheme to reliably detect marker protein. The GFP-LC3 transgenic colony was maintained by crossing heterozygous GFP-LC3 mice with the wildetype C57BL/6 background strain. Resulting offspring were imaged using the vendor protocol. Standard region of interests (ROI) were placed over the paws on the unmixed GFP component image, summed for each mouse, and used to predict the presence of the transgene. Based on the frequency distribution, a group of mice ($n = 16$) was tail clipped with count levels spanning from 2 to 85 counts per second and genotyped using RT-PCR. Mice with signal levels > 40 counts per second and < 28 counts per second were confirmed gene +ve and gene –ve, respectively. However, imaging results were inconclusive for the mice with signal levels 28 to 40 counts per second and genotyping is required to confirm the presence of the transgene. Even though the described method has some limitations, it can scan approximately 20 mice per hour which is 12 times faster than conventional RT-PCR with an approximate cost reduction per mouse of 75%. Moreover, prompt analysis allows efficient cage use which reduces housing cost. Reported method provides rapid, reliable, cost effective and noninvasive alternative for the conventional genotyping method.

P86 Evaluation of a Rodent Individually Ventilated Cages Microenvironment during a 14-Day Cage Change Cycle

A Xenos[*]

Comparative medicine, Genzyme, Framingham, MA

The frequency at which rodent cages are changed can contribute to the health of the animals and experimental results. The *Guide for the Care and Use of Laboratory Animals* suggests a 7-d cage change interval for rodents and requires performance-based data to justify a longer cage change cycle. Technological advances in the development of individually ventilated cages (IVC) may allow a cage change cycle up to 14 d. The objective of this study was to provide experimental data to support the longer change cycle. Levels of ammonia, CO_2, and relative humidity were evaluated. In addition, cage temperature, fecal quantity, body weight, and health issues were monitored to identify any additional cage variables. These parameters were monitored and recorded over a 2-wk change cycle 3 times per week. The primary endpoint was to maintain intracage ammonia concentration below 50 parts per million (PPM) when cages were ventilated at 70 air changes per hour. In addition, CO_2, temperature, and humidity levels, as an average of all the cages on the rack, were measured in the exhaust plenum and compared with room levels. The quality of feces was classified as to whether it was scattered uniformly or compacted to an isolated portion of the cage. The moisture of the bedding was also scored. The mice were weighed on days 0 and 14. On day 14, the maximum intracage ammonia level was 10 PPM in a small IVC housing 4 adult mice and 25 PPM in a large IVC housing 5 adult mice. Temperature and relative humidity were approximately equal to the room ambient levels. CO_2 was approximately double the room levels, averaging 1000 ppm, but within acceptable limits. There were no health issues or changes in body weight during this 14-d cycle. The results provided support a performance standard allowing 14-d as an acceptable cage change interval for rodents housed in IVCs.

P87 Withdrawn

P88 An Inexpensive Approach to Social Housing Enrichment for Laboratory Rabbits and Guinea Pigs

A Rose[*], CE Ferrecchia, A Howell, L Presson-Jennings, K Jensen, R Van Andel

Office of Laboratory Animal Care, University of California, Berkeley, Berkeley, CA

Environmental enrichment is an essential aspect in the daily lives of laboratory animals. Enrichment for social species in particular has gained more attention recently with the release of the eighth edition of the *Guide for the Care and Use of Laboratory Animals* and AAALAC's Position Statement on Social Housing. Our current enrichment protocols were analyzed in order to develop and implement social experiences for our rabbit and guinea pig colonies. This analysis was performed in conjunction with husbandry, veterinary, and investigative staff in order to foster a team approach. In addition, the switch to social housing for our guinea pigs occurred at an opportune time for our investigators as they were interested in forming harem breeding groups for their animals. Several social housing options were explored in terms of materials, ability to sanitize and perform regular husbandry, as well as ease of implementation and potential duration of the social experience over the course of the work day. To comply with social housing requirements for rabbits, a canine exercise pen was purchased from an online pet supply store, while guinea pigs were socially housed in plastic equine feed tubs purchased from a regular vendor. Both methods of social housing have been highly successful for both species in terms of enriching the lives of the animals, generating enthusiasm among care staff, as well as ease of implementation. The authors conclude that these options for social enrichment in laboratory rabbits and guinea pigs should be considered at other institutions seeking potential options for housing of social species.

P89 Environmental Enrichment Use in Multispecies Facilities

AR Weller[*]

VP Research, University of Toronto Mississauga, Mississauga, ON, Canada

The CCAC Guidelines defines environmental enrichment as "additions to an animal's environment with which it can interact." This tool helps reduce an animal's stress, and enhance their psychologic and physiologic wellbeing. Without environmental enrichment, inappropriate or abnormal behaviors such as stereotypies, depression, and aggression could be displayed. By providing enrichment, animals are able to express species-specific natural behaviors, have cognitive stimulation, and have control over their microenvironment. This is a direct benefit to the animal, and ultimately the research itself. Types of enrichment for most traditional laboratory animal species have been well established and practiced. However, many vivaria are now using nontraditional animals that have not had much quantitative research done on enrichment efforts to ensure these species are in fact benefiting from the strategies

being provided. Our institution's Arts and Science departments house a variety of traditional and nontraditional laboratory animals, all of which require environmental enrichment. Using personal observations, pilot studies, and literature reviews, normal and abnormal behaviors for over 33 individual or groups of mammals, birds, reptiles, amphibians, fish, and invertebrates used within 4 vivaria are recognized. Researching the 5 main enrichment categories used in successful enrichment programs, I will describe the various University Animal Care Committee-approved enrichment strategies and personal tips used for the vast variety of species throughout the 3 campuses, including nontraditional animals such as naked mole-rats, hummingbirds, saltwater crocodiles, and fruit flies.

P90 Creative Tool for Vivarium Space Management

A Carte*, J Kiesel, RK Uthamnathil

Comparative Medicine, Fred Hutchinson Cancer Research Center, Seattle, WA

Managing a vivarium often means finding ways to improve the usability of every square inch of space. Each new special device needs a place and colonies fluctuate in size, so keeping space managed can be a monumental task. In order to keep up, you frequently need to know the capacity of each room, whose animals are where, what studies are active, and where equipment is located. Spreadsheets can show the numbers and normal maps can give you some perspective but when it comes to brainstorming they fall short. We needed to contrive something to broaden our ability to recognize areas of underutilized space and provide the means to virtually walk staff through cascading changes. We have created a tool that represents our entire vivarium and allows us to easily manipulate space and conceptualize changes by using a large magnetic dry erase board, craft tape, and movable equipment icons. This manipulatable vivarium mockup has enhanced communication and strategizing by providing a forum that evolves with the idea.

P91 Recovery of Murine Cage Flood Victims

ML Wick, J Kiesel, A Carte*

Comparative Medicine, Fred Hutchinson Cancer Research Center, Seattle, WA

Following cage floods, we observed mouse coats sometimes became infiltrated with wet, ground up feed which, when dry, resulted in an "armor-like" coating they were unable to groom out. Additionally, it prevented them from fluffing up their coats to conserve body heat. The resulting hypothermia occasionally resulted in deaths, particularly in animals that had been in wet cages for a longer period of time. We decided to proactively recover wet mice we came across in our daily cage checks. Initially, we rehoused the mice in a fresh cage with more than the normal amount of nesting material. Next, we elected to manually dry the animals with sterile paper towels and warm them under a heat lamp or on a warm water circulating pad. This procedure improved our survival rate; however, the problem of the armor-like coating persisted. We decided to try bathing the animals in warm, sterile water to remove the debris. We heated water to a comfortable bathing temperature, bathed the mice, dried them, and placed them in a fresh cage under a heat lamp for 30 to 45mins. Every 10 to 15min, the animals were evaluated and the cage moved further from the heat source. Eventually the cage was removed from the heat source entirely and returned to the housing rack. We found this procedure successful because it removed the wet, ground up food coating animals' fur, allowing us to dry them and enabling the mice to begin grooming almost immediately. The external heat source, carefully monitored, aided in recovery from hypothermia. We have increased our survival rate due to the implementation of this recovery process.

P92 Applying a Lean Management-Based Material Replenishment System in a Veterinary Research Clinical Pathology Laboratory

A Mikkola*[1], A Stepanek[1], A Vernet[1], D Brown[1,2]

[1]Center for Comparative Medicine, Massachusetts General Hospital, Boston, MA; [2]Pathology, Massachusetts General Hospital, Harvard Medical School, Boston, MA

Research clinical pathology laboratories require consistent workflow to provide timely, accurate results. This workflow includes numerous multistep processes, daily workload fluctuation, and reagents with variable expiration dates, complexities which can impact quality and efficiency. To address workflow efficiency, a systematic Lean approach to problem-solving through root-cause analysis, known as plan-do-check-act (PDCA), was applied to a laboratory's material replenishment system (MRS). Refrigerated reagent inventory was chosen to analyze the existing MRS because it comprises 30% of total laboratory inventory and includes 36 different reagents with variable demand and expiration dates. A value stream map (VSM) was implemented in the first step of PDCA ("plan") as a tool to provide detailed process analysis and expose nonvalue added steps, or waste, in the MRS. The process was found to have 43 steps and required 31 min to complete per week. Using data for actual reagent usage and expiration gathered over the prior 12 mo, pilot countermeasures were implemented (the "do" step) to reduce wasted steps, or unnecessary movement. These included: 1) establish minimum/maximum quantities of inventory based on reagent usage; 2) specifically partition refrigerator space per reagent; and 3) create visual cues for each reagent. To "check" the success of the countermeasures, a new VSM was evaluated 6 mo into the pilot. The new process required 8 steps and 14 min per week. Based on the improvements identified in the pilot, the refrigerated laboratory inventory was fully diverted to the new MRS ("act"). In conclusion, data-driven inventory system analysis allowed for application of PDCA to a clinical pathology laboratory MRS. Specific waste, in this case unnecessary movement, in the existing MRS was exposed and eliminated. Countermeasures improved workflow efficiency through the elimination of 35 steps in the MRS, saving 17 min of staff time weekly for refrigerated reagent inventory alone.

P93 Train the Trainer: Challenge in a Foreign Language

A Knipe*, S Duarte-Vogel, IM Brun Del Re

Division of Laboratory Animal Medicine, University of California Los Angeles, Los Angeles, CA

Training new researchers in procedures and safety protocols ensures good animal care and safety for people working in our facilities. Many investigators are still in the process of learning the English language, which complicates participation and comprehension of the training material. These English language learners (ELL) may benefit from different training styles and teaching approaches rather than a traditional training style. A training class was designed to simulate the experience of ELL students for the trainers in our veterinary department in the hope of increasing empathy for these students. The trainers were given a lecture and a novel task in a foreign language. The participants were then asked to fill out a questionnaire and participate in a discussion about how to improve the educational delivery to ELL students. While this training was initially developed as a method to increase trainer awareness of potential trainee learning difficulties, many new ideas were also generated during these discussions which focused on improving the institution's current training program. The discussion and ideas that resulted from the training will help to improve our program in effectively delivering information to ELL students as well as improve our training program as a whole.

P94 Development of Performance Standards for Mouse Husbandry Practices: How Often Is Often Enough for Cage-Change Frequency?

AM Mexas*, A Caro, T Hillanbrand, AK Brice, DJ Gaertner

University Laboratory Animal Resources, University of Pennsylvania, Philadelphia, PA

Changes in rodent housing influence the outcome of research findings and the wellbeing of research animals. The *Guide* emphasizes performance standards as the ultimate measures for colony management. The frequency of cage-changes must strike a balance between minimally disturbing the mice in their environment and providing clean bedding, and low ammonia and CO_2 levels. According to the *Guide*, "soiled bedding should be removed and replaced with fresh materials as often as necessary to keep the animals clean and dry and to keep pollutants, such

as ammonia, at a concentration below levels irritating to mucous membranes." No engineering standards for acceptable levels of ammonia are indicated in the *Guide*. To determine if current husbandry practices adequately maintain mouse cages, we completed 2 studies. First, we evaluated nasal histopathology in clinically sick and healthy mice, for evidence of microspcopic changes consistent with high ammonia levels. In these cohorts, we compared the severity and frequency of microscopic nasal lesions between mice in static, microisolation cages and mice in individually ventilated racks. Then, we measured ammonia and CO_2 levels during 3 cleaning cycles before, during, and after breeding in mice housed in pairs, trios, or groups to determine the relationship between housing density and gas levels in static, microisolation cages. In our colony mice, there were no significant differences in the severity or frequency of nasal histopathology of mice housed in static compared with individually ventilated cages. These data suggest that cage-change frequency of once per week in mice housed in static cages does not result in increased nasal pathology (when compared with mice housed in individually ventilated cages with the same cage-change frequency), despite having levels of ammonia that might be considered to be excessive. Our data provide the basis for the development of performance standards that can be used to evaluate adequate husbandry practices for mice housed in static filter-top cages.

P95 Rodent Anesthesia Rental System: Process Improvement for a Rapidly Growing Program

J Shannon, A Brinkley*, N Zielinski-Mozny

Northwestern University, Chicago, IL

Animal resource programs provide a variety of services to support animal biomedical research including rental of equipment. At our University, the provision of rodent anesthesia systems to researchers was identified as an unmet need to fulfill a rapidly growing use of rodent inhalant anesthesia in experiments. Investigators needed anesthesia systems for the occasional experiment but, not on a long term basis. Thus, the need to purchase an anesthesia system was not required by the laboratories. To support this need, our department initially purchased 2 anesthesia systems. Based on the impact, the number of rental systems increased to 8. With this increase in equipment, came a need to have an efficient and effective end user reservation and servicing system. Initially, an emailing system was used to communicate a reservation need between the researcher and the veterinary unit coordinators. This process required multiple email and phone communications between research staff and the coordinators delaying the approval of anesthesia systems use. This process has now been improved to provide a self-service component while still providing customer service. We adopted an online reservation system through an existing University platform for reserving lecture halls. This online software provides the users direct access to the anesthesia systems' availability and convenient scheduling options as well as the ability to edit and/or cancel reservations independently and in real time. In conjunction with the online reservation system, a key card access system has been implemented to allow users to retrieve the reserved anesthesia system. The revision of the reservation system has improved efficiency and workflow. Our users are self-efficient in completing their research requiring rodent anesthesia. Our rodent anesthesia program coordinators work more efficiently while facilitating reservations and providing assistance to users.

P96 Comparison of 2 Bedding Types (Corncob and Cotton Pads) on the Microenvironments of Static and Individually Ventilated Cages of Mice

A Williams*

University of Houston, Houston, TX

Bedding is one of the most important components within the microenvironment of laboratory rodents. It provides warmth, maintains the environment of the cage, and adds to the overall welfare of the animals. The purpose of this study was to compare the environmental conditions in static and individually ventilated cages (IVC) with either corncob or cotton pad bedding. The parameters analyzed included intracage temperature, humidity, ammonia levels, and bedding weight. Five adult mice per cage were housed in 2 groups of 12 in either caging system (n = 12). The static cages were measured on day 0 and day 4, due to the cage change-out schedule. The IVC were measured on day 0 and day 7. The temperature readings and levels of ammonia showed statistical significance when bedding types were compared. In particular, corncob bedding had more effect on these 2 parameters, which were both substantially lower in IVC cages. This finding is likely attributed to the airflow design of the IVC caging style. Although we found no significant effects on the weight difference, it is critical to point out that cotton pads are considerably more lightweight and have a lower overall waste volume which improves the ergonomics involved in waste disposal. In conclusion, the cotton pads bedding was comparable based on the parameters that were measured. The cotton pads also have advantages over the traditional corncob type in the following areas: hypoallergenic, free of metals, chemicals, aflatoxins, and phytoestrogens. Cotton pads also serve as a form of environmental enrichment and provide a material for nest building which is ideal for rearing young pups.

P97 Process for Receiving and Maintaining 70 Transgenic Mouse Lines

A Gyles*, Y Galeas, C Brobst-Wormell, J Woo, B Fisher

SoBran, NIAMS, Bethesda, MD

Operating and maintaining a transgenic mouse colony in a small lab animal facility can be quite challenging. With effective strategic planning and appropriate renovation, 70 transgenic mouse lines were successfully imported, and are maintained at our institution's animal facility. Here we demonstrate that with precise planning you can maximize the animal facility space, to accommodate 8 additional animal racks and account for every animal's location through a web-base inventory system. With no additional added square footage our facility was modified to change the location of an animal and procedure room. This added the necessary space to house additional animals. Facility modifications included moving a wall to add one additional animal room. Renovation process did not impact daily operations. The additional racks were added into circulation during completed phases of the project. After completion of renovation, within a 3-mo timeframe, 70 transgenic mouse lines were imported, averaging approximately 800 transgenic mice per month. All animals are now maintained in a web-based inventory management software which provides optimum animal inventory management. Through the web, animal users have access to real-time information inclusive of animal rooms, racks, and animal cage locations. In conclusion, with a few structural modifications we are now able to house an additional 700 mouse cages, and provide the researcher's with convenience and more research support.

P98 Effect of Corncob Bedding on the Feed Efficiency in a High-Fat Diet-Induced Prediabetic Model in C57Bl/6 Mice

A Ambery*[1], L Tackett[2], B Penque[2], J Elmendorf[2]

[1]School of Medicine, [2]Cellular and Integrative Physiology, Indiana University, Indianapolis, IN

Commercially available bedding substrates for mice include wood chips, paper products, and corncob. Corncob bedding is inexpensive and able to keep cages drier with less ammonia buildup. However, observations that mice eat the bedding lead to concerns that its use can interfere with dietary studies. This study evaluated the effect of bedding on the feed conversion of mice. Four groups of mice (n = 6 per group) were housed in an individually ventilated caging system: 1) high-fat chow (45% kcal from fat) housed on corncob bedding, 2) high-fat chow housed on paper bedding, 3) low-fat chow (10% kcal from fat) housed on corncob bedding, and 4) low-fat chow housed on paper bedding. Mice arrived at the facility at 4 wk of age and were started on regular rodent chow. Two weeks later, all were started on the low-fat chow. Four weeks after arrival, the mice were housed on either corncob (groups 1 and 3) or paper (groups 2 and 4) bedding. Five weeks after arrival, the high-fat diet was initiated for groups 2 and 4. Body weight and feed consumption were measured weekly for 9 wk. After 4 wk on high-fat chow, the mice on high-fat feed housed on corncob bedding showed a significant decrease in feed conversion and change in percent body weight per week compared with mice on high-fat feed housed on paper

bedding ($P < 0.0001$ and $P = 0.0011$, respectively). A similar decrease in feed conversion and change in percent body weight was also seen in the mice on low-fat feed housed on corncob compared with mice on paper bedding, yet it did not reach statistical significance. There was no significant difference in the average daily feed consumption between the 4 groups ($P = 0.17$). In conclusion, corncob bedding decreases the efficiency of feed conversion in mice on a high-fat diet weight gain model and other bedding choices should be favored in these models.

P99 Centralized Management of a Gnotobiotic Research Animal Facility

BR Theriault*[1,2], A Vest[1,3], C Olivares[1,3], C Mathieu[1], G Langan[1,2]

[1]Animal Resources Center, [2]Department of Surgery, [3]Charles River Laboratories, The University of Chicago, Chicago, IL

In recent years there has been a new wave of research interest exploring the role that the host microbiome may play in multiple areas of health and disease. Using the powerful combination of genetically engineered mice maintained germfree (axenic) or with defined flora (gnotobiotic), researchers have an enhanced ability to dissect the multifactorial influences contributing to disease pathways. To assist researchers interested in conducting studies using gnotobiotic and germfree mice at our institution, we developed a centrally managed gnotobiotic research animal facility (GRAF). Although a number of academic institutions have principal investigators managing and maintaining mouse colonies in gnotobiotic and germfree isolators, few operate facilities that are entirely managed and operated by the centralized animal resource program. Several key components have contributed to the success of our centrally managed program. Institutional support has provided space allocation and capital resources for space renovation, equipment purchase, and staffing expansion to meet program needs. Redundancy in critical equipment such as dedicated autoclaves and chemical sterilant delivery components ensure uninterrupted facility operations. A GRAF faculty advisory committee has provided guidance on facility size, design, and capabilities based on scientific objectives and projected facility use. A dedicated facility veterinarian overseeing all aspects of the gnotobiotics program has been pivotal in forming relationships and implementing programmatic practices across and between all program components. All facility activities are substantially documented contributing to quality control and operational success. A highly motivated, specially trained husbandry staff with top level AALAS certification providing colony management and technical/procedural support has facilitated limiting isolator activities and facility access. Our goal is to share our experience in maintaining a centralized gnotobiotic research animal facility and to highlight key components of the program that have contributed to its development, expansion, and ongoing success.

P100 Sanitization Effectiveness: A Novel Hands-on Training Approach to Reinforce Dog Kennel Cleaning Procedures

B Callahan*, G Marks, CM Symonowicz

Bristol-Myers Squibb, Wallingford, CT

Compliance with proper sanitization procedures of animal rooms is critical in maintaining healthy animals and meeting legal requirements of a laboratory animal facility. Our current process for dog kennel sanitization involves using hot water, a degreasing foamer, chlorine bleach, and a quatricide disinfectant. Staff are trained in proper dog kennel sanitization procedures and often do not fully comprehend why it is so important to follow step by step procedures, as well as chemical concentration and water temperature parameters. To ensure that staff fully comprehend the reasons for the proper use of each component in the sanitization process, we worked with our local chemical distributor to help create a novel hands-on training. We devised different cleaning scenarios for our dog kennels and assigned staff to participate in each of the scenarios. Each scenario changed a different component of the sanitization process including water temperature and chemical combinations. Following each sanitization scenario, microbiologic monitoring using agar plates was performed and bacterial colonies were counted for each test scenario. The results reinforced our current process for dog kennel sanitization and also provided a visual illustration for staff to understand why proper sanitization procedures must be followed. The results were also used in a training presentation for all staff in our department to demonstrate and reinforce the importance of following standard operating procedures regarding water temperature and chemicals when sanitizing dog kennels.

P101 Buccal Swab Analysis as a Refinement for Biopsy Sampling in Genetically Modified Mice

CM Symonowicz*, G Hirschfeld, J Huang, C Witter, T Zima, P Kayne

Bristol-Myers Squibb, Wallingford, CT

Genetically modified mice are produced through various methods. The animals resulting from these manipulations must be analyzed to determine if the genetic modification (knockout, knockin, etc.) have been passed down through Mendelian segregation. To assess genotypes, samples of tissue (biopsy) are obtained from young animals by ear snips, toe clip, or tail sampling. This tissue collection may be considered to be a surgical procedure. The analysis of buccal swabs to collect saliva from the cheek of mice was evaluated to determine the feasibility of obtaining accurate genetic results, while eliminating tissue sample collection. Swab samples were taken using different size swab material, different size samples, and processed with various lysis and qPCR protocols. Conditions were determined that would yield identical results when compared with tissue-samples–based genotyping from the same mice for several strains of animals. Results showed that buccal swabs can be substituted for tissue samples and provide a refinement to the genotyping process by obviating the need for surgical procedures when characterizing genetically modified mice.

P102 Customization of Standard Rabbit Dividers Provides Enhanced Socialization for Singly Housed Rabbits

C Hedrick*, M Rowley, S Lewis

University Laboratory Animal Resources, Office of Research, The Ohio State University, Columbus, OH

Social animals should be housed in stable pairs or groups of compatible cohorts whenever possible. Rabbits are a social species and providing species-appropriate social interaction including auditory, olfactory, tactile, pheromonal and visual cues from conspecifics that are not compatible for pair or group housing due to factors such as age, sex, or protocol restrictions can prove challenging. Our institution currently uses a commercial side by side caging system that allows for all sensory interaction, with the exception of tactile, when the divider is in place. One way to provide tactile interaction between individually housed animals and to maximize social interaction is to provide a cage divider that allows for nose touching but restricts further physical contact. To achieve the enhanced social interaction between 2 individually housed rabbits, 70 holes were drilled measuring 1 in. in diameter and 2 in. apart on center into the standard commercially available clear acrylic 31.5 in. × 12.13 in. divider. The holes were made using a 1-in. boring bit, smoothed with a dremel, and deburred. The determined hole size allowed for nose touching but restricted further interaction between cohorts. Hole placement was determined by evaluating the upper and lower lips of the divider slide and if the rabbits were on or below the loft/shelter thus allowing access to cohort on both levels. Upon placement of the modified divider, rabbits were immediately observed using the holes in the divider to nose touch. Uniform results were noted between same-sex side by side caging. By customizing the commercially available standard rabbit cage dividers we were able to provide a simple, economic method of effectively maximizing the social interaction between singly housed rabbits that would otherwise have limited social contact with cohorts housed on the same level of the caging system.

P103 Foraging Tray Customization for Cynomolgus Macaques (*Macaca fascicularis*) Optimizes Novelty and Sanitization

C Hedrick*, M Rowley, S Lewis

University Laboratory Animal Resources, Office of Research, The Ohio State University, Columbus, OH

Developing novel environmental enrichment devices that promote species-specific behaviors can be challenging as variability, safety, ease of sanitization, and device cost all must be considered. Foraging is a natural behavior in macaques that can be promoted by using a finger board foraging tray in a laboratory setting. Commercially available finger boards can be expensive and offer limited variability within a single product design and can be difficult to mechanically sanitize. In an effort to provide increased novelty with easier sanitization, our institution developed a high density polyethylene foraging board that was cut to measure 7 in. L × 1.75 in. W × 0.75 in. H with 11.75-in. holes 1 in. apart on center in an alternating pattern using a 0.75-in. ball-end mill bit, sanded with a dremel, and deburred. Board size was designed to allow its use with commercially available and institutionally customized holders. Compared with flat-bottomed hole designs most commercially available trays have, our unique rounded bottom concave holes were based on cynomolgus macaque digit size with space to allow for natural "scooping" and "picking" motions. The concave holes were more effectively sanitized in a standard tunnel washer than the commercially available flat-bottom design as determined by an organic material detection system test (ATP) performed after placing various types of typical food enrichment in both designs and then sanitizing them in a standard tunnel washer. Inhouse production expenses and supplies resulted in an 87.5% cost decrease per unit compared with commercially available products, thus allowing for the development of additional trays and designs. Further customization of the standard tray design being explored includes variation of number, pattern, depth, and diameter of holes, which provides greater novelty and can potentially be adapted for multiple nonhuman primate species. The inhouse customization of the foraging tray allows for more effective sanitization than commercially available products, was cost-effective, and promoted the natural foraging behavior in macaques housed in a laboratory setting.

P104 Protocol for Eliminating Murine Norovirus from a Small Transgenic Breeder Colony

C Chiedi*[2], M Dillon[2], TJ Ruckwardt[4], JP Gorres[3], AM Malloy[4], AA Taylor[1], BS Graham[5], SS Rao[1]

[1]Laboratory Animal Medicine, Vaccine Research Center, [2]Laboratory Animal Medicine, Vaccine Research Center, SoBran, [3]Laboratory Animal Medicine, Vaccine Research Center, Kelly Services, [4]Viral Pathogenesis Laboratory, Vaccine Research Center, [5]Clinical Trials Core Laboratory, Viral Pathogenesis Laboratory, Vaccine Research Center, National Institute of Allergy and Infectious Diseases, Bethesda, MD

Murine norovirus (MNV) is an emerging pathogen in many laboratory mouse facilities, causing severe disease in immunocompromised mouse strains and persistent infections in immunocompetent mice. While infections in immunocompetent mice are generally asymptomatic, they can potentially alter host immunology and affect experimental outcomes of vaccine and therapeutic studies. Measures for eliminating or preventing MNV have included depopulation and decontamination of racks or positive rooms; culling positive animals, or cross-fostering neonates. Depopulation eliminates MNV but is often impractical, and culling has been previously shown to be ineffective. Cross-fostering pups from MNV-positive mothers to MNV-negative ones have yielded MNV-free strains that are viable for breeding; however, the logistics of executing this vary among facilities. Here, we describe the details of the process of cross-fostering to eliminate MNV infection in our small animal facility. MNV was first detected by screening transgenic animals received from a university breeder source. MNV-positive mice remained in quarantine and were cross-fostered with BALB/c wildtype mice. Strict sterile practices, sera and fecal PCR testing, and monthly sentinel screening (during quarantine and after) were also implemented. Five of 5 strains successfully left quarantine and were released into the general facility population. Although long-term duration of elimination has not yet been tested, continued diligent monitoring has ensured there is no reversion to MNV-positivity; this is especially critical for a facility housing immunodeficient animals. These results suggest that cross-fostering combined with good husbandry practices, testing, and monitoring is an effective alternative to eradicate MNV from a small transgenic breeder colony.

P105 Effects of Trimethoprim and Sulfadiazine on Weight When Administered to Mice in the Diet

C Hoffman*[2], L Zitzow[1], M Yabes[2], C Olivares[2], K Sanders[2], R Van Mynen[2], G Langan[1]

[1]University of Chicago, Chicago, IL; [2]Charles River at University of Chicago, Chicago, IL

Sulfamethoxazole and trimethoprim (SMZ-TMP) oral suspension is frequently added to drinking water and provided to immunocompromised mice, such as those that have been irradiated. It is also used as a therapeutic agent for mice with dermatitis or other infections. A recent price increase in SMZ-TMP prompted us to look for alternatives. One potential alternative is the use of a commercially available standard rodent chow mixed with sulfadiazine and trimethoprim (SDZ-TMP), which is molecularly similar to SMZ-TMP. In order to determine if administration of SDZ-TMP would have negative impact on mice, 4 age-matched CD1 breeding pairs were set up. Two pairs (control groups) were fed standard 18%-protein rodent chow and 2 pairs (experimental groups) were fed standard 18%-protein rodent chow with 4,100 ppm of SDZ-TMP. Adult mice were weighed at the start of the study and then once weekly, along with the pups. Pups were weaned at 21 d of age and placed on the same diet as their parents. Mice were housed in positively ventilated IVC cages, fed and watered ad libitum, and supplied with enrichment consisting of cotton and paper nesting material. Cage changing was done at 14-d intervals. Based on the results, the standard 18%-protein rodent chow with 4,100 ppm SDZ-TMP appears to have no effect on the weight of adult mice that are placed on it. However, pups born to dams fed the SDZ-TMP diet failed to gain weight as rapidly after weaning when compared with the pups of the same age on the control diet. Additional testing will be performed to make a final determination whether a standard 18%-protein rodent chow with 4,100 ppm of SDZ-TMP can be used as a substitute for SMZ-TMP oral suspension.

P106 Animal Fact Sheets: Meeting the Animal Welfare Training Expectation

CS Coke-Murphy*[1], R Meyer[1], D Molnar[2]

[1]Division of Animal Care, [2]Office of Animal Welfare Assurance, Vanderbilt University Medical Center, Nashville, TN

The eighth edition of the *Guide* states "All personnel involved with the care and use of animals must be adequately educated, trained, and/or qualified in basic principles of laboratory animal science to help ensure high-quality science and animal wellbeing." Increased emphasis on employee training, by the *Guide* and the AAALAC, has created the need for species-specific trainings. Numerous institutions with a variety of species have been tasked with developing, implementing, and documenting species-specific trainings. Our institution has developed animal fact sheets to serve as supplemental training materials to be kept in the animal room and/or suite or online for quick reference. The animal fact sheets were created by the Environmental Enrichment Coordinator (EEC) and approved by the Attending Veterinarian for our institutions Animal Care and Use Program. The animal fact sheets purvey the following for each species housed at our institution: 1) morphology, 2) social organization, 3) reproduction and mating system, 4) communication, 5) normal and abnormal behaviors, 6) common postures, and 7) environmental enrichment plan requirements. The EEC will work in conjunction with the Training Coordinators to implement animal fact sheet trainings for animal care, welfare and research personnel. Animal fact sheet trainings will be conducted for both new and current animal care, animal welfare and research personnel. All trainings will be documented and recorded within the animal care and welfare training matrix and/or within our web-based protocol platform. We expect the animal fact sheets to become a valuable addition to our training program fostering increased knowledge and interest in the animals housed at our institution.

P107 Lean Transformation of a Facility Sanitation Program Using ATP Bioluminescent Verification

CR Sikes*, DW Brammer, LR Gray, JM Distefano, N Beckford, E Allen, DE Frigo

Animal Care Operations, The University of Houston, Houston, TX

The use of ATP bioluminescent surface sanitation surveys applied within the framework of a standardized cleaning regimen can be used to improve the efficiency and effectiveness of a routine facility sanitation program. Two key components of surface sanitation are routine cleaning and sanitation verification. Routine cleaning is typically scheduled to be performed at intervals which are either closely linked to husbandry routines or the maintenance of facility cross-contamination prevention. Sanitation verification is often performed at intervals independent of facility activities which are the drivers for the performance of sanitation. Cleaning facility areas at frequencies which are not supported by a verification method is inefficient and wastes not only staff effort but also materials. To ensure that routine cleaning is efficient, cleaning should be performed only as needed to meet area standards. To ensure that area standards are met and as a component of efficiency, a verification method should be employed which yields real time results based upon area specific standards. The application of the lean management principles of standardization and waste elimination to a facility sanitation program has led to the development of sanitation processes that have improved both the effectiveness and the efficiency of facility sanitation. As a component of a standardized sanitation program, the use of ATP bioluminescent swab assays provide a real-time metric for setting area standards, establishing area sanitation frequencies or time to failure data, and evaluating sanitation effectiveness. Analysis of daily ATP sanitation verification tests has led to the development of a sanitation index that may be used to establish area sanitation frequency, the identification of high failure zones or sanitation "hot spots" which require more stringent sanitation criteria, and the reduction of sanitation frequencies throughout the facility required to maintain established passing levels.

P108 Promising New Treatment for Ulcerative Dermatitis in Mice

CS Pater*

CP Consulting, Edgewood, MD

Ulcerative dermatitis (UD) is a common skin condition of unknown etiology that mostly affects mice on a C57BL/6 background. It is a spontaneous and progressive condition characterized by the appearance of scabs and crusts on the skin that are often severely pruritic. Animals tend to scratch the lesions, causing additional ulceration and inflammation. The lesions range from mild to severe and can eventually progress to necrosis and fibrosis. Morbidity is high in severe cases. Movement discomfort, pain, weight loss, dehydration, which are commonly associated with UD, have the ability to confound results obtained from ongoing research. Some of the commonly used interventions are as follow: ibuprofen, vitamin E, nail clipping, triple antibiotic ointment, maropitant citrate, injectable antibiotics, and antibiotics in the water. Most of these treatments are external, labor intensive, and take a long time to show an effect. None have been consistently curative so the intent is to minimize negative effects on research through prevention or spread of the disease. We propose the use of common rodent diet with doxycycline (an antibiotic) along with lidocaine (analgesic). This new treatment modality for UD has proven to relieve the symptoms and prevent spread of the disease between cage mates or within cages on the same rack in a majority of the mice treated. Treatment is easy, short term and not labor intensive.

P109 Withdrawn

P110 Waste Bedding as a Fuel Source

C Cosgrove*

The ElmCos Group, Charlottetown, PE, Canada

The majority of animal facility waste bedding is disposed of in landfills. A novel process has been developed to convert waste bedding into energy. Bedding, enrichment products, and other waste are homogenized through a shredding process which also reduces moisture content. The material passes through a cyclonic separator and is processed into briquettes. Testing of the briquettes indicates they are able to produce 4.7 kWh of energy per kilo. Using this method a 10,000 cage facility is able to divert 82,000 tons a year of material from landfills and produce 325,000 kWh of energy, the equivalent of 36,890 L of oil. The energy produced can be used for heating water or in a wide range of heat exchange processes resulting in an investment payback period of 2.3 y.

P111 Adventures in Middle School Outreach

C Huber*[1,2], DS White[1]

[1]Sinclair Research Center, Columbia, MO; [2]Agriculture Education and Leadership, University of Missouri, Columbia, MO

Outreach in the laboratory animal science community has become increasingly popular. Middle school aged students provide an ample opportunity for exposure to science, due to their desire to learn as well as their increased interest in developing personal opinions. A prepackaged educational program is not readily available to those employed in the laboratory animal science community for this age range. A middle school outreach program was developed to expose students in grades 5 through 8 to principles used in the laboratory animal science industry. The main intent of this program was to improve perceptions of the laboratory animal industry. The program was delivered to students in grades 5 through 8 in the gifted program of a middle-sized rural school district in the midwest. Students were introduced to the drug approval process, and guided through the study development of a recently discovered flu strain. To determine the impact of the program on students' perceptions, pre- and posttests were administered rating: 1) preferences of science and animal based careers, 2) perceptions of laboratory animal science, and 3) factual knowledge regarding regulations, laboratory terminology, and procedures. Through initial analysis of descriptive statistics, several trends were observed. The presentation had a positive effect on students' perceptions of animal research, especially on those who did not previously have an opinion. With respect to factual knowledge, students had a clear benefit and correctly responded to 79% to 100% of the statements. Overall the program met 2 of the 3 objectives, but will need future development to improve deliverability.

P112 Implications of Water Quality Differences between Vivaria at One Institution

CE Ferrecchia*, K Jensen, R Van Andel

Office of Laboratory Animal Care, University of California, Berkeley, Berkeley, CA

After a recent bedding switch from reclaimed wood pulp to a proprietary processed corncob bedding material, animal care staff and laboratory personnel began reporting a gradual coat color change in their animals. Some animals even experienced contact dermatitis. Oddly, these changes only occurred in one of the vivaria on campus. Several steps were taken to deduce the cause of this coat change, including water quality testing in the vivaria, housing of animals on various other bedding substrates, and topical treatment for dermatitis when indicated. A pilot study was performed with animals in 2 of the main vivaria using 3 different bedding substrates. Animals housed on reclaimed wood pulp and irradiated corncob bedding substrates remained clinically normal, while animals housed on the proprietary processed corncob bedding substrate showed signs of coat color change and dermatitis. Histologic analysis was performed. The results of this pilot study, in addition to water quality testing results, established that the water quality between the 2 facilities differed greatly in terms of pH. The animals were essentially experiencing a topical bleaching effect. This finding led to the implementation of more robust routine water quality testing in all facilities across campus and the discontinued use of the proprietary processed corncob bedding material. This report demonstrates the necessity of water quality testing in each individual vivarium within multivivaria institutions.

P113 Hanging Habitats Take Enrichment to New Heights

C Kite*

UTMB, Galveston, TX

The *Guide for the Care and Use of Laboratory Animals* is not specific on environmental enrichment for exotic species. Ferret enrichment in a lab animal setting, while maintaining compliance with sanitation and sterilization policies and keeping costs controlled, can be challenging. Some livestock enrichment not in use met a creative technician's mind and the result is the 3Rs doubled. A 6 in. × 6 in. × 6 in. triangle was cut out midway in the side of a 10-in. hard polyethylene ball. The edges were sanded smooth to prevent injury to the ferrets. A hole was drilled into the top of the ball, and a stainless steel eyebolt with a stainless steel chain was used to suspend the ball from the top of the cage. The chain length was adjusted so the ferrets could access the hanging ball without it resting on the cage floor or hanging too high for access. We expected the ferrets would play inside the balls and return to plastic boxes placed on the floor of their cage for sleeping. Instead, the ferrets preferred sleeping and nesting in the hanging habitat, and no longer used the box or cage floor for sleeping.

P114 Larger Biohazard Bags Add up to Big Savings

C Boehm*, N Meiring, D Hobson, DL Hickman

LARC, School of Medicine, Indiana University, Indianapolis, IN

Our facility was in the middle of a pinworm and fur mite outbreak, and our animal husbandry technicians (AHTs) were spending considerable amounts of time bagging up cage bottoms, filter tops, and wire bar lids in biohazard bags that could only hold small amounts of each. The AHTs reported they were spending up to 90 min per room per day bagging, while the cage wash staff reported that they were spending up to 60 min unbagging the autoclaved equipment. To solve this problem, we located larger bags that were 66.5 × 110 in. We found that by layering a bag on the bottom of an autoclave rack, pulling a second bag down over the rack of dirty cages, and folding the 2 bags together, we could cover all of the cages and prevent contamination on the way to cage wash where the rack and equipment were autoclaved. This significantly reduced the amount of time the AHTs spent bagging up dirty equipment. The cage wash staff used a knife to slice open the large bag after autoclaving, resulting in no increased labor for their processing. We calculated that we were able to realize a savings of over $7,000 over the 12 wk that we implemented this new procedure. These savings were so significant that we have expanded the use of these bags to other areas where containment is required, such as biohazard. This procedure modification would also be useful for autoclaving clean caging for delivery to animal rooms without cross-contamination.

P115 Use of Environmental Swabs for Pinworm DNA Pre- and Post-decontamination during a Pinworm Outbreak

C Boehm*, R Crisler-Roberts, D Hickman

LARC, Indiana University, Indianapolis, IN

The introduction of PCR-based screening for pinworm DNA has enhanced our ability to diagnose pinworm infestations by using feces and environmental swabbing to screen rodent colonies. When our facility discovered pinworms in one mouse housing room, we needed to find out how many other rooms were affected. We collected samples from the exhaust plenums in rooms. Nine of the 20 rooms came up positive. These positive environmental samples were confirmed with visual confirmation of either eggs or worms in colony animals in subsequent testing. Animals were treated with fenbendazole, using published dosing regimens, and post treatment assessment of the colony animals confirmed the treatment was successful. The housing rooms, including the racks and hoods, were decontaminated with chlorine dioxide gas. The filters of the racks were changed. To confirm the effectiveness of the decontamination with the chlorine dioxide gas, the exhaust plenums were sampled using PCR, and 6 of 37 were still positive. To test if the test results were due to residual contamination from noninfective DNA, positive exhaust plenums were swabbed with a cotton square, and naïve sentinel mice exposed to the cotton square. The sentinels tested negative via PCR for pinworm DNA at 12 wk postexposure. While PCR is an excellent methodology for detecting pinworm DNA, it is best used in conjunction with a larger screening program and not relied on as a sole agent.

P116 Improving the Standard Operating Procedure for Water System Changeouts Prevents Fungal Growth

CM Brown*, F Pruitt, E Helman, M Bolding, SC Cartner

Animal Resources, University of Alabama at Birmingham, Birmingham, AL

We have observed mouse cages with bacterial or fungal growth between the hard plastic shield and the water bag of the watering system on cages. The current cage change procedure requires the watering system to be changed when visibly dirty or once the water runs out. To determine if the observed fungal growth was caused by the watering system and create a standard operating procedure to stop the growth of fungus, we proposed to change all components weekly. Six cages of mice (5 mice per cage) received a fresh cage with clean watering system (shield and water bag). Four cages kept on the current water system change out had one kibble of food placed between the hard plastic shield and the water bag itself to mimic the conditions of "dirty" cages, whereas 2 control cages did not have any food placed inside the hard plastic shield. Water shields were swabbed and tested for fungal growth once per week for 8 wk. Only normal bacterial flora was present during the first 2 wk. By week 3, fungal growth was observed visually, although none grew in culture. At week 5, fungal growth was confirmed in culture and identified as *Rhizopus* as well as *Aspergillus niger* and *A. flavus*. At the end of 8 wk, one mouse from each cage was necropsied to evaluate animal health. We did not observe any adverse effects of fungal growth in the mice during this study; however, fungal growth was easily prevented by changing out all components weekly. Because many research projects involve immunodeficient animals, this confirms the need to change out the watering system frequently.

P117 Improving Efficiency in Animal Care Training for Trainers and Trainees

CL Madura*, T Childers

University Animal Care, University of Arizona, Tucson, AZ

Training in animal care can often be chaotic with all the aspects of animal care technicians must learn. In our department, one of the primary obstacles our trainers have had to overcome was that information necessary for training was located in many and various places. Essentiallly, none of the training or information was in an organized, complete format. For the trainers, recording training was in a paper packet. This packet proved to be very inefficient because it was often in the hands of another trainer or the technician themselves. The disorganization of our system led to retraining and staff meetings filled with constant reminders of how work should be done. Our organizational disorganization led to vast amounts of time being spent in "managing" the flow of information. To reverse this spiraling disorganization, our department committed to the goal of revising our processes, procedures, and training to present an organized and stuctured content. To accomplish this task, we standardized the way each room is set up so they all have the same supplies and equipment in the exact location. SOPs were standardized to the species and refined and condensed, eliminating unnecessary and repetitive information. As a result of our simplified SOPs, training is concurrently simplified. To assist the trainer and trainee, a technician handbook is being developed to contain information technicians will need to do their jobs, including pictures of room and hood set up. Additionally, recording of training is now on a database, replacing the old paper packet. This eliminates the need to search for the paper training records and enables all trainers and coordinators to access the records very quickly. Technicians now have access to investigators' protocols giving them the freedom to learn more about the study of the animals if they choose to do so. We are in the implementation proceess of revamping our training. Some changes have been implemented, and others are still being tested. The changes enacted to date have increased training effectiveness. Further enhancements to our system will continue to reap benefits to the department and to the employees.

P118 The IQ Consortium 3Rs Leadership Group: Overview of the 2012 3Rs Benchmarking Survey Results

C Hoorn*[1], LV Medina[2], WJ Underwood[3], NA Bratcher[2], GJ Gaito[1], GA dal Negro[4], C Petursson[5], M Vasbinder[4]

[1]Comparative Medicine, Pfizer, Groton, CT; [2]Animal Welfare & Compliance, Abbvie, North Chicago, IL; [3]Eli Lilly and Company, Indianapolis, IN; [4]Animal Welfare, Ethics and Strategy, GlaxoSmithKline, Research Triangle Park, NC; [5]Veterinary Science, Bristol-Myers Squibb, Princeton, NJ

The concept of the 3Rs (refinement, reduction, and replacement) is fundamental to the ethical use of animals in research, and there are legal and scientific drivers for expanding the use of methods that replace, reduce, and refine our work with animals as well. The 3Rs Leadership Group (LG) of the International Consortium for Innovation and Quality in Pharmaceutical Research (IQ Consortium) was formed in January 2012 by approval of the IQ Board of Directors. One of the first initiatives of the 3Rs LG was to create a Benchmarking Working Group (WG) to develop a 3Rs survey to establish the depth, breadth, and range of our 3Rs initiatives across the industry. The 3Rs benchmarking survey was created with input from the Benchmarking WG members and the 3Rs LG members, which represent a total of 20 biopharmaceutical companies. The WG members reviewed a 2009 to 2010 3Rs survey conducted by the European Federation of Pharmaceutical Industries and Associations (EFPIA) and used some of the same questions, but included other questions to broaden the focus of the survey. Fifteen of the 20 member companies (75%) responded to the survey, sharing details on their internal management of the 3Rs, technical applications of the 3Rs, scientific applications of the 3Rs, and how they promote and communicate about the 3Rs internally and externally. We provide examples of some current 3Rs practices and areas of consistency across companies, areas of disparity and opportunity, and potential next steps for the 3Rs LG based on the survey results. The primary learning is that there is a very strong commitment from the biopharmaceutical industry to adopt and promote alternatives and high standards of laboratory animal welfare. In addition to being a regulatory mandate and benefitting science, pursuing the 3Rs is perceived as an ethical imperative and "the right thing to do." As a result of this survey, the 3Rs Leadership Group has identified several opportunities to encourage a more strategic and harmonized approach to adopting and promoting the 3Rs across our member companies.

P119 Successful Eradication of Furmites and Pinworms from Mice with a 4-Week Ivermectin Treatment Regimen

CL Besch-Williford*[1], J Palmer[2], B Bauer[1], R Livingston[1]

[1]IDEXX RADIL, IDEXX BioResearch, Columbia, MO; [2]ClearH20, Portland, ME

A study was performed to evaluate effectiveness of a 4-wk ivermectin treatment regimen in eradicating mouse fur mite and pinworm infections. Eight weeks of continuous ivermectin treatment is currently recommended for fur mite eradication. However, a 4-wk regimen would offer significant cost savings in terms of supplies, labor, and research productivity. Three groups of female mice, 20 per group, that were naturally infected with fur mites and pinworms were housed 2 per cage in a ventilated microisolation rack. Group 1 mice were treated with ivermectin for 8 wk, group 2 mice were treated for 4 wk and group 3 mice were untreated controls. Ivermectin was delivered continuously to mice via a medicated gel containing 12 ppm ivermectin in a 4-oz cup that was used in place of a water bottle. Fur and fecal samples were collected prior to onset of the study, at 2-wk intervals throughout treatment, and at 4-wk intervals afterward for a total of 6 mo. At each time point, fur plucks, feces, and perianal tape tests were subjected to nonmolecular diagnostic examinations, and feces and fur swabs were tested by parasite PCR assays. Evaluation of fur plucks for mites revealed no evidence of mites in groups 1 and 2 by day 28 of the study. Nits were no longer observed on hairs after 84 d and PCR furmite tests were negative after 112 d post treatment. Tape tests and fecal floats were negative at day 14 and pinworm PCR assays were negative at day 28 in samples from both treatment groups. At study end, mice were euthanized for examination of the pelt and large intestinal contents for parasites. All treated mice were parasite free. Results of the study verified that 4 wk of continuous ivermectin treatment successfully eliminated fur mite and pinworm infections. Nonmolecular parasite exams can be used to monitor treatment efficacy for the first 3 to 4 mo post treatment, and afterwards, PCR assays would be useful for long-term intermittent screening.

P120 Cage Changing Nonhuman Primates on a Shoestring Budget Excites African Green Monkeys

C Douglas*

Office Of Animal Resources, University Of Missouri, Columbia, MO

Cage changing time for Nonhuman primates can be challenging at times and can be stressful to the animal technicians and the animal. We have always used a collar and pole system to change NHPs from the dirty cage to the clean cage. With African greens, this system proved to be very stressful on the animal as the collar started causing lesions on their neck and they became terrified of the pole when we tried to catch them. The collars had to come off and we needed another way. We searched for a tunneling system from different companies, but US$10,000 to US$15,000 was out of our price range, as we are working on a limited budget. Our solution was to construct a tunnel to transfer the NHPs from dirty to clean cage. Everything we needed was found at our local hardware store for around US$200. Using the tunnel had positive results and exceeded our expectations. The African greens were healthier and their stress level was at an all-time low. The tunnel actually is a part of their weekly environmental enrichment. In the beginning we had to use treats to entice them through the tunnel. Now, no sooner is the tunnel attached to the dirty and clean cage, the African greens open the door themselves and fly through the tunnel at lightning speed. They love their new toy!

P121 Training to Improve Postprocedural Recordkeeping

D Molnar*, W Kahn, KJ Salleng

Vanderbilt University, Nashville, TN

Medical records are a key element of the veterinary care program and are considered critical for documenting animal wellbeing as well as tracking animal care and use at a facility. IACUC semiannual inspections and post approval reviews identified failure to maintain adequate postprocedural records as one of the top 5 items of noncompliance in research laboratories at our institution over the last 5 y. Administration of postprocedural analgesics is required per protocol. Absence of records equates to no direct evidence of analgesic administration. The IACUC deems the lack of documentation as a reportable noncompliance. All those involved in animal care and use must comply with federal laws and regulations regarding human and veterinary drugs, to include drug records, treatments, and storage. While documentation requirements for nonUSDA and USDA regulated species differ at this institution, USDA record maintenance has been appropriate, but the nonUSDA rodent users' records continue to be inadequate and are a subsequent noncompliance concern. Development of required recordkeeping training for nonUSDA rodent users, planning and initiation, along with the individuals impacted will be described. Training efforts included the implementation of a customized online course that highlights the regulatory requirements and basic principles of recordkeeping, an optional didactic lecture, continued education for the IACUC members, and increased attention on records during semiannual inspections and post approval monitoring visits. Since implementation of the required nonUSDA rodent recordkeeping course, the semiannual inspections and routine post approval monitoring visits have shown a decrease in noncompliance under the surgical and/or postoperative/procedural care records for nonUSDA rodent. Through the combined efforts of education and the required completion of the course prior to protocol approval, post procedural recordkeeping has improved.

P122 Revisiting Guinea Pig Biomethodology: What Is Effective and Humane?

E Ahearn, T Kawamoto, S Reda, DE DeOrnellis*, K Astrofsky

Laboratory Animal Services, Novartis Institutes for BioMedical Research, Cambridge, MA

The introduction of guinea pigs to our facility prompted the Laboratory Animal Services (LAS) training team at our Institutes for BioMedical Research in Cambridge, MA (NIBR Cambridge), to research common research techniques (compound administration, blood collection, etc.) for implementation. Despite being an established research model, guinea pig biomethodology continues to be limited and, in some cases, thought to be challenging. After extensive literature review and collaborations with industry colleagues, there appears to be a wide range of opinion and conflicting information regarding appropriate Guinea pig biomethodology. Our skilled training team set out to evaluate and define optimal techniques, ensuring data/sample quality and humane animal welfare standards. As a result, we determined that compound administration (intramuscular, subcutaneous, intraperitoneal, and intravenous) and blood collection (retroorbital) can be successfully and safely performed. This poster will have multimedia content.

P123 Does Bedding Type Have an Effect on Breeding Performance in Mice?

D Yoakum*, M Mackay, K McCafferty, P Willhite, B Peters

Laboratory Animal Care, La Jolla Institute for Allergy and Immunology, La Jolla, CA

Few studies have directly assessed the influence of different contact bedding types on mouse breeding performance and offspring survival and growth. In this study, we evaluated breeding performance of C57BL/6J mice monogamous paired at 8 wk of age, by comparing 2 different contact bedding types: wood chip and corncob. There were 40 cages on wood chip and 40 cages on corncob. We monitored the following variables over 6 consecutive months: number of pups at birth, number of pups weaned per litter, average weight of weanlings at 21 d, and the number of days between litters. Testing for equivalence, we followed standard procedures to test for equivalence of experimental variables. Briefly, we first determined a level of difference that we considered significantly different (for example, a 20% change in the average number of animals at birth between bedding conditions). Then we calculated 90% confidence interval of the experimental variable under one bedding condition assuming a normal distribution, and asked if that entire confidence interval lies within ± 20% of the mean for the other bedding condition. If that is the case, then we can conclude with $P < 0.05$ that the means are indeed equivalent at the predefined cutoff for significant differences. The purpose of this study was to evaluate contact bedding types and assess if these conditions have an impact on breeding performance. This study supports that there were no significant differences for any of the study parameters between wood chip and corncob bedding. With these results, selection of bedding type may be based on factors other than breeding performance.

P124 Expanding Vivarium Capabilities on a Shoestring Budget

D Adams-Fish*

ATCC, Manassas, VA

In support of ongoing and future contract and commercial commitments we found it necessary to expand our vivarium usage from an only rodent facility to one that could house rabbits and potentially ferrets. The challenge was to increase our capacity while minimizing costs and facility changes. The ultimate goal would be to bring inhouse contracts that we were previously outsourcing due to our constraints. Outsourcing was costing us time and money. One solution, using caging that traditionally houses rabbits, would require the facility to renovate the entire cage wash facility. This was too costly and would interfere with ongoing work. At an AALAS conference the facility manager noticed that some facilities were using pens as enrichment for their traditionally housed animals and fortunately the pens could be used as permanent housing. We developed a plan, with IACUC approval, that demonstrated with minimal capital investment the facility was able to expand its animal housing capabilities. We found that for under US$500 we could expand our animal facility from housing only rodents to now housing rabbits and potentially housing ferrets. After implementation we found that the alternative housing not only provided greater enrichment for the animals, but it also reduced stress on the animals and the staff as well. There was a reduction in cost of feed and bedding as less was needed to house the same number of animals as traditional housing. Stepping outside the box allowed the facility more flexibility and a greater use of the vivarium space.

P125 Filter Top Testing of Ventilated and Static Mouse Cages for Detection of Fur Mites

D Isbell*, C Killingsworth, E Dohm, SC Cartner, J Cadillac

Animal Resources Program, University of Alabama at Birmingham, Birmingham, AL

To determine the incidence of fur mites in our facility, we tested individual colony animals using commercially available PCR testing. This direct testing of animals was time-consuming and potentially stressful to mice that were restrained to swab the fur. During this time, others reported testing the shelf-exhaust manifolds of individual ventilated cages. We launched a pilot study to test the hypothesis that swabbing the surface of the filter top would detect fur mite DNA without handling the mice. Using the same commercial laboratory, initial PCR testing involved positive mice (*Radfordia affinis*) that were housed in a building with individually ventilated caging or a different building with mice that tested positive for *Radfordia affinis* but were housed in static caging. Sterile flocked swabs were rubbed over the surface of the filter top that was directed towards the mice. Separate swabs were rubbed against the lay of the hair on the dorsal and ventral body surface. Initial testing involved 1 swab with pooled samples from 10 cage lids and one swab from 10 mice maintained in static caging. The same procedure was repeated with pooled samples from 10 cage lids and 10 mice in individual ventilated cages. At the same time, one swab per mouse from the same 10 mice and one swab from each of the 10 mouse cage lids in the ventilated system were also tested for fur mite DNA. The pooled sample from the lids in the static cages was positive, but the pooled mouse swabs from the same 10 static cages was negative for fur mite DNA. However, the pooled swab sample from the lid and the pooled sample from the 10 mice in the ventilated system were both fur mite positive. Of the individual cage lids tested in the ventilated system, 5 of 10 lids were fur mite positive whereas only one individual mouse was PCR positive. In addition, one mouse was positive but the lid was negative. Further testing is necessary to confirm fur mite positive mice by PCR testing with subsequent positive direct observation of live mites with parallel testing of caging lids in both ventilated and static air conditions. However, these divergent data suggest that simple sampling of the cage lid is not an accurate method to detect fur mites in mice housed in individual ventilated cages or static cages.

P126 Use of Mechanical Shoe Cleaners in an Animal Vivarium

D Molk*

[1]Department of Comparative Medicine, University of Washington, Seattle, WA; [2]Center for Comparative Medicine, Massachusetts General Hospital, Boston, MA

The our institution began using motorized shoe cleaners at the entrance of several animal vivariums in 2006. Each cleaner had a HEPA vacuum system, as well as multiple internal brushes, so when a shoed foot was placed inside, particles would be removed from the soles of users' shoes. It was believed that such a device could minimize the tracking of mud and dirt into the animal vivariums, as well as minimize any pathogens that might be carried in with such substances. While the cleaning devices were in place for several years, their efficacy had not been tested. A study by Hickman-Davis and colleagues employed the use of florescent contamination powder to measure the effectiveness of footwear sanitation practices at their institution, specifically the use of shoe covers. Using a similar product, we set out to determine the efficacy of our mechanical shoe cleaners. Motorized shoe cleaning devices are advertised for use in cleanrooms and production settings, but not necessarily animal vivariums. Yet, the use of such devices in a vivarium could potentially be a substantial cost savings over the use of

other shoe disinfectant practices, such as shoe covers; it could also be a "greener" option. In our study, contamination powder, which simulated mud, dirt, and debris from outdoors, was applied to the soles of the shoes of volunteers that then placed their feet in a motorized cleaner for time periods ranging between 5 and 30 s. The shoes were viewed under ultraviolet light before and after being placed in the cleaner and photographs documented the powder's fluorescence. In summary, no difference was noticed before and after the use of the shoe cleaner at any time interval tested. In our hands, the shoe cleaner did not seem efficient at sufficiently removing fluorescent powder from the shoes of our volunteers in a reasonable amount of time. These findings altered the shoe disinfectant procedures at our institution; the use of the mechanical shoe cleaners was discontinued.

P127 Microisolation Filtered Cage Lids: Verification of Sanitation Standards

DM Harrison*, B Smith, CC Hofer, J Petty, M Nicolaus, VK Bergdall, J Hickman-Davis

University Laboratory Animal Resources, The Ohio State University, Columbus, OH

According to the eighth edition of the *Guide for the Care and Use of Laboratory Animals*, enclosures and accessories, such as [cage] tops should be sanitized every 14 d. Extended changeout times have been proposed for cage lids on individually ventilated cages (IVCs) that do not directly contact the animal. Sanitation levels of mouse IVC cage lids were tested within the facility every 14 d up to 180 d using contact plates and ATP monitoring system. Based on the literature, pass standards were defined as 0 to 15 colonies for contact plates and 0 to 30 RLU for ATP. The cage lid was tested to determine the capacity to act as a barrier to the spread of compounds from within the cage to the outside and for the dirty lid to act as a fomite for transmission to new animals within the cage. Lid testing included standard barrier and sterile barrier areas. Standard cage lids resulted in a pass rate of approximately 70% at 14 d, approximately 50% at 30, 90, and 120 d and failure at 150 and 180 d. Sterile lids resulted in a pass rate of approximately 80% at 120 and 150 d. There was no difference between 14 and 180 d performance of cage lids as defined by biologic testing. Currently, approximately 8% of cage lids are sanitized every 14 d. Sanitation of all IVC lids on a 14-d schedule would increase labor cost by more than 90%. These data indicate that extending the sanitation interval for nonanimal contact IVC lids is reasonable for some housing conditions and represents a significant cost savings.

P128 Novel Device to Deliver Carbon Dioxide for Rodent Euthanasia

D Mabunga*, B Batiste, J Vigil, K Cataline, C Roberts, LG Carbone

Lab Animal Resource Center, UCSF, San Francisco, CA

The 2013 AVMA Guidelines on Animal Euthanasia list CO_2 asphyxiation as acceptable with conditions when CO_2 displaces room air in the euthanasia chamber at a rate of 10% to 30% of volume per minute. Meeting this standard requires knowing the volume of the euthanasia chamber and the flow rate, not the pressure, of the CO_2. Adjustable flowmeters used for this purpose require adequately trained and attentive operators. We developed devices to deliver the correct volumes, either 20% per minute or 30% per minute, of CO_2 to a standard mouse cage, to a standard rat cage, and to a chamber that holds several mouse or rat cages. Operator-independent volume of flow is determined by calculated apertures that deliver approximately 20% or 30% displacement per minute at 30 or 40 psi, respectively. Adult mice (n = 32) were randomly assigned to be euthanized in one of 4 conditions: 20% displacement per minute for a single 7.6-L mouse cage (20-cage), 20% displacement per minute for a mouse cage set into in a large (75.44 L) chamber (20-chamber), 30% displacement per minute for a single mouse cage (30-cage), and 30% displacement per minute for a mouse cage set into in a large chamber (30-chamber). Mean times to respiratory arrest for each condition were 166 s for 20-cage, 208 s for 20-chamber, 157 s for 30-cage, and 169 s for 30-chamber. We conclude that the novel device with defined diameter apertures reliably delivers flow rates that achieve the AVMA recommendation of 10% to 30% exchange per minute without requiring adjustable flowmeters. Respiratory arrest at fill rates recommended in the 2013 AVMA Guidelines requires at least 166 s for adult mice, but may take longer in chambers that contain several cages or other obstructions to free gas flow.

P129 Big Results Arrive from Small Changes: Water Bottle Evaluation

DG Paquin*, C Ranns

Comparative Medicine, Pfizer, Cambridge, MA

A Comparative Medicine (CM) department in Cambridge Kendall Square opened a new facility in May 2012 using rat disposable caging. Due to the compact size of the facility and the need for a flexible layout, disposable caging was selected over other industry IVC options. The original rat water bottle had a smooth exterior and a blue cap with an approximately 2-mm diameter hole. Upon inversion of the bottle, a stream of water naturally escaped until surface tension built, holding the flow until the animal actively touched the exterior hanging drip. Upon implementation of a new commercially available rat disposable IVC system, approximately 20% of cages required a cage bottom spot change prior to the weekly scheduled changeouts due to excessive moisture in the cage. Technicians observed a large volume of fluid escaping from the water bottle upon inversion of the bottle. This lost volume was measured at approximately 10 mL. In an effort to extend cage bottom life, the water bottles were evaluated to determine if they were contributing to the unexpectedly high percentage of spot changes. This poster details the evaluation of several parameters altered between a new and old water bottle design which included cap hole diameter, vacuum inversion technique, and a new plastic bottle configuration. With the new water bottle configuration, leakage was reduced to approximately 3 mL. With proper utilization of a vacuum flipping technique the leakage of water reduced by an additional 1 to 3 mL each time the water bottle was inverted. One lesson from this experiment is the importance of continuous improvement with industry vendors to optimize animal caging systems. The new plastic water bottle design substantially reduced the amount of water released upon bottle inversion even in the absence of a vacuum flipping technique. Combined, these 2 changes resulted in a substantial reduction in the number of spot changes and associated cost.

P130 How Safe Is Your Facility?

F Karani*, F Warren

Office of Laboratory Animal Medicine, University of Delaware, Newark, DE

Animal facilities have the advantage of providing everything that an animal needs. This includes food, water, sanitation, air, and lighting. The disadvantage of providing lighting is that animal facilities are constructed without windows that allow natural light to enter. If there happens to be a power failure, an animal facility can be an extremely dark place. Navigating around a completely dark facility cannot only be intimidating, but dangerous. Large equipment would become hazardous if personnel were trying to find their way out. In our facility, we have installed simple, cost effective features that promote safety in the event of a power failure. In each hallway, there are LED rechargeable flashlights that illuminate in the event of a power failure. These flashlights are plugged into electrical outlets, and are constantly charging. In the darkened hallway, the flashlight is the brightest object seen. These battery powered flashlights also emit a beeping sound after 3 min to make them easier to find. All animal rooms and support areas have luminescent strips on the door frame, placed directly next to a pen-style flashlight mounted to the wall. The strip serves to locate the door, as well as to find the flashlight. The flashlight can then be used to safely guide individuals out of the room, into the hallway to illuminated facility maps. In addition, all office areas and hallways immediately outside of the animal facility have large rechargeable flashlights mounted to the walls. In each hallway, there is a facility map that provides information in regard to fire extinguishers, eyewash stations, and directions to the nearest exit, in the event of an emergency. The maps have luminescent strips around the edges. The strips become a vibrant green, should the lights go out, making the map easy to find. In the event of a power

failure in our facility, it is possible to see in the dark. Using simple, inexpensive items, such as glow in the dark tape, we have made safety a first and last priority.

P131 SCORHE: A System for Automated Video-Based Assessment of Activity and Behavior for Mice Housed in a Home-Cage Environment

G Salem*[1], J Krynitsky[1], M Garmendia-Cedillos[1], S Pajevic[1], JD Malley[1], JU Dennis[2], T Furusawa[3], T Deng[3], M Bustin[3], J Gillet[4], MM Gottesman[4], A Sowers[5], JB Mitchell[5], TJ Pohida[1]

[1]Division of Computational Bioscience, Center for Information Technology, National Institutes of Health, Bethesda, MD; [2]Center for Biologics Evaluation and Research, Food and Drug Administration, Bethesda, MD; [3]Laboratory of Metabolism, [4]Laboratory of Cell Biology, [5]Radiation Biology Branch, Center for Cancer Research, National Cancer Institute, National Institutes of Health, Bethesda, MD

The System for Continuous Observation of Rodents in Home-cage Environment (SCORHE) was developed to quantify activity levels and behavior patterns for mice housed within a commercial ventilated cage rack. The SCORHE in-rack design provides daytime and nighttime monitoring with the stability and consistency of the home-cage environment. The dual-video camera custom hardware design makes efficient use of space, does not require home-cage modification, and is animal facility user-friendly. Given the system's low cost and suitability for use in existing vivariums without modification to animal husbandry procedures or housing setup, SCORHE opens up the potential for wider use of automated video monitoring in animal facilities. SCORHE potential uses include day-to-day health monitoring, as well as advanced behavioral screening and ethology experiments, ranging from assessing short- and long-term effects of experimental cancer treatments to evaluation of mouse models. When used for phenotyping and animal model studies, SCORHE aims to eliminate concerns often associated with many mouse monitoring methods, such as circadian rhythm disruption, acclimation periods, lack of nighttime measurements, and short monitoring periods. Custom software integrates the two video streams to extract several mouse activity measures. The results of studies comparing activity and behavior profiles for knockout mice (HMGN and ABCB5) and their respective C57BL parental mice are reported. A study of the effect of the antioxidant acetaminophen on mice activity is presented.

P132 Social Housing Program for Sexually Mature Male Macaques

G Kuhlman*

Covance, Evansville, WI

To ensure both regulatory compliance and high animal welfare standards a program was initiated in our facility to allow sexually mature male macaques the opportunity for social housing. Historically, there had been concern over social housing of these animals. It was believed they were more prone to fight amongst themselves causing great bodily harm or even death. At initiation of the current project, primate behavioralists were consulted to determine the best plan for introducing the animals to each other. Several long-held beliefs regarding animal introductions and housing practices were modified. The initial introductions of the animals to each other were found to be the most important aspect in successful long-term social housing. A road map was developed during this project that can be implemented by other facilities to allow for successful social housing of their sexually mature male macaques. Since initiation of our new strategy, over 125 pairs of sexually mature male macaques have been created. Only a single animal has been excluded from social housing due to overly aggressive behavior.

P133 Withdrawn

P134 See PS87 for Abstract

P135 An Innovative Method of Providing Water to Mice

JL Taylor*, W McClelland, A Langford, P Noel, B Mickelsen, D Bird

Office of Comparative Medicine, University of Utah, Salt Lake City, UT

The Office of Comparative Medicine at our institution has developed an innovative method for providing water to mice using a disposable water pouch and valve system. This method was designed to be used with our grommeted cages, but may be adaptable to other cage systems. In order to use this method, the cages are placed on shelf racks or in IVC racks with the grommet facing out. We designed and produced a proprietary pouch holder that also serves as a cage card holder. The holder is mounted on the front of the cage and the water pouch is placed in it so that the pouch valve enters the cage through the grommet. The cage card slides into the front of the holder. The position of the holder does not hinder the observation of animals in the cage. This method of providing water is a tremendous time and labor saver. Since the water pouches are clearly visible on the outside of the cages, technicians can quickly assess the condition of all of the pouches in the rack without taking the cages from their position. This eliminates trips to the change hood. Additionally, the pouches can also be removed and replaced while the cages remain in position. Again, this saves considerable time over the conventional method of taking the cages to a hood to change the water bottles.

P136 Maternal Weight Gain as a Predictor of Litter Size in 3 Mouse Strains

JB Finlay*[1], X Liu[2], R Ermel[1], TW Adamson[1]

[1]Division of Comparative Medicine, [2]Information Sciences, City of Hope, Duarte, CA

One of the significant tasks facing both researchers and animal core facilities is producing a specific number of mice for a given project. The inherent biologic variability of mouse reproduction and litter size further exacerbates the challenge of effective research planning. A lack of precision in project planning is a contributing factor to the high cost of animal research, over production (thus waste) of animal life and facility/personnel shortages experienced by many institutions. The aim of this project was to examine to what extent (if any) prepartum maternal weight gain (measured daily) can predict the litter size in three commonly used mouse strains (BALB/c, Swiss Webster, and C57BL/6). To examine this specific aim we weighed 25 pregnant females from each strain every day starting from the morning they were found to have a vaginal plug (day 0). The morning the females were found to have delivered their litter, we weighed the mother, weighed all pups together, and counted the number of pups born. Litter sizes ranged from 1 to 9, 2 to 12, and 2 to 11 for BALB/c, Swiss Webster, and C57BL/6, respectively. A linear regression model (based on weight change from day 0) of these mouse strains demonstrated that maternal weight change at day 11 (BALB/c and Swiss Webster) or day 12 (C57BL/6) is a significant predictor of litter size ($P \leq 0.05$). These data indicate that it is possible to determine with a high level of probability the number of pups that will be born based on weight changes at 2 time points. Armed with this knowledge breeding strategies can be altered to minimize needless loss of animal life and maximize research funds, personnel efforts, and facility space.

P137 Physical and Visual Indicator for the Presence or Absence of an Automatic Watering System in Rodent Cage Racks with Quick Disconnect Automatic Watering System

JD Cox*[1,2], B Coop[3,2], RA George[2]

[1]Vivarium, [2]Scientific Services, [3]Instrument Design and Fabrication, HHMI/JFRC, Ashburn, VA

Automatic watering valves for ventilated cage racks come in a variety of styles. These include cage-mounted automatic watering system, fixed-rack–mounted automatic watering system, and rack-mounted automatic watering system with quick disconnects. At our institution, we use rack-mounted automatic watering system with quick disconnects. When automatic watering system are removed from racks to be cleaned and autoclaved to prevent cross-contamination, this creates the potential for placement of a cage in a location that does not have a automatic watering system, thereby not providing water to the animals inside. To prevent this from occurring, we designed an "automatic watering system stop" that provides a visual and physical barrier to alert staff and researchers when a cage location on a ventilated rack does not have an automatic watering system in place. The barrier is a 2-in.

diameter red aluminum disc with white letters that says "no water" to both visually alert staff that no automatic watering system is installed and to prevent a cage from being docked on the ventilated rack. The use of the "automatic watering system stop" was implemented as part of the daily cage checks. Animal care staff are responsible for ensuring a cage location either has a cage in place or if there is not a cage, they would remove the automatic watering system and place the automatic watering system stop over the quick disconnect for the automatic watering system. When a new cage is placed on a ventilated rack, the "automatic watering system stop" is removed and an automatic watering system is installed before the cage is placed on the ventilated rack. By preventing inadvertent placement of cages in rack positions lacking an automatic watering system, all animals are ensured access to water.

P138 Easy Enhancement to Prevent Water Bottle Removal by Macaques

J Gatke*, CE Ferrecchia, K Jensen, R Van Andel

Office of Laboratory Animal Care, University of California, Berkeley, Berkeley, CA

Water bottles are often used to monitor intake of water or medications in macaques. Their ability to remove the bottles from the cages before they are empty confounds these goals, as it is unclear how much water has been consumed. We developed an inexpensive and easy inhouse solution to this problem which prevents the water bottles from being removed from the front of the cage, while still using the existing water bottle holder provided by the caging manufacturer. The solution involves modifying a bicycle spoke to hold the bottle in place. Since its initial trial on several cages, none of the water bottles have been removed prematurely by the recipient nonhuman primate. We believe that this modification is an easy and inexpensive solution to a common problem that can be readily applied in similar situations at other institutions using water bottles for nonhuman primates.

P139 Beta Testing of a Water Flow Monitoring System on Mouse Racks with Automated Watering

JL Steele*[1], RP Reynolds[1], V Lanphier[2]

[1]DLAR, Duke University, Durham, NC; [2]Edstrom Industries, Waterford, WI

Automated watering is the preferred method of providing high quality water to mouse racks. A leaking drinking valve with automated watering will cause the bedding to become moist and may lead to standing water within an animal's cage. As a result of the increased moisture, the animals can become hypothermic and ultimately die if the condition is not addressed promptly. An automated means to detect a constant flow condition of a valve can significantly improve animal welfare. A water flow monitoring system was installed on the automated watering system, between the rack supply water and the racks on 2 mouse ventilated racks, to determine if the system would alert facility staff of the leaking valve before it was actually noted by visual inspection. The system was configured to monitor and alarm when the water flow continues through the animal cage rack after a time delay of 40 min. The monitoring system will alert designated staff by way of pager or email through the environmental monitoring system. During a 90-d beta testing period, there were 4 alarms sent by the system. These incidences were not evident during routine health checks, but were verified after receiving the alarm. The number of drops of water per minute was recorded for each incident. There were no false alarms. The health of the mice in all incidences was not compromised. In conclusion, the water flow monitoring system, in conjunction with an environmental monitoring system, identified leaking water valves at the rack level and notified the designated personnel in a timely manner. This allowed the animal care staff to provide immediate attention to the cages.

P140 Zebrafish Facility Improvements

J Muster*[1,3], GE Sanders[2], RT Moon[1,3]

[1]Pharmacology, [2]Comparative Medicine, University of Washington, Seattle, WA; [3]Howard Hughes Medical Institute, Chevy Chase, MD

In 2006, our institution's School of Medicine designed and built a new zebrafish (*Danio rerio*) facility with a capacity for 416 tanks. The design of this facility, specifically the recirculating system and husbandry components, was advanced for the time; however, system control, husbandry, and research procedures could be better optimized. In 2011, this facility was redesigned to accommodate additional investigators with a capacity for 1,616 tanks. We used this opportunity to improve upon the original facility design by incorporating new technologies in the system to reduce the use of disposable elements, increase the monitoring and control of multiple system parameters in real-time, increase the efficiency of routine husbandry practices, and facilitate research efficiency. One of the new technologies used in this system was a computer controller capable of sending passive, important, and critical system alerts by detailed email as well as by telephone. This controller also allows the user to remotely access the system to view the real-time status of the system controlled parameters such as pH, conductivity, total gas pressure, dissolved oxygen, and temperature, to view the water levels for multiple reservoirs and sumps, and have the ability to control various pumps and system components. These remote monitoring and adjustment options were not possible with the prior system. An additional sink, multiple dishwashers, and redesigning the standard operating procedures for the facility improved the routine cleaning and disinfection of husbandry equipment. And finally, expansion of the existing facility included an additional suite of specialized research rooms connected to the fish housing area to minimize transport distances of live fish and provide enhanced resources to the investigators. These facility improvements have reduced the time spent on standard husbandry 3-fold, expanded the facility fish housing 4-fold, expanded the specialized research space 5-fold, improved the quality of animal welfare, and therefore, improved the efficiency with which the research is conducted.

P141 Four Custom-Made Devices Used for Rodent Restraint and Gaseous Anesthesia

J Johnson*[1], S Jankie[2], L Pinto-Pereira[2]

[1]School of Veterinary Medicine, [2]Department of Paraclinical Sciences, The University of the West Indies, St Augustine, Trinidad and Tobago

To conduct proper experimental procedures, adequate restraint of test animals is of paramount importance. To date, research is still plagued somewhat with concerns regarding increased animal and human stress levels or adverse effects due to inadequate or prolonged handling and restraint. In addition, risks to the human handlers from bites, scratches, urine, feces, or other bodily fluids, are of continual concern especially with the existence of zoonotic diseases and increased occurrence of severe allergic reactions to animals by humans. Over the years, numerous restraint devices have been created and developed and a large plethora is available commercially. However, they may have limitations, which may only be identified when purchased and used by the investigator. Additionally, they may be costly and some add nonrecyclable materials to environments when discarded. We present 4 devices that were handmade and customized for their intended use and for our research animals. A restrainer for tail snips/intravenous cannulation, a device for restraint for parenteral injections, an improvised isoflurane anesthetic machine, and a chamber for induction of anesthesia were created and tested on animals. All devices created consisted of recycled materials, such as plastic drinking bottles, clear borosilicate glassware, discarded intravenous fluid lines, and others. The materials used were inexpensive, easy to obtain and easily sanitized after use.

P142 Husbandry and Veterinary Care of Nutria (*Myocastor coypus*) as a Laboratory Animal: A Process of Trial and Error

JK Pepping*[1,2], JF Bova[1,2], N Fowlkes[1], JS Ialeggio[3], AM Gaither[4], RA Malbrue[5], B Sharp[2], J London[2], C Redditt[2], DG Baker[1,2], RW Stout[1,2]

[1]Department of Pathobiologic Sciences, School of Veterinary Medicine, [2]Division of Laboratory Animal Medicine, School of Veterinary Medicine, [3]Department of Renewable Natural Resources, Louisiana State University, Baton Rouge, LA; [4]Division of Veterinary Medicine, Tulane

National Primate Research Center, Covington, LA; [5]Department of Clinical Sciences, School of Veterinary Medicine, Tuskegee University, Tuskegee, AL

The nutria (*Myocastor coypus*), also known as the coypu or river rat, is a semiaquatic rodent of the family Myocastoridae. Nutria were first introduced to Louisiana in 1930 and have since flourished due to the ideal wetland habitat. The damage and destruction they inflict on the Louisiana wetlands has become an increasing problem and is contributing to coastal erosion. Neonatal, juvenile, and adult nutria were housed at our institution's School of Veterinary Medicine for the purpose of conducting a study to determine their vegetation feeding preferences. Husbandry and veterinary care were provided by the Division of Laboratory Animal Medicine. We encountered numerous husbandry issues and problems pertaining to their shelter, feed, bedding, water, and neonatal care. Since nutria have been used infrequently in research, we wanted to establish the ideal husbandry and veterinary care practices for these animals. Through a process of trial and error, we established improvements that can be implemented in their husbandry and veterinary care practices. At the end of the study, we sedated the nutria and performed various blood sampling techniques in order to collect blood for CBC and chemistry panels. Complete necropsies and parasite evaluations were also performed. The improvements we implemented and the information we gathered proved to be useful in establishing a frame of reference for the care of nutria as a laboratory animal. If nutria prove to be a useful and unique rodent model in the future, this information will be essential.

P143 Invisible Bottles Made Visible

N Montalto, J Kiesel*, A Carte

Comparative Medicine, Fred Hutchinson Cancer Research Center, Seattle, WA

Animal care technicians working with rodents housed on racks with water bottles must be diligent in providing and checking the fill level of water bottles. During the period of changing out cages it is very important to switch the used water bottle for new clean water bottle. If a bottle is forgotten the animals can suffer from dehydration or even die. If an animal care technician gets distracted, adding the water bottle could be missed during the change out process. To prevent this from occurring, we came up with the process of double checking for water bottles before leaving the room. This helped, but we found that it was sometimes hard to recognize a missing bottle because everything blends together making it difficult to discern the difference between the cage lid and the bottle in a visual sweep of the housing rack. We went back to the drawing board and decided to try marking the bottles to give them a visual distinction from the lid. Three types of permanent markers (A, B, and C) were selected based on their ability to withstand high temperatures for cage washing and autoclaving. After it passed our testing, we chose marker C to mark all of the water bottles. We have found this procedure to be successful; having no incidents of missed water bottles after all water bottles were marked. The markings on the bottles have held up to the washing process well with replacement lines only needing to have been done on a minimal number of bottles after 4 mo. This marking has been a permanent solution to an equipment-specific challenge.

P144 Teaching Students and Improving Animal Welfare through Socialization in a Higher Education Facility

JK Willis*[1], JM Cavarra[2], K Bruner[3], LV Kendall[3]

[1]Biology, Colorado State University, Fort Collins, CO; [2]George Mason University, Washington, DC; [3]Laboratory Animal Resources, Colorado State, Fort Collins, CO

At higher education institutions, there are an abundance of students eager for practical experience with animals and laboratory animals that can benefit from receiving such attentions. We recognized this need and began a multilayered approach to developing a program that would improve the lives and experiences of both students and animals. Our aim was to provide a structured setting where students could learn and apply scientific principles of learning, enrichment, and the human-animal bond with the laboratory dogs. The student were required to commit 2 h per week for a semester. The students had bimonthly training sessions to evaluate their progress and improve their skills. They had an online course where they could document the progress made with each animal individually and develop a sense of community among other socializers. Video tutorials we made on different training topics were available for students to review. Care staff were trained each semester so they could use the newly trained cues in daily husbandry. The dogs were evaluated for temperament before and after the program and we found that temperament improved significantly enhancing their poststudy adoptability. Additional outcomes were a decrease in barking and improvement of cooperation and handling by daily caregivers. The students stated pride in accomplishment and an improved sense of wellbeing. We expanded our program to include cats and rabbits in subsequent semesters. Our most recent semester of student participation numbered 75 students, for a total of 150 h of weekly training and enrichment time across species. The budgets of most laboratory facilities could not support that level of training and enrichment if they relied solely on paid staff. Higher education institutions should apply this model for improvement animal welfare and student learning.

P145 Effects of Enrichment and Environment on the Behavior and Serum Corticosterone Levels in *Xenopus laevis*

JA Scott*, D Taylor

Division of Animal Resources, Emory University School of Medicine, Atlanta, GA

Xenopus laevis is a commonly used research animal for which well-accepted enrichment strategies have not been established. Our overall objective was to identify enrichment strategies that are most beneficial to *Xenopus* as a step toward creating housing standards that effectively promote wellbeing. To measure preference for housing conditions, 16 *Xenopus* were housed in a single tank with half white and half black background. Additionally, lily pads and PVC pipe enrichment were added to each individual side, and then to both. Animals were observed and videotaped for 10 min under each condition. On average, frogs spent 64% of the time on the black side of the tank regardless of enrichment location, suggesting a preference for the dark background. When a frog purposely used the enrichment as cover for at least 5 s during the 10-min observation, it was considered one enrichment interaction. Out of 192 10-min observations, only 19 enrichment interactions occurred. With preference determined, 12 *Xenopus* were then placed into either black tanks with enrichment (DE) or white tanks with no enrichment (WN), with 6 animals per housing condition in groups of 3. Twelve frogs were also individually housed in DE and WN environments, with 6 per housing condition. After 7 d, blood was collected and is being analyzed for corticosterone levels. Out of 42 observations, singly housed *Xenopus* used enrichment daily with 37 enrichment interactions, whereas grouped housed *Xenopus* preferred to crowd together rather than use enrichment with only 9 enrichment interactions. Behavioral data collected to date, suggests that *Xenopus* prefer a dark background over a light background. When group-housed, interaction with enrichment is minimal compared with singly housed, suggesting that enrichment provisions are important for singly housed animals and less important for group-housed animals.

P146 Assessment of Issues Related to Exportation and Importation of Nonvendor Approved Animal Shipments

JM Cadillac*[1], SA Lyons[2]

[1]Animal Resources Program, [2]Center for Clinical and Translational Science, University of Alabama Birmingham, Birmingham, AL

Based on local observations that too many animals are at risk to unregulated conditions, adverse events can occur that are sometimes fatal. We designed a web-based survey to determine the number of adverse events and other variables related to the exportation and importation of nonvendor approved animal shipments. Fifty institutions that we had either exported to or imported animals from over the past 3 y were contacted. Twenty-seven respondents from 25 institutions completed

the survey. Fifty percent of the institutions have less than 100,000 gross square feet in the facility. Seventy percent of the institutions have a rodent population up to 75,000. Nonvendor approved animal species exported and imported include aquatics, invertebrates, mammals, and reptiles. Sixty-two percent of institutions use 20% to 40% of a full time equivalent (FTE) to prepare shipments. Most institutions use a combination of ground and air transportation for exportation and importation. Thirty-three percent of respondents export animals 6 to 20 times per year; 46% of respondents export over 50 times per year. Approximately 52% of institutions import animals less than 50 times per year, whereas roughly 48% import animals more than 50 times per year. Each shipment may include a single animal to many hundreds. Of the number of shipments exported and imported, 43% of respondents experienced no adverse events. Fifty-seven percent have experienced one to 5 adverse events in a year. Adverse events included natural disaster, extreme temperatures, length of time in transit, logistics, or other issues described by the institution. Risk insurance and material transfer agreement processes were similar across institutions polled. In summary, approximately half of the institutions that responded to the survey experienced adverse events during the exportation or importation of nonvendor approved animal shipments, thereby constituting a significant problem in the transport of nonvendor animals that may be preventable.

P147 Establishment, Implementation, and Refinement of a Large Animal Sanitation Monitoring Program

J Petty*, T Jones, S Lewis

ULAR, The Ohio State University, Columbus, OH

The eighth edition *Guide for the Care and Use of Laboratory Animals* recommends regular evaluation of sanitation effectiveness in both manual and automated processes. While a rodent sanitization monitoring program has been in place at our institution, establishment of a formal large animal sanitation monitoring program was necessary. Numerous components from transport cages/carts, rabbit cages, environmental/food enrichment devices, and common shared equipment (scale, feed bowls, etc.) were cleaned using standard rack and tunnel washer procedures. Large animal enclosures were foamed and rinsed using a commercially available alkaline-based cleaner, then foamed, scrubbed, and rinsed using an acid-based cleaner, and finally a disinfectant was applied and rinsed. All of the components were evaluated prior to and after sanitization to determine the efficacy of the current standard operating procedures, cost analysis, and evaluate the new testing plan using both microbiologic culture (contact plates) and an organic material detection system (ATP). According to the product information guide, contact plates plate sanitation thresholds for critical contact areas are 0 to 15 colonies per plate and less than 30 relative light units for ATP. Results of preliminary testing showed that the majority of our current sanitization processes are effective and manufacturer recommended pass standards are adequate, but potential exists for refinement and optimization. Both contact plates and ATP must be employed regardless of cost, as some unique equipment design does not allow for contact plates testing (that is, automatic watering system). A smaller subset of areas could be tested and still provide enough information to evaluate proper sanitization. Using both the contact plates and ATP testing on a quarterly basis is a convenient, efficient, affordable way to establish and implement a large animal sanitation monitoring program.

P148 Quantifying Cold-Stress in Laboratory Mice with Thermography: a Noninvasive Imaging Technique

JM David*, D Stout

Molecular and Medical Pharmacology, David Geffen School of Medicine, University of California, Los Angeles, Los Angeles, CA

Routine vivarium temperatures are well below the murine thermoneutral zone, causing a net loss of thermal energy to the environment. When cold-stressed, laboratory mice physiologic compensate via nonshivering thermogenesis, which primarily occurs in interscapular brown adipose tissue (BAT). Nonshivering thermogenesis is dynamic physiologic process that changes from moment to moment. Quantifying this dynamic physiologic process in the context of the mouse's native vivarium is very difficult. Yet understanding the cold-stress experienced by laboratory mice inside the vivarium is critical to developing proper husbandry practices to support murine thermoregulatory needs. We developed and validated a quantitative method to measure nonshivering thermogenesis in mice using thermography. Thermography is a technology that captures images of emitted infrared heat signatures. By exposing C57BL/6J and Nu-Foxn1Nu mice to various environmental temperatures, measuring emitted nonshivering thermogenesis of BAT with thermography (ΔT BAT), and using a nonlinear mixed effects model, we demonstrate ΔT BAT can be used to calculate energy expenditure, as measured by classic indirect calorimetry (entropy production rate, EPR, calories/min) according to the following formulas: ♀ C57's EPR (calories/min) = 3.138 + 3.781× ΔT BAT ♂ C57's EPR (calories/min) = 2.879 + 3.781× ΔT BAT ♀ Nu-Foxn1Nu EPR (calories/min) = 4.228 + 3.781× ΔT BAT ♂ Nu-Foxn1Nu EPR (calories/min) = 3.969 + 3.781× ΔT BAT. Thermography is a portable technology with high-sampling frequency that can quantitatively measure cold-stress in mice while they reside in the vivarium. Using thermography, we can assay cold-stress.

P149 Effect of Caging System and Bedding Sterilization on Intracage Ammonia Accumulation with Time

JA Maher*[1], JP Rodriguez[2], SA Mischler[1]

[1]Comparative Medicine, Pfizer, Pearl River, NY; [2]Vision IT, Philadelphia, PA

Cages holding rodents accumulate ammonia and fecal material over time and need to be periodically changed and washed. Cage changing can cause distress to the rodents and expose the lab personnel to allergens and infectious agents; cage washing, furthermore, is resource intensive and expensive. Extending the cage change interval, and/or using disposable cages are possible alternatives to traditional cage changing and washing. The present study sought to: a) determine if using autoclaved bedding (AB) lowered the rate of intracage Ammonia production compared with regular bedding (NAB), and b) Compare the ammonia removal efficiency of two ventilated caging systems, CH and CV, in which the air injection and exhaust ports, and the predominant air flow direction, were horizontal and vertical, respectively. Twenty mouse cages, each with 5 CD1 females (28 to 30 g of body weight), were allocated to 4 treatment combinations: CH-AB, CH-NAB, CV-AB and CV-NAB. Ammonia levels were measured daily as the indicator of Intracage environmental quality. Intracage Ammonia levels were lower in CV than in CH cages: 9 of the CH cages had ammonia levels of 50 ppm or more by day 7 of the test while 4 of the CV cages had ammonia levels of 12 ppm or less by day 14. Bedding sterilization had no discernible influence on ammonia levels under the current testing conditions. Attempts were also made to describe the evolution of intracage ammonia for predictive purposes: an exponential growth model closely described the changes in ammonia with time ($P < 0.001$, $R^2 = 0.55$); a Kaplan–Meyer survival analysis model was used to estimate the probability of a cage's survival (not reaching 50 ppm) with time and to compare cage survivability rates among treatments.

P150 Two of a Kind or a Full House? Alloparenting and Reproductive Suppression in Laboratory Mice

K Pritchett-Corning[1], BN Gaskill[1], J Garner*[2]

[1]Research Models and Services, Charles River, Wilmington, MA; [2]Department of Comparative Medicine, Stanford University, Stanford, CA

Alloparenting (when individuals other than the biologic parents act in a parental role) is seen in many mammalian systems. In wild female house mice, alloparental care is seen when familiar sibling females simultaneously immigrate to a male's territory. When unfamiliar wild mice are trio mated in the laboratory, one female will typically be reproductively suppressed. In contrast, laboratory mice have long been thought to alloparent regardless of familiarity, and their behavioral plasticity, combined with the constraints of housing and husbandry, have led to the common (but untested) wisdom that mating 2 females with one male increases overall production per cage. In a small-scale study, albino and pigmented C57BL/6 and CF1 were used to test that

assumption. We housed pairs or trios of mice with nesting material in disposable ventilated cages and followed them for 16 wk. Females in trios were dominance tested at the start of the experiment, and also when their pups were 5 d old to determine whether a female's dominance shifted with the birth of pups. By mating pigmented and albino females with albino males of the same stock or strain, maternal parentage was easily determined. Pigmented mice nursed albino pups and vice-versa, indicating that group nesting and alloparenting occurs. However, when overall production of the mice and cages was examined, reproductive suppression was seen in trio cages. Results showed a significant difference in production between B6 and CF1 ($P < 0.0001$), and that on a per-mouse basis, pair mating outperformed trio mating ($P = 0.02$). No infanticide was noted, so the likely mechanism of reproductive suppression is through estrous cycle suppression. We are now using data from breeding rooms to replicate the study and quantify the large-scale implications of this result.

P151 "Nip" It in the Bud: Handling the Feisty Ferret

J McMahon*, M Crain, M Tucker

Comparative Medicine, Pfizer, San Diego, CA

Ferrets are a new species at our institution and were brought in to support vaccine research in developing a universal influenza vaccine. Our enrichment program incorporated items in their housing that caters to their species' specific behaviors. Also, to help dissipate the ferret's natural instinct to bite from fear of being handled, we implemented a program of regularly scheduled acclimation. Here, we present our ferret enrichment and socialization program. The ferrets are housed in compatible groups of 2 to 3 animals per cage. Our enrichment program is designed to provide an opportunity for the ferrets to hide, burrow, play, and gnaw. Enrichment items are rotated to maintain novel interest. Each cage will be provided with one hammock, at least 2 toys, and one item for hiding/sleeping. Recently, we have had much success with adding a paper bag into the cage once a week for 24 h for animals to play, shred, hide and burrow with the bags. All other items are changed at least once a week. To promote curiosity and maintain interest to novelty, item rotation is staggered and new items are introduced in the cages at least 3 times a week. A prepopulated enrichment calendar is used to record items given. In addition, our socialization program allows interaction with a technician at minimum of 3 times a week for 5 min per animal. Ferrets are acclimated to handling and restraining with positive reinforcement as well as with time to play with the technicians. A well-developed relationship between humans and ferrets encourages behaviors that ensure the health and wellbeing of both. The regularly scheduled acclimation program reduces the stress level of the ferrets and their inclination to bite. In turn, this improves staff productivity, conserves resources, and increases data quality.

P152 Withdrawn

P153 Impact of Cage Change Interval on Frequency of Animal Health Report Observations in Laboratory Mice

K Lencioni*, AR Douglas, JF Baer

Office of Laboratory Animal Resources, California Institute of Technology, Pasadena, CA

Routine animal health observation of laboratory mice is generally limited to a daily cage side exam (CSE). As cage manipulation is an unwanted variable, cages are not typically opened or handled daily unless an obvious health issue is apparent. A more thorough health exam is possible during routine cage changing (RCC) when the animal is handled. With the advent of ventilated cages, the cage change interval has been extended from one to 2 or more weeks in many facilities. As an extended cage change interval provides fewer opportunities to thoroughly examine animals, we hypothesized that some animal health issues may remain unidentified between cage changes. The frequency of animal health reports on RCC days compared with daily CSE was evaluated to test this hypothesis. In our facilities, RCC is performed at least once every 7 d. CSE is performed at least once daily on the remaining 6 d of the week. Over a 6-mo interval, 1,290 animal health reports (AHR), including animals found dead, were categorized as identified during RCC or CSE. A significant proportion of AHRs were reported during RCC compared with CSE (43% compared with expected 14.28%, $P < 0.0001$). Observation of specific animal health issues differed significantly between AHRs identified during CSE compared with RCC. Preputial gland enlargement (3 of 23 AHRs, $P < 0.0001$), malocclusion (22 of 87 AHRs, $P < 0.0001$), and masses (16 of 49 AHRs, $P < 0.0001$) were reported less frequently during CSE than RCC. The AHR frequency for other animal health issues did not differ significantly from expected during CSE compared with RCC (for example, ocular issues, including microopthalmia and blepharitis, were identified at close to the expected frequency (58 of 73 AHRs reported at CSE, $P = 0.088$)). In summary, we conclude that extending the cage changing interval may negatively impact animal welfare due to decreased opportunity to closely observe the animals.

P154 "Bunny Cam:" The Use of Wireless Video Baby Monitors as a Tool for Socializing Rabbits

K Marshall*, L Martin, M Holmes

ONPRC, Beaverton, OR

Enriched rabbit exercise pens were established as part of our enrichment program to facilitate socialization of our singly housed rabbits. Rabbits are placed in adjacent pens for 4 h/wk, allowing social interaction through the bars as well as additional exercise opportunities. The excitement of this enriched social housing was tempered with concerns regarding aggression and injuries that could occur when rabbits were left unattended in the room. A wireless video baby monitor capable of both video and sound was purchased to alleviate this concern. The camera has a large range which allows the staff to take turns watching (or listening) to the rabbits while working in other areas of the facility. The cameras are situated in a manner that allows visualization of the entirety of both pens. The researcher, while fully supportive of social housing their rabbits, feels a bit more secure knowing that the animals are monitored while being socially housed.

P155 Compressed Gas Carts: Improving Safety and Increasing Efficiency

K Marshall*, L Martin

ONPRC, Beaverton, OR

CO_2 euthanasia and portable anesthesia machines (PAM) both require the use of compressed gas. In many facilities portability is the key element for using these techniques. Working with compressed gas carries some risk and care must be taken to ensure that the tanks are properly secured to prevent falling. One method of transporting the smaller 'E' sized tanks for the support of PAM units and CO_2 euthanasia stations uses small wheeled stands. These stands have 2 wheels and 2 pegs that allow the stand to remain motionless when fully upright. The stands, being small, have some potential stability concerns. It is possible for the stands to tip when being moved through corridors. It is also possible to trip over the tubing or the stands themselves creating a potential safety hazard. Moving both the stands and the associated equipment can be a challenge for one person to do alone. We have combated these challenges and increased the safety of working with the smaller compressed air tanks by modifying a durable plastic cart. Working with our facilities staff, 2 holes slightly larger than the circumference of an 'E' tank were cut into the top shelf of a plastic 3-shelved cart. A PVC cap was secured onto the second shelf, directly below each hole. With this modification we are now able to place an 'E' sized compressed air tank into each the holes, securing them to the cart. This enables users of either the PAM or the portable CO_2 station to move both the gas and other required equipment in a safer and more efficient manner. Having 2 tanks available on the cart ensures that users always have an ample supply of compressed gas, eliminating stress to the animals when gas runs out during procedures. By implementing a simple, inhouse modification to our existing equipment we were able to increase our efficiency and improve safety while still using the portability compressed gas tanks provide.

P156 Do It Yourself: Conversion of Single Rabbit, Plastic Caging to Paired Caging

K Marshall*, L Martin, M Holmes

ONPRC, Beaverton, OR

The eighth edition of the *Guide for the Care and Use of Laboratory Animals* clarified its language regarding housing social species, specifying that, "Single housing of social species should be the exception…" This is a challenge for many institutions as often new housing needs to be purchased but financial support may not be present. Our unit considered several options to accommodate our rabbits: creating a door-mounted tunnel that connected adjacent cages; creating a door-mounted tunnel that allowed 2 cages to connect face-to-face; or creating an internal tunnel that connected the cages. Some concerns with the concept of this type of tunnel included rabbits not being able to move the tunnel such that they could get trapped between the cages; material needed to be easily sanitizable; prevention of injury to staff or the rabbits and, manufacturing in a manner that would be cost effective. After exploring other materials, a stainless steel square was developed measuring 10 in. It is secured by a carabineer that slides into a single hole at the top of one side. The other side has an enlarged, flattened, face that prevents the tunnel from slipping into the cage. The tunnel has 2 caps that act as doors, one for each cage. They are secured by a tight spring, threaded between the caps. While humans have the dexterity and strength to manipulate the caps free, bunnies are less able to do so. Holes for the tunnels are cut through the plastic cages using a template to standardize placement. This template ensures that the cages can be removed from the rack and shifted to another location without misalignment. This gives us the flexibility to have both compatible and incompatible pairs on one rack, maximizing our space. The tunnels have been placed in a forward position, ensuring visualization of both rabbits in the room and through the use of our "bunny cam," a wireless video baby monitor. Modifying our existing caging has allowed us to meet the requirements of the new *Guide* in a cost-effective and environmentally friendly manner. We hope other facilities facing similar challenges might find our work-around helpful.

P157 Going Green and Staying Clean: Using Accelerated Hydrogen Peroxide as a Disinfectant in Rodent Facilities

KM McDonald*, M Mihalik, L Bihler

Division of Laboratory Animal Resources, Unversity of Pittsburgh, Pittsburgh, PA

Our institution, which has historically used chlorine dioxide as a disinfectant during rodent cage change procedures, recently piloted an alternate disinfectant, accelerated hydrogen peroxide (AHP). The product we evaluated contains a surfactant to improve biofilm penetration and cleaning ability, has a shelf life of 90 d as compared with chlorine dioxide's shelf life of 14 d, has an improved safety profile, and is biodegradable. To evaluate efficacy in our cage change procedures, we tested AHP and chlorine dioxide, in 6 rooms, on 3 occasions, before and after cage change. Adenosine triphosphate (ATP) was measured using a luminometer. Samples were taken from each room's animal transfer station, the exterior of a mouse cage, and from the ventilated caging rack. We observed no statistically significant difference in ATP values following cage change, between AHP and chlorine dioxide; both were effective disinfectants. Subsequently, we evaluated AHP in a fogging application, an off label use of the product. We selected 2 rooms, and using contact plates, tested 5 room surfaces, before and after fogging. Significantly fewer colony forming units were observed after fogging ($t(9)=1.89$, $P < .05$), and our Environmental Health and Safety department supported our use of AHP for fogging in animal biosafety level 1 and 2 areas. Finally, we surveyed staff. Some reported that the surfactant in AHP made the mouse boxes slightly more slippery to handle, and that streaks were sometimes left on the cages if they were not wiped thoroughly. Overall, the majority of staff preferred AHP over chlorine dioxide, stating that their gloves deteriorated less frequently, and that they appreciated the "green" nature of AHP. Due to the positive pilot results, and because our institution will observe cost savings of US$0.55 per gallon of disinfectant, we are transitioning from chlorine dioxide to AHP, in all of our rodent facilities.

P158 Mouse Dystocia in an Academic Research Facility: Is Construction a Driving Influence?

KA Bennett*, BJ North

CCMR, Dartmouth College, Lebanon, NH

Although a relatively common occurrence in many, if not most, research mouse colonies, the reported prevalence of mouse dystocia varies between institutions and facilities. This variation may be due to differences in mouse strains, breeding strategies, husbandry practices, facility environment and other factors which may or may not be easily identifiable. Most academic institutions experience construction of some type. As such, the associated vivariums are often exposed to some level of unanticipated and undesirable vibration and noise. This situation at our institution has raised concerns regarding vivarium-based mouse dystocia prevalence rates. In response we studied the situation over a 24-mo period. The data demonstrated an increased rate of dystocia during some construction. However, similar fluctuations were seen during similar seasonal nonconstruction periods. Further analysis indicated increased dystocia rates positively correlated with seasonal changes regardless of construction activity. Specifically, more than 60% of the dystocias occurred between March and August regardless of construction. Additional positive dystocia correlations were made with older female breeding age (57% of dystocias occurred in female mice > 6 mo of age) and female age at first breeding (bred for the first time when > 4 mo of age; 67% prevalence). Although modern animal housing eliminates many seasonal effects, there are still seasonal fluctuations that appear to influence breeding success in our facility. Our data strongly suggests that increased dystocia rates are associated with seasonal change, female breeding age and the age at first breeding. Our data further suggest that in our situation there was not a direct correlation between construction and dystocia rate.

P159 Increased Cage Height Does Not Impact the Wellbeing of Rabbits

KL Stewart*, D Guilfoyle, W Preisser

Freimann Life Science Center, University of Notre Dame, Notre Dame, IN

In the eighth edition of the *Guide for the Care and Use of Laboratory Animals*, the recommended rabbit cage height was changed from 14 to 16 in. In contrast, the majority of our rabbit cages, purchased in 1985 after the USDA prescribed rabbit cage size requirements, provide 15 in. of interior cage height. A review of the literature failed to identify published data that support an advantage to rabbits having 16 in. of cage height compared with 14 or 15 in. The study described here evaluated the benefit of this minimal change in cage height for rabbits by comparing the effect of the cage height on the health, growth, and overall wellbeing of the rabbits. Groups of 10 New Zealand white rabbits were housed in cages that provided either 15 or 18 in. of interior cage height. The rabbits were observed for 25 1-h periods over 7 wk and various behavioral parameters scored. In addition, rabbits were weighed weekly and general clinical health assessed. After 4 wk, the groups were switched to the alternate housing. No significant differences were observed in body weight gain or behavioral parameters between groups housed in cages of different heights, nor were significant differences observed in groups of rabbits when moved from one cage type to the other. In addition, all rabbits remained clinically healthy through the course of the study. These results demonstrate that minimal changes in interior cage height neither benefit nor harm rabbits.

P160 Simple Techniques to Train Mouse Weanlings to Use Automated Watering Systems

KA Lynch*

Division of Laboratory Animal Medicine, University of California, Los Angeles, Los Angeles, CA

Many transgenic pups are often small and unsure at 21 d. The separation from their home cage can create anxiety and fear that often leaves these animals huddled in the corners of their new home. By day 2 after

weaning, they are typically roaming and exploring the cage looking for food and water. Those weaned onto automatic water systems sometimes do not know how to locate and use the water automatic watering system. This lack of experience often leaves many weanlings helpless and dehydrated within the first 3 d of weaning. In an effort to train these animals to use the automated system, weanlings were divided into 4 groups. Within each group, different techniques were implemented to help assist the animals to gain strength, understanding, and confidence to use the automated system. One technique included guiding the animals to the water by priming the automatic watering system for 3 consecutive days postwean. Another technique involved smearing a mixture of a commercially available water gel pack and powdered feed onto the automatic watering system. We observed some animals gravitating to the automatic watering system after it was primed, but did not return after the residual water had disappeared. Mice that failed to learn to drink from the automatic automatic watering system within the first 3 d accounted for 11% of the total animals investigated and were excluded from the project. These animals were found hunched and weak at which time they were given additional support such as moist food at the bottom of the cage. The vast majority of the animals successfully learned to use the system with the implemented training techniques. Whichever process or training technique is chosen, one must realize that it takes at the very least a 3-d follow-up period to ensure that the animals are maintaining proper hydration.

P161 Which Nest Is Best? A Study to Harmonize the Happiness of Both Mouse and Animal Care Technician

K Rule*, VA Hill, P Lester, J Lofgren, RC Dysko

Unit for Laboratory Animal Medicine, University of Michigan, Ann Arbor, MI

The eighth edition *Guide* and recent publications have highlighted the significant role appropriate nesting material can play in nest quality, breeding performance and mouse feed conversion efficiency. We designed a study to determine which commercial nesting material resulted in the best nest quality but also allowed the animal care technician to effectively evaluate the animal's health. Three different nesting materials were evaluated: crinkled paper strips, cotton squares, and compact pucks of paper strips. Control cages were not provided nesting material but instead given an equivalent weight of extra bedding. Each study group contained 4 cages of two 6- to 7-wk-old albino C57 mice of the same sex; males and females were equally represented. In this study, we scored the nests using a previously described nest scoring system, evaluated the amount of food consumed per cage-change cycle (2 wk) and recorded the ease of health checking using a score-based method. This study was conducted over a 3 cage-change cycle (42 d). The crinkled paper strips group demonstrated significantly higher ($P < 0.05$) nest scores than either the cotton square or compact puck groups and was also the only group that did not show significant nest quality deterioration over the 2-wk cycles. Mice offered the crinkled paper ate significantly less food ($P < 0.05$) over the entire study period than either the control group or the other nesting material groups. The compact puck of paper strips and the control groups were significantly easier ($P < 0.05$) for the technicians to health check than either the cotton square or crinkled paper strip groups. In the end, the crinkled paper strips allowed for the best quality and most durable nests and significantly reduced feed consumption however, it was also among one of the two nesting materials that decreased the visibility of the mice and therefore decreased the ease of health checking. In conclusion, when deciding upon the optimum nesting material to use for mouse enrichment, each institution must weigh the benefits to the animals, as well as the impact on major staff job functions, such as daily health checks.

P162 Novel Mouse Feeders for Measurement of Daily Food Consumption

KR Hess*, MM Kocher, RP Myers, TD Morris

WIL Research, Ashland, OH

Obtaining accurate food consumption measurements for mice is often difficult when using a standard glass feeder and lid, particularly when a project requires daily feedings of small amounts of food. During the conduct of a project requiring 7 g of food to be fed on a daily basis, it became clear that the mice may not be able to reach all of the food. However, removing the lid became problematic as food spillage increased and mice began inhabiting the jars, often moving bedding into the jars. In addition, urine and feces contamination of the food resulted in loss of consumption data. It became time consuming for technicians to separate feces and bedding from the food in an attempt to obtain accurate measurements. To remedy this issue for future projects, a new feeder was designed, using a small glass dish, a custom platform that elevates and holds the glass dish, and a commercially available stainless steel mouse feeder shield that fits over the platform and dish. The feeder shield has an opening on the top large enough for a mouse to reach the bottom of the feeder, but small enough that an adult mouse cannot crawl all of the way into the apparatus. This prevents the mouse from digging out the food and causing spillage. Use of the new feeder resulted in less food spillage and increased accuracy of food consumption measurements. Other benefits included improved sanitary conditions for the animals, less food wasted, and less time spent weighing feeders; thus making this feeder a more efficient and reliable way of feeding small amounts of food to mice.

P163 Robots: The New Era of Water Innovation

L Miller, K Serrano*, E Czarra, K Cooper, LJ Hughes

Animal Health Care Section, NINDS/NIH, Bethesda, MD

Water is an "essential nutrient" in any animal research facility. Our facility is an all water bottle facility, so the need to supply an adequate number of bottles per day is a top priority for the cage wash staff. Also of paramount importance is the need to achieve this daily task most efficiently. In our facility, we are privileged to own a robotic water filling system as well as a manual water filling system. Through our daily observations, we have found that the robotic system is more efficient and produces less ergonomic stress for the operator whereas the manual system is more time consuming, more labor intensive while producing a lower output. We will offer the advantages and disadvantages of both the robotic water filling system and the manual water filling system and show that the advantages of the robotic system far outweigh its disadvantages. We looked at 3 key areas in our comparison; number of water bottles processed, time spent, and ergonomic friendliness. In summary, the robotic water filling system is the more efficient device with a high level of ergonomic friendliness which is of key importance to those working in the cage wash area. It is also a time-saver as it provides flexibility for cage wash staff to perform other tasks while bottles are being processed.

P164 Achieving Global Animal Welfare Compliance

KS Frazier*, MM Perez, TL Condet

Animal Welfare Compliance, Zoetis, Kalamazoo, MI

Achieving animal welfare compliance in a new global corporation can be complex based on multiple country requirements and regulations (for example, Animal Welfare Act, EU Directive, Australia Animal Welfare Act, Brazilian Animal Welfare Regulations). In addition, a global corporation may have its own internal corporate policies, as well as a desire to achieve other accreditations (for example, AAALAC, Canadian Council on Animal Care). Development of a global multiteam compliance program with clearly defined roles and responsibilities helps to achieve animal welfare compliance. Our institution, a new global company, has implemented a multiteam approach. Teams within the program consist of an Animal Welfare Board (creates global policies), IACUC Council (harmonizes animal ethic committees and implements policies), Global Risk Assessment Team (assesses risk for external CROs, vendors and transportation companies), Global Accreditation Readiness Team (prepares sites for accreditation visits) and local animal use committees (foe example, IACUCs, Ethics Committees). Each team is responsible for specific activities that complete the animal welfare compliance program. In summary, the responsibilities of the different teams within our program and how they partner together helps to achieve animal welfare compliance by fostering effective communication and assisting with harmonization.

P165 Behavioral Management in the Digital Era

K Rappaport*

UCSF, San Francisco, CA

The eighth edition of the *Guide for the Care and Use of Laboratory Animals* emphasizes the greater need for detailed records in behavioral management and social housing. Paper records are a storage burden, requiring manpower, time, and resources to retrieve and share information. Despite advances in both technology and animal behavior, the current market for behavioral management database programs is limited by both its affordability and effectiveness. Most institutions are highly impacted by the economy and budget cuts, making the cost of a system overhaul difficult. We designed a versatile interface program using a cross-platform relational database that reduces the tedious act of manual searching, copying, and redacting. Our goal was to offer employees an effective program for documenting behavioral management for a wide variety of species including rodents, sheep, pigs, dogs, cats, rabbits, ferrets, squirrel monkeys, and macaques, while providing a user friendly interface for optimal transitioning and record maintenance. Our findings show that modifications to the specific institute needs increased usage and compliance along with reducing the time it took to fill record requests with or without redaction. The program allows for a variety of behavior data entry including arrival behavior evaluation, monthly baseline evaluation, weekly follow-up notes on special needs animals and daily notations on critical cases. A simple data entry interface reduced the level of computer skill required, allowing users to enter data more frequently and with greater detail. We will review the features of this new database, that would be useful in a range of facilities, making regulatory compliance simpler and more importantly, allowing better tracking of animals' needs. Transferring from paper to electronic documentation is a daunting task, even more so when it is a large institution that needs to transition. All institutions looking to refine their animal care methods will eventually hit this hurdle.

P166 Toe Tattooing: Small Rodent Identification That Lasts

K Diven*, S Richardson

IACUC, Johns Hopkins University, Baltimore, MD

Long-term small rodent identification can be problematic when using various popular techniques. Ear tags or ear notches can be pulled out or torn, fur that is shaved or stained will grow out and implanted transponders are expensive. Identification of most rodent pups can be problematic as they are too immature or small to ear tag, ear notch, or implant a transponder. Historically the solution to identifying pups was to clip toes, but many institutions discouraged or only conditionally allowed this method. While toe tattooing is an effective means for identification it was often not considered as a long-term choice due to the perception that it is not permanent. To address this problem, refining toe tattooing to last for months or longer was investigated. Toe tattooing can be accomplished with simple tools, which include a lancet, tattoo paste, an object to hold the paste and an alcohol wipe; and it requires only a little practice. Young pups are cupped in the hand; older animals are placed in a restrainer. The toe is cleaned with an alcohol wipe, the lancet tip is dipped in the tattoo paste and the desired toe pad is poked with the lancet introducing the paste into the skin and leaving a visible mark. The discomfort is momentary and does not require analgesia. Since longevity of the tattoo is our goal, in the following experiment we evaluated the time course of tattoo fading. Fourteen litters of mouse pups were tattooed at age P5 to P15 and the tattoo's visibility was followed for 5 to 10 mo. Each pup's tattoo was periodically scored 1 through 5 with 1 being good tattoo visibility and 5 being not visible. All pups had visible tattoos through the duration of the scoring or up to 8 mo of age. Some tattoos did slowly fade which would allow ample time to retattoo the toe. We conclude that, toe tattooing is a reliable method for long term identification of small rodents and it is an excellent replacement for toe clipping. Also, there are no age limitations for toe tattooing, it is inexpensive, easy to learn, and the numbering pattern can be customized to match a previously used numbering system.

P167 Canine Tooth Modifications in Mature Male Macaques

K Gerbick*, A Wathen

Covance, Madison, WI

Due, in part, to updated guidelines in the USDA's Animal Care Policy no. 3 stating that tooth reduction that exposes the pulp cavity does not constitute appropriate veterinary care while maintaining balance of concern for human safety, we updated our nonhuman primate dental procedures. Previously, we performed root canals and pulp cap procedures on mature nonhuman primate canine teeth, which are now not ideal per the Animal Care Policy update. These practices were originally put into place due to our need to hand catch the majority of our nonhuman primates inhouse for procedures while providing safety to our staff. At times, these procedures caused tooth root abscesses that resulted in canine tooth extractions. After consulting with a specialist in veterinary dentistry, staff veterinarians, technicians that handle nonhuman primates, and the Animal Care Policy no. 3, we determined the best practice would be to perform only tooth blunting on sharp mature canine teeth. The result of this refined blunting procedure is that the sharp point and surfaces are removed, which decreases opportunity for typical slashing-type injuries during manual restraint. This method is also an advancement over previous methods as the dentin is not left exposed but instead is sealed using a bonding agent and light curing to ensure that there is no postprocedural tooth pain for the animal. In the rare case that the pulp cavity would be inadvertently exposed or in cases of traumatic injury, we also updated our pulp cap procedure. Improvements included with this updated procedure are: use of high speed irrigated dental drill to prevent trauma to the dental pulp, superior filling compound with increased effectiveness and longevity, a bonding agent to achieve a good coronal seal to prevent leakage and postprocedural pain while reducing the potential for secondary bacterial infection. Taken together, our process refinements for nonhuman primate teeth blunting and pulp cap procedures have led to a zero incidence of negative sequelae in our population of animals undergoing the procedures. We are in compliance with the updated Animal Care Policy no. 3 as the pulp cavity is not exposed routinely in our new procedure. Additionally, a high level of safety for our staff is maintained where hand catching nonhuman primates is a necessity.

P168 Strategies to Increase Compatibility among Pair-Housed Rabbits

L Young*, R Lin

Animal Care Department, San Jose State University, San Jose, CA

The eighth edition of the *Guide for the Care and Use of Laboratory Animals* (the *Guide*) states that appropriate social interaction among members of the same species is essential for normal animal development and wellbeing. For research animals to exhibit normal behaviors is an underlying necessity which can affect their health and influence research data in a positive way. Rabbits are social, yet territorial animals, and present many challenges for laboratory animal management to maintain in a communal setting. In efforts to improve compatibility, female rabbits were procured as littermates at 3 mo of age from the vendor and pair-housed in opened pens situated on the animal room floor. Care was taken to allow ample space for animals to roam about the enclosure and interact with strategic locations for hide boxes and areas that the animals could be solitary. Rabbits were observed for 19 wk and attention was paid to the use of space, timing and presentation of each feeding, style and placement of furniture, and availability enrichment items. The space provided for each pair was congruent with all housing standards defined by the Animal Welfare Act. With this pilot approach, husbandry and veterinary care personnel observed a notable increase in normal behaviors with the rabbits, such as standing on hindlimbs, hopping, foraging, and stretching; an increase in body tone, as well as a decrease in fighting and reported morbidity overall compared with individually caged animals. This approach to pair housing of rabbits greatly improved compatibility within a small sample size. However, this method of housing the rabbits may not be considered the most cost-effective, particularly for larger rabbit colonies.

P169 Evaluation of Communal Nesting to Enhance Breeding Efficiency in C57BL/6 Transgenic Mice

LB Eurell*, M Hatch, AH Battles

Animal Care Services, University of Florida, Gainesville, FL

Communal nesting (2 females and 2 litters in a single cage) has been used to increase survivability in neonatal pups by allowing females to share in caring for the litters. In a standard mouse cage of 75 in.^{2}, this form of housing is outside of the recommendations of the *Guide*. To determine if communal nesting would increase the breeding efficiency of transgenic mice and to satisfy the requirements to evaluate the performance standards for nonstandard housing required by the IACUC, communal nesting was compared side-by-side with singly housed breeding females in 3 lines of mice (A, B, and C). Litters were observed until 21 d of age when they were weaned and weighed. The following parameters were recorded: dates of birth, number of pups at birth, number of pups at weaning, and the weight of the litter at weaning. Production index (PI) was calculated for each cage (number of pups weaned per female per week) and the means compared using an unpaired t test. Communal nesting cages showed a lower PI in line A compared with single housing (0.71 compared with 1.20, $P = 0.049$). Lines B and C showed no significant difference (0.88 compared with 1.13, $P = 0.2737$ and 0.74 compared with 0.47, $P = 0.4078$, respectively); however, line C demonstrated a trend towards increased PI in communal cages. Pup weights were significantly less in communally raised animals in line A ($P = 0.0375$) and showed no significant difference in lines B and C (P =0.4086 and 0.5229, respectively). Communal nesting may represent a useful technique to improve breeding efficiency in transgenic mice; however, not all mice benefit from this form of housing. Due to the high variability in the production index of individual females, further studies and additional animals may be required to demonstrate the impact of communal nesting in standard cages on breeding efficiency in transgenic mice.

P170 Environmental Enrichment for Newly Weaned Calves

LV Anglin*, PA Mount, A Wagner, J Robinholt, P Madlinger, A Carter, A Dardenne

SCCR, Cardiovascular Research Foundation, Orangeburg, NY

Environmental enrichment is an essential part of the daily care of animals in research. In caring for 3-mo-old Holstein calves, it was recognized that our standard methods of enrichment could be improved in order to diminish unwanted behaviors. On arrival, calves were unwilling to drink water from buckets and were noted to vocalize throughout the day, a sign of postweaning stress. Initially the veterinary staff hand-fed milk replacer or water from a slip-on nipple bottle; however, this becomes time consuming. A novel environmental enrichment device was created to address these issues. A 4-ringed jolly ball was customized to allow a slip-on nipple bottle to fit inside the ball with the nipple protruding outside the ball. The novel device was hung from a stainless steel chain at an easily accessible height for the calf. This enrichment device takes advantage of a calf's natural instinct to nurse. The device has proved beneficial in administering milk replacer, water, and oral medications when needed. It was also noted that the calves enjoyed drinking water from the bottle; resulting in overall improved water consumption and decreased vocalization. Disassembly and sanitization of the device was an easy process as all of the items are amenable to being processed through a cage rack washer. In creating this device, a cost-effective method of managing postweaning stress and fluid consumption was implemented. This novel enrichment device avoided more costly and space-occupying options such as commercially available automated calf feeders. Both the calves and veterinary staff benefitted due to the positive interaction gained by using this device.

P171 Performance of 3 Types of Rodent Bedding in Static and Ventilated Caging Systems

L Steiner*, E Vernasco-Price, CJ Maute, RC Dysko

ULAM, University of Michigan, Ann Arbor, MI

A previous study at our institution evaluated 3 types of bedding in a polyuric mouse colony, assessing cage changing frequency as well as labor and bedding costs. A more detailed study with the same 3 bedding types was performed, comparing cages of polyuric mice to mice with normal urination rates, this time also determining ammonia levels, as well as comparing static cages, cages receiving house ventilation, and cages receiving blower pack ventilation. Five cages, ranging in housing density from 1 to 5 mice, were assigned to one of 18 groups, and were evaluated over a 2-wk period. Bedding 1 (B1) was combination corncob bedding, bedding 2 (B2) consisted of compressed cellulose, and bedding 3 (B3) was pelletized corncob that swells and breaks apart when it becomes saturated. For each static cage, 300 mL of B1 and B2 and 250 mL of B3 were added to the cages. For each ventilated cage, 225 mL of each type of bedding was used. Cage dimensions were 7.5 × 11.75 × 5 in. A colorimetric ammonia sensor was suspended in one cage in each of the 18 groups, and sensors were moved between cages and groups upon cage change. Each cage was observed daily for bedding saturation levels and the color of the ammonia sensor was noted. The cage was changed if at least one-quarter of the bedding appeared wet or if the ammonia sensor registered more than 25 ppm. There was no difference in the number of cage changes for normal urinating mice for all 3 bedding and housing types. For polyuric mice, there were 58 additional cage changes for B1 during the 2 wk, 67 additional changes for B2, and 52 additional changes for B3. Regarding ammonia levels, B1 had 6 readings over 25 ppm; B2 had 18 readings over 25 ppm; B3 had 2 readings over 25 ppm. Based on our data for all cage ventilation and bedding types, the house ventilation system performed better than static housing or blower packs. Overall, B3 performed best regarding ammonia levels and cage changing frequency. However, the dust created by B3 posed an increased risk of blocking the ventilation port (located at the cage bottom) and interfering with the water source (either sipper tube or watering valve), causing cages to become saturated; this development should be taken into consideration when selecting a bedding type.

P172 Use of Fluorescent Powder to Determine the Protective Value of Disposable Gowns During Mouse Cage Changes

T Martin, LV Kendall*

Laboratory Animal Resources, Colorado State University, Fort Collins, CO

Personal protective equipment (PPE) requirements vary between facilities, but gowns or lab coats are frequently required for work in rodent rooms. Fluorescent powder was used to judge the ability of disposable gowns to protect animals and animal users from pathogens. Fluorescent powder was mixed in mouse bedding and mice were allowed to sit in cages for at least 5 min. Mice were then changed into a clean cage where they were again allowed to rest. This was repeated twice for 5 clean cages each time. Mice, cage changers' hands, cages, and water bottles were checked for fluorescence between each cage change. Fluorescence was detected on gloves after 5 clean cage changes, but cages no longer fluoresced by the third cage change and mouse fluorescence was drastically reduced by the fifth change. In a second experiment, fluorescent powder was mixed in the bedding of 5 cages and a caretaker was asked to change all cages while wearing a disposable gown and gloves. This was repeated with 4 different caretakers. The room, change station, rack, and caretakers' PPE and clothing were checked for fluorescence. Fluorescence was detected on gloves, sleeves, and chests of all caretakers. One caretaker had minimal fluorescence on scrub top after gown removal. These studies show that caretakers can act as fomites for long periods of time when changing cages. They also show that disposable gowns can prevent particles such as allergens or pathogens from reaching caretakers' skin and scrubs.

P173 Occupational Therapy Assessment of Laboratory Animal Resources

S Lavey[2], A Severino[2], K Atler[2], M Roll[2], M Fletcher[2], A Rogers[2], LV Kendall*[1]

[1]Laboratory Animal Resources, [2]Occupational Therapy, Colorado State University, Fort Collins, CO

Work place injuries from repetitive motion and routine procedures are common place in laboratory animal facilities. To mitigate against the procedures and routines that result in work-related injury and lost productivity, the Assistive Technology Resource Center (ATRC) within the Occupational Therapy Department was sought to provide an evaluation of work place practices within Laboratory Animal Resources. The preassessment evaluation consisted of baseline video analysis of common procedures such as cage changes and cage wash. Animal care staff completed a preassessment Canadian occupational performance measure (COPM), an employee-supervisor needs assessment, NIOSH ergonomic awareness worksheet, and NIOSH manual material handling worksheet to gauge employee awareness of occupational risks and were interviewed by an occupational therapist. Following preassessments employees received training in risk factor awareness through presentations, video discussions, demonstrations of simulated tasks, and repetitive motion demonstrations and discussions. Postassessment using the COPM was completed following the training which demonstrated employees had an increased awareness of job risks and how to mitigate them. The most problematic tasks identified included scraping bedding, removing lids from water bottles, transferring mice to and from cages, and loading cages onto carts. Recommendations for mitigation were provided by the ATRC with employee involvement included alternating tasks, use of specialized tools and equipment, and frequent breaks with stretches. This assessment provided an opportunity for employees to become cognizant of their work place environment and how to mitigate the risk as well as identified training opportunities for the animal care staff.

P174 Design of a 2-Lift, Spring-Loaded Ergonomic Cart for Laboratory Animal Operations

K McGregor[2], N Savig[2], J Abdulrazzaq[2], T Donahue[2], LV Kendall[*1]

[1]Laboratory Animal Resources, [2]Mechanical Engineering, Colorado State University, Fort Collins, CO

The design of animal facilities often requires equipment be transported throughout the facility. This is often performed on carts with a fixed platform. When moving equipment on and off the carts, personnel will frequently have to bend outside of the optimal work zone, waist height to elbow height, which can result in repetitive bending and consequently increases the risk for work-related injuries. Following an ergonomic evaluation of Laboratory Animal Resources, there was a need for a cart that maintained a platform height within the optimal work zone to facilitate the movement of cages throughout the facility for cage changing. To this end, the College of Engineering was sought to have students in the senior design course design a concept cart that would maintain the optimal work zone while loading and unloading the cart. The 2-lift, spring-loaded cart was successfully created from this collaboration. Constructed of sanitizable and chemical-resistant materials, the cart has a locking mechanism to lock the platform in the down position to facilitate moving stacks of cages onto the cart. There are 2 separate platforms such that clean cages could be unloaded, while dirty cages are loaded. The design allows animal care personnel to work in an optimal work zone and minimizes repetitive bending reducing work related injuries.

P175 Positive Reinforcement Training Effects on Hemogram and Chemistry Parameters in Chimpanzees

SD Breaux, JJ Breaux, M Fontenot[*]

Behavioral Sciences, University of Louisiana at Lafayette New Iberia Research Center, New Iberia, LA

Positive reinforcement training (PRT) is used at various biomedical research facilities and zoos in order to facilitate animal husbandry activities as well as veterinary procedures. Previous research suggests that the use of PRT helps alleviate stress during potentially distressing events, such as anesthesia for physical examination, as indicated by changes in certain hematology values including white blood cell (WBC) counts, neutrophils (NEU), hematocrit (HCT), and glucose (GLU) levels. However, decreases in these values resulted from trained subjects who cooperated with sedation procedures rather than involuntarily receiving a darted intramuscular injection. Here we consider more fully the effects of PRT by comparing hematology levels of untrained subjects compared with trained subjects both prior to and following PRT for voluntary injection. The subjects were chimpanzees that had no prior experience with PRT (injection only (Inj); n = 11, 11F; 12.67 to 26.83 y; mean = 18.71) or had experience with PRT for urine collection (urine and injection (U&I); n = 35, 14F, 21M; 3.5 to 15.17 y; mea$n$ = 8.33) and were then trained to present a thigh for voluntary IM injection, while others remained naïve to PRT (untrained (Unt); n = 27, 10F, 17M; 3.33 to 19.08 y; mean = 14.22). Data analyzed were obtained from two annual physical examinations for each subject. For trained subjects, one data point was collected prior to onset of voluntary injection training while the other was collected after the subject had reached training criteria. Analysis of covariance, covarying age and preinjection training baseline blood values, indicated that among trained subjects WBC levels were significantly lower (U&I<Unt, $P < 0.05$), as were NEU levels (U&I<Unt, $P < 0.05$; Inj<Unt, $P < 0.05$), while GLU (Inj>Unt, $P < 0.05$; U&I<Inj, $P < 0.05$) and HCT (U&I>Unt, $P < 0.05$) were significantly higher. No effect of cooperation with injection was observed. These results suggest that the use of PRT for voluntary injection lowers the stress responses of chimpanzees to sedation procedures, whether or not the subjects voluntarily present for injection.

P176 Benefits of an Enhanced Enrichment Program for a Canine Research Colony

M Sposato[*]

VivoPath, Worcester, MA

The overall benefits of an enrichment and exercise program for research canines has been well documented and is a required element, by the United States Department of Agriculture (USDA), of any institution's Animal Care and Use Program. However, some research animals which are sent out for adoption after being released from a research program can show signs of various social and adjustment issues after being bred and raised in a laboratory setting. These differences from "normal" behavior indicate an underlying level of stress that may have an impact on multiple baseline physiologic parameters in these animals. By adding additional enrichment aspects to our Canine Exercise and Enrichment Program we have seen valuable changes in the overall behavior and physiologic stability of our canines, which are valuable for the preclinical modeling work done with these animals. We also anticipate that this will translate into the animal's ability to adjust faster and more effectively to the various new aspects of life outside the laboratory environment. In order to enhance the overall care, wellbeing, and stability of our canine colony we house them socially in pairs and in large runs that are 3 times the standard size required per animal. Our housing also includes added elements such as raised canvas beds, soothing environmental background noise, and chew toys. We have also added training aspects to our program which includes using puzzle toys for mental stimulation, "hunting game" and digging for olfactory stimulation, stair training, grass mat for bathroom training, as well as basic leash and behavioral commands. These elements are all in addition to the standard requirements of exercise and human interaction with staff. The addition of these aspects into the daily program and care of our canine colony has resulted in a group of healthy well-behaved animals that can more reliably represent normal physiology, and therefore, more precisely model the disease state.

P177 A Training Program Using Video and Plastinated Murine Model

ML Streber[*1], MA Ramirez[2], L Ladron de Guevara[3]

[1]Exp Research and Lab Animal Unit, INCMNSZ, Mexico; [2]Lab Animal Unit, CENASA, SENASICA, SAGARPA, Tecamac, Mexico; [3]Support Services for Diagnostics, SENASICA, SAGARPA, Mexico

Our training program is based on the Mexican Norm NOM-062-ZOO-1999, and one of the species used as a requirement are small rodents. Most of the time, students have different animal experience and different levels of manual skills. We refined this program using classroom presentations, a training video in Spanish, and a plastinated murine model. We used 30 adult nude mice, no longer needed as reproducers. They were euthanized by CO_2, internal organs were removed, and cotton balls were placed inside. The bodies, including head and

skin or the skeleton, were fixed by immersion in 10% buffered formalin. After several weeks of fixation, bodies were washed with tap water, allowed to dry with paper towels, and the cotton balls were removed. Immediately, the bodies were placed in a glass jar with acetone. After 2 changes, the bodies were immersed in glycerin. The process was finalized when the skin was soft and translucid. Plastinated nude mice were used to learn handling as well as different routes of administration (ocular, oral, intraperitoneal, intramuscular, and subcutaneous). This is particularly important because the nu/nu strain is very expensive and sensitive. Also, the murine models could be used to learn blood and tissue sampling. Each plastinated mouse has a soft consistence, no bad odor, and could be reused as many times as students need. The course is 25-h long and each wet lab lasts 4 h and is composed of 15 students. Survey questions were asked before and after training. The learning curve using the video, plastinated models, and anesthetized animals was reduced. The students felt comfortable to have the opportunity to learn a lot in a short period of time and to have the experience before using live unanesthetized animals. This format has the potential to improve animal welfare and promote high quality research, avoiding accidents like bites and harming the animals.

P178 Effect of Light Intensity as Determined by Cage Rack Position on Tumor Growth in a Mouse Model of Melanoma

M Suckow*[1,2], S Wilhite[1], WR Wolter[1], GE Duffield[2]

[1]Freimann Life Science Center, [2]Department of Biologic Sciences, University of Notre Dame, Notre Dame, IN

Within the typical laboratory animal housing facility, animals may be exposed to varying intensities of light as a result of cage type, cage position, light source, and other factors. While evidence exists that light contamination during the dark phase of the light cycle can impact the growth of tumors in laboratory rodents, no studies evaluating the differential effect of light intensity during the light phase on tumor growth have been published. The effect of cage face light intensity as determined by cage rack position was evaluated in the C57Bl6 mouse model of melanoma using transplantable B16F10 cells. Animals were housed in individually ventilated cages placed at the top, middle, or bottom of the rack in a diagonal pattern so that the top cage was closest to the ceiling light source, 10 mice per light exposure group. Cage face light intensity was measured with a digital illuminance meter to be 3.1 lx (bottom), 169.0 lx (middle), and 320.8 (top) lx. Following a 2-wk acclimation period at the assigned cage position, animals were administered 1.3 × 106 B16F10 melanoma cells subcutaneously. Tumor diameters of mice were measured with a digital caliper at days 12, 15, and 18. ANOVA analysis of tumor diameters showed the middle light intensity group to have significantly smaller ($P < 0.001$) tumors on every day they were measured compared with high and low light groups. Likewise, when mice were euthanized 18 d after tumor cell administration, mean tumor weight was significantly ($P < 0.001$) less in middle light intensity mice (1.21 ± 0.79 g) compared with high (6.32 ± 2.74 g) and low (5.98 ± 3.25 g) light intensity mice. In summary, the light intensity to which animals are exposed may vary markedly with cage location and can significantly influence experimental tumor growth, thus supporting the idea that light is an important experimental variable.

P179 Challenges with Meeting Recertification and Training Requirements in ABSL3 Facilities

MM Loll*, T Wilder-Kofie, R Moore, J Magrath

CMB, NIAID/SoBran, Bethesda, MD

In the ABSL3 environment, research and animal care pose unique challenges, including the implementation of the regulations that must be followed. Depending on the ABSL3 agent, several regulatory entities may be involved, such as NIH, CDC, USDA, and OSHA. Recently, our facility underwent renovations and upon completion approximately 18 mo later, staffing had changed significantly due to new job opportunities and promotions. In addition to retraining experienced staff, we had to ensure proper training for new staff and laboratorians. We also had to provide trained individuals to perform the required work. All of the required training had to occur with the knowledge that due to the testing requirements for annual recertification of ABSL3 areas, we may be required to shut down again in a year. This would entail retraining all individuals with access to the ABSL3 vivarium.

P180 Comprehensive Method for Monitoring Individual Rodent Health in a Research Setting

M Reed*, CB Volpe

Division of Lab Animal Medicine, UCLA, Los Angeles, CA

Current Animal Welfare Act policy allows for animals in genera *Mus* or *Rattus* to be excluded from health monitoring. Despite the exclusion, the efficacy of individual rodent health monitoring has been gaining traction in recent years. A web-based and mobile device system has been developed which allows Animal Health Care Staff and Veterinarians to record individual rodent health cases, present standardized response options for consistency in communication, and enhance dialog between principal investigator and animal care staff. The system was originally deployed in 2001 and went through major revisions, based on user input, to increase efficiency of use. The data is comprised of over 72,000 individual rodent health cases that were classified by diagnostic assessment and includes all related subjective, objective, and treatment plan logs from case inception to resolution. Collected health data can be easily analyzed by commonly used spreadsheet software to help ensure compliance with the Institution's Animal Welfare guidelines, contribute to the facility health monitoring goals as well as aid in the collection of retrospective research data.

P181 Ammonia Level Testing in Various Cage Types to Standardize Changing Frequency

M Rainey*, R Leal, E Ferrel, J Toloza, R Soares, E Jaime, L Dalisay

Animal Biology, Bristol-Myers Squibb, Redwood City, CA

Our vivarium is currently using 3 different cage types and 2 different bedding combinations to maintain our mouse colonies. The *Guide* recommends cage change frequency for solid-bottom mouse cages of at least once per week, which may be extended in certain circumstances. Our goal was to see if we can standardize our cage change intervals and to determine an appropriate cage change frequency based on ammonia monitoring within the cage. In order to determine this, we used a patented, passive, direct-read autogenic ammonia exposimeter. The monitor measures an exposure range of 4 to 300 ppm/h via a color change. Using blue painter's tape, these monitors were taped inside the lids of the individually ventilated cages. For all 3 cage types, we placed monitors in cages 7 d after a cage change and left them in for 1, 4, and 24 h. We tested cages containing 1 through 5 adult mice, and cages containing breeding pairs with varying number and age of pups. The same cages were similarly tested at the 14-d time point after cage change, using all parameters above except breeding cages. Apart from the breeding cages, we saw no color change on the monitors after 1 wk. At 2 wk, there was only a color change at the 4 to 20 ppm/h range. Dividing this ppm/h exposure by the 24 h the monitor was in the cage, the mice exposure to ammonia is less than 1 ppm/h. Published references state that the human level of 35 ppm/h can be used as the permissible exposure limit for mice, and we are well below that. Based on the readings we obtained, we present findings to support a 14-d cage change interval, except for breeding cages with litters and those visually deemed in need of change at their daily check. We have presented these findings to our IACUC, and we will summarize the outcome of that presentation in the final poster.

P182 Noisy Construction Does Not Have to Generate Noisy Data

M Posadas*, T Neubauer, CA Buckmaster

Center For Comparative Medicine, Baylor College of Medicine, Houston, TX

Our institution opened the Jan and Dan Duncan Neurologic Research Institute (NRI) in November of 2010. This facility houses a team of world renowned researchers working with mice and rats to develop

treatments for childhood neurologic disorders. The second and third floors were designed for vivarium use; however, the second floor was not begun until February 2012. In an effort to minimize the impact of noise and vibration from construction on ongoing behavioral and physiologic studies, the Center for Comparative Medicine tested noise and vibrations levels, using different shock absorbent floor mats and drill bits in a mock animal holding room. High frequency microphones and vibration sensors were used to evaluate and select the best materials and construction began. Vibration sensors were placed at the base of every ventilated rack and microphones were suspended from each ceiling. A computer system sent automatic email alarms to team members if noise or vibration exceeded set thresholds, prompting the manager to check the room and then contact the construction manager to stop or modify work when necessary. We were relieved to discover that our animals set off more alarms at night, during their active period, than the construction itself. The vibration pads mitigated noise and vibration and allowed construction and research to happen simultaneously.

P183 Sustainable Nonhuman Primate Enrichment on a Budget

MJ Williams-Fritze*[1,2], J Cratic[2], M Thomas[2], B Leverett[2], L Moredock[2]

[1]Veterinary Services, CBSET, Lexington, MA; [2]Lab Animal Services, Georgia Regents University, Augusta, GA

The USDA Animal Welfare Act Regulations and the *Guide for the Care and Use of Laboratory Animals* mandate that environmental enrichment be provided to research animals to promote psychologic wellbeing. These documents encourage housing of animals in enclosures that allow adequate freedom of movement and postural adjustments, cognitive activity, species-typical behavior, and social interaction. Ideally, environmental enrichment should be well-conceived and provide animals with choices and a degree of control over their environment, which enhances their ability to cope with environmental stressors. Provision of species-directed enrichment can decrease expression of stereotypic behaviors and improve animal welfare. Typically, animals are socially housed and offered environmental enrichment devices to promote cognitive activity and species-typical behavior. In an effort to enhance our environmental enrichment program to better meet the welfare needs of our NHPs, we implemented a natural, sustainable, and cost-effective source of enrichment: bamboo. Bamboo is high in dietary fiber and can easily be placed inside many foraging devices. Recycled plastic containers and inexpensive planting materials were used to create a renewable enrichment resource at our institution. Here we describe the development and implementation of a container bamboo farm for nonhuman primates on a US$100 budget.

P184 Distribution of Health Conditions in Mice (*Mus musculus*) in a 9-Year Period as Reported by Veterinary Check Requests

N Koewler*, VH Monterroso

Department of Comparative Medicine, OHSU, Portland, OR

At our institution animal health concerns are brought to the attention of the veterinary staff by submission of a veterinary check form. Basic veterinary check data has been collected in a database format since 2003. At the present time the database is being used to obtain information on reported animal health conditions. The objectives of this study were 1) to determine the overall distribution of veterinary checks for all species housed within the facility, 2) to determine the percentage of treatable conditions from those requiring immediate euthanasia, and 3) to determine the major health problems within the mice population (the largest animal population within the vivarium). The study included entries (n = 23,269) from August 2003 to September 2012 with each entry considered an independent event. Incomplete entries were not considered in the analysis. Using the number of entries per species and the total number of entries, the percentage of entries corresponding to each species was calculated. The same procedure was used on mice data to calculate the percentage of cases needing euthanasia, treatable cases, and the analysis of health conditions. Results showed that 85% of entries corresponded to mice (n = 19,814). Also, 63% of the 19,814 mice entries corresponded to mice requiring euthanasia or found dead, 20% to skin conditions, and 17% to miscellaneous conditions. In conclusion, the overall veterinary health checks within the facility are done on mice (85%; 19,814 of 23,269). From mice entries (19,814), skin related conditions comprised the largest proportion of mice health problems requiring veterinary attention (20%; 3,935 of 19,814). Limitations of databases such as incomplete information, lack of flexibility, difficulties in correlation to the true populations, human errors, and other concerns were evident during the study. Finally, these results will be used to streamline veterinary concerns and treatment protocols that can be implemented immediately upon examination.

P185 Streamlining Tumor Size Assessment with a Novel Device

N Shomer*, K Demers

Merck Research Labs-Boston, Boston, MA

Tumor size is a common humane endpoint for mouse oncology studies. Our IACUC recently updated our criteria for euthanasia of mice with iatrogenic tumors to include a primary tumor size of 2 cm in diameter. At our facility, the procedure for evaluating large tumors involved a member of the veterinary staff using calipers as a measuring device. Not only do the calipers require cleaning after each use in order to ensure biosecurity, but they also pose an ergonomic risk to the user, as well as an animal safety risk due to their sharp edges and the delicate nature of the tumors. We successfully streamlined this procedure while simultaneously reducing the risks involved. Many alternatives to the caliper method were considered; we found that the most practical device was a stainless steel washer with an internal diameter of 2.0 cm, an external diameter of 2.3 cm, and a width of 0.08 cm. These washers were custom made and purchased from a vendor. This new device has allowed us to save time and minimize the number of steps involved by giving the husbandry staff the ability to evaluate tumors before veterinary staff is contacted. Though custom made, each ring's cost is equivalent to that of a disposable hypodermic needle, making them inexpensive enough to be single-use and disposable. However, they can also be autoclaved, reused, and recycled. The tumor sizing rings have proven to be an economic, safe, and efficient alternative to the caliper method of tumor assessment.

P186 Decreasing Stereotypic Saluting Behavior in a Singly Housed Male Rhesus Macaque

DR Lopez, NR Turner*, KB Gilbraith, R Brownlee, C Doane

University Animal Care, University of Arizona, Tucson, AZ

One of seven adult male rhesus macaques, singly housed due to behavioral restrictions, was observed to exhibit mild stereotypic saluting behavior. To effectively decrease saluting stereotypy in this male it was hypothesized that doubling cage space, L-tryptophan supplementation, and doubling environmental enrichment by adding video in addition to an established sensory enrichment program. Ethograms were collected in January and May of 2013, before and after these changes were implemented. For consistent ethogram behavior monitoring, saluting was defined as pressing the periocular region with his thumb. Stereotypy was recorded as 1 or 0 for occurrence within a 5-min interval, positive occurrences were measured for duration in seconds, and the total instances within each 5-min interval were recorded. In January 2013, the male was observed on average saluting 1.8 times per 5-min interval, the average duration of the salute was 6.9 s, or 151 s/h (n = 1). In May 2013, following changes to cage space, L-tryptophan supplementation, and increased enrichment, instances had decreased on average to 0.3 times per 5-min interval, with an average duration of 6.7 s, or 32 s/h (n = 1). The joint effects of L-tryptophan, enhanced enrichment, and increased cage space effectively decreased his saluting behavior by 79%.

P187 Chlorine Dioxide Gas Renders *Syphacia* Ova Nonviable with a 4-Hour Exposure Period

J Czarra, JK Adams, C Carter, WA Hill, PN Coan*

Office of Laboratory Animal Care, University of Tennessee, Knoxville, TN

We evaluated the efficacy of chlorine dioxide gas for environmental

decontamination of *Syphacia* spp. ova. *Syphacia* spp. ova were collected by perianal cellophane tape impression of pinworm infected mice. Slides with attached ova were exposed to chlorine dioxide gas for 1, 2, 3, or 4 h. Slides containing ova not exposed to chlorine dioxide gas were designated as controls. Chlorine dioxide gas was delivered to the chamber at a concentration of 360 ppm/h. Both control and treated slides were incubated in a hatching medium. The 4-h chlorine dioxide gas exposure rendered 100% of *Syphacia* spp. ova nonviable. Conversely, only 17% of ova on the 4-h negative control slide were rendered nonviable. Other exposure times resulted in variable effectiveness. These data suggest that chlorine dioxide gas at a minimal exposure time of 4 h is effective for surface decontamination of *Syphacia* spp. ova.

P188 Relative Humidity in the Microenvironment of Mice

P Noel*, JL Taylor, D Bird, B Mickelsen

Univeristy of Utah, Salt Lake City, UT

When providing adequate environmental conditions for mice, the macroenvironment is used to establish acceptable conditions in relation to relative humidity. In regions where humidity is extremely high or low, mechanical devices are implemented in an attempt to achieve the accepted range of 30% to 70%. Often, these devices are unable to maintain humidities within the accepted range. Measuring humidity in the microenvironment, the rodent cage, one finds a significant difference in humidity relative to the macroenvironment. Over a span of 5 mo, with the relative humidity below 20%, measurements were taken in both the macro- and microenvironments of a traditionally housed static mouse colony. Our results suggest microenvironment conditions to be significantly different from the macroenvironment and thus a much better representation of animal exposure in terms of humidity and temperature. For instance, on 14 December 2012, the room temperature was measuring 69.3 °F and the temperature inside the cages was 76.05 to 77.03 °F. The humidity was 17% in the room and the cages were ranging from 40% to 51% humidity inside the cage. According to the *Guide*, we were out of range for humidity in the room, but looking at the inside of the cages, we were well within perimeters. This trend was repeated for every week over the 5 mo. Thus, asking the question, what does the macroenvironment really tell us in comparison to the microenvironment and the wellbeing of the animal?

P189 Determining the Cost of a Good Laboratory Practice per Diem

R Sanchez*

Comparative Medicine, University of Maryland School of Medicine, Baltimore, MD

Due to our historical adherence to federal regulations, policies, and guidelines (for example, USDA, PHS, AAALAC) and the numerous professional experts we employ in laboratory animal medicine, our department is in a strong position to implement and oversee preclinical studies intended for submission to the FDA, requiring good laboratory practice (GLP) compliance, under 21 Code of Federal Regulations (CFR) part 58. In order to recover the costs of maintaining a GLP compliant program, we were tasked with determining a GLP per diem. In order to begin undertaking the cost analysis procedure to determine a nonhuman primate (NHP) GLP per diem, a multiyear study, with an animal census greater than 100 animals was used as the reference point. Direct labor was calculated by interviewing all husbandry, veterinary, and GLP administrative staff. Discreet tasks were then documented based on all applicable SOPs, as well as the time spent on each discreet task during a 40-h work week for work above and beyond standard animal husbandry and technical support (that is, GLP effort only). The cost for all employee effort over a given year was calculated and based on the annual animal census for that study, an NHP GLP per diem cost was determined. It should be noted that this did not include costs of maintaining a quality assurance unit or the cost of certifying/calibrating equipment. The cost analysis model we have implemented establishes an NHP GLP per diem that can be functional in recovering our costs, yet must be reviewed frequently within a given year to initiate adjustments so that revenue is still captured for required work that is not reduced by a decline in animal census.

P190 Public Outreach: Do Not Forget Your Kids

RE Gump*[1,2], L Fitts[2]

[1]BioMed, SoBran, Silver Spring, MD; [2]Veterinary Services Program, WRAIR/NMRC, Silver Spring, MD

Bring your child to work day (BYCTWD) is an annual event at our institution that allows school age children inside our facilities to learn about, and in some cases experience, typical days in the multifaceted work of biomedical research. BYCTWD is a perfect opportunity to combine research education with fun. It is sometimes difficult to explain to family, especially kids, what we do or why we do it. The messages sent by animal rights group can often confuse issues, and even have family question what we do. We held a BYCTWD with varied activities that were both fun and educational for children 7 to 14 y old. Items related to animals and further educational opportunities were given out with a goodie bag. Along with these items, kids left with smiles and knowledge from participating in hands on learning stations. They also saw and experienced researchers as fun and caring individuals, not as animal abusers. It is possible to partner fun and learning with family or children to promote further positive discussion and/or study about using animals in research. An outreach event like this could easily apply to company open houses, picnics, or other public forums.

P191 Plant Naïve Mice: A Husbandry Conundrum

R Kavanaugh*, K Wilcox, R Davis

URAR-LS, The University of Georgia, Athens, GA

Our institutional's Monoclonal Antibody Facility (MAF) develops antibodies for research use at customer requests. Currently, the MAF is participating in a project endeavoring to create a library of monoclonal antibodies against plant cell wall carbohydrates. We have had considerable success working with materials from legumes and other dicot plants, but have been encountering difficulties generating a strong immune response against corn and grasses, which are monocot plants. In fact, with many of the monocot antigens, we were unable to detect ANY antibody response at all. As corn is the chief component in both standard mouse diet and bedding, we theorize that the mice are tolerant to the monocot plant cell wall components and therefore will not recognize these antigens as "foreign." In order to generate a detectable immune response, the mice might need to be on a diet and bedding not containing plant material. We will describe the husbandry issues associated with housing and maintaining a plant free environment for mice. Our staff had to research all materials that the mice are in contact with and come up with substitutions that are plant free. It was surprising to realize how many products are associated with plant material, from bedding to gloves the staff wear during cage changes. We will describe our substitutions and procedures to assure that the mice were not exposed to any plant material. The results of the antibody production are pending as our first set of production mice are currently being exposed; however, we are confident that our procedures will assure a positive outcome.

P192 Lean Processes: The Use of Different Colored Huts to Reduce Unnecessary Cage Changes

R Franceschi*, D Fong

National Jewish Health, Denver, CO

Supplying clean cages for room-to-room transfers and rodent colony management, such as breeding and weaning, is a daily activity performed by animal care and cage wash personnel. It was noted that animal care staff would change cages that had been just set-up by investigators the previous day. Emphasizing lean processes, we developed a system of colored huts to eliminate these unnecessary cage changes. First, we identified the number of cages used by investigators and animal technicians daily for colony maintenance practices and animal transfers. We then use blue huts to differentiate the colony maintenance and transfer cages from the red huts used for regularly scheduled cage changing. This color visual allows the animal technicians and research-

ers to easily identify the colony maintenance and transfer cages so that the correct cages are used. During cage changing, the blue hut cages are identified and assessed for changing. If a static cage will not exceed an 8-d change, it is not changed until the next weekly scheduled change. Over 1 mo, in 6 animal rooms with approximately 2,000 cages, changes were reduced by approximately 11%, with larger reductions in large breeding rooms. Based on our cage change rate per hour, over 55 h of labor per year would be saved in just these 6 rooms. Additional savings include associated costs, such as bedding, autoclaving, and cage wash labor. Furthermore, this color visualization system allows management and animal technicians to ensure weaning guidelines are followed, closely observe the health of newly weaned cages, and confirm the accuracy of room censuses, transfers, and breeding reports. The contrasting colored huts have been a positive step in our lean vision to improve efficiency and accountability within our vivarium.

P193 The Biggest Bang for Your Buck: Environmental Enhancements for Group-Housed Macaques

SL Nelsen*, D Diaz, R Escobar, J Bernal

SNBL USA SRC, Alice, TX

Environmental enhancement programs for nonhuman primates are mandated to promote the best psychologic wellbeing for primates in research settings. The goals of the program should include preventing and responding to abnormal behaviors, while also improving the quality of life of these animals by encouraging natural behaviors and enriching environments that are biologically meaningful to the species that are part of the program. This is best accomplished by developing a program that not only addresses the 5 categories of enrichment (that is, social, occupational, structural, sensory, and nutritional) but also realistically, effectively, and completely addresses the needs of all primates onsite. At our institution, the majority of resident macaques are group housed in indoor/outdoor enclosures, which does address their social needs. However, there are still 4 other categories of enrichment we try to provide. As our animal population can be large (average 2,500), this can be a somewhat daunting task; therefore, we have developed enrichment options that provide the "biggest bang for the buck." For sensory enrichment, color and design have been added to the enclosures by painting the walls to visually stimulate the animals. For structural and sensory enrichment, colored barriers have been added to provide visually appealing complexity to the enclosures that also helps maintain stability within the groups. For occupational and nutritional enrichment, artificial turf boards have been strategically placed at various heights throughout the enclosures to turn supplemental feeding into more complex foraging bouts. For structural and nutritional enrichment, large eye-hooks have been added to the walls of the enclosures to create a climbing wall effect as well as places to hang nutritional enrichment. Last but not least, pools have been added for occupational enrichment, as well as a means to keep the animals cool during hot weather. Through these enrichment options, we are able to fully address the psychologic needs of our animals on a large scale, while remaining cost-effective.

P194 Behavioral and Functional Assessments in Mice

N Libal, A Casper, SJ Murphy*

Anesthesiology and Perioperative Medicine, Oregon Health and Science University, Portland, OR

Behavioral and functional assessments are commonly used to characterize rodent phenotypes and to determine experimental outcomes in different disease models. However, obtaining valid results requires a detailed knowledge of how to perform the tests and correctly analyze and interpret the findings. When selecting behavioral tests, it is important to use the most appropriate behavioral tests for the research model and rodent species being studied. We use examples from our departmental stroke research program to illustrate some of the specific challenges of behavioral and functional testing in rodents. We explain how several commonly used sensorimotor and cognitive tests in rodents are conducted as well as address the advantages, potential pitfalls, and limitations of the different tests. Since many behavioral tests are scored subjectively, we also address the impact of the evaluator on behavioral and functional outcomes. Tests described include those to assess overall general health (neuroscore), sensorimotor impairment (rotarod, open field), forelimb asymmetry (paw preference or cylinder test), and cognitive function (novel object recognition, passive avoidance learning, Morris water maze). A better understanding of behavioral tests and their applications can benefit the research community when designing experiments involving functional outcomes in rodents.

P195 Reproductive Performance of Pair-Housed Zebrafish (*Danio rerio*) as a Function of Tank Size

S Frederickson*, DA Castranova

Charles River/NIH/NICHD-Contractor, Bethesda, MD

Within the field of zebrafish (*Danio rerio*) research, it is common to house one or 2 fish within a tank when they are genetically distinct or being tested for individual differences (for example, fin clips). Fish housed in this manner are often of great importance to researchers because they are carrying mutations and/or transgenes of interest. Many research projects require consistent spawning and large clutches from individually or pair-housed fish. Anecdotal evidence suggests that there may be differences in reproductive performance of pair-housed fish depending on the size and shape of the holding tank. There are 3 different tank sizes used for this purpose on the Bethesda campus of our institution (0.8-, 1.0-, or 1.8-L tanks). There are multiple variables between the tank sizes that may influence fecundity, behavior, and the overall health of zebrafish including different ratios of volume to surface area, different replacement rates, and different flow patterns. To test the effect of tank size on reproductive performance we will place pairs of fish in 10 tanks of each size (0.8, 1.0, and 1.8 L) and give them a 1-mo acclimation period before setting them up in individual pair-wise crosses every 2 wk for 4 spawning events. At 1-d postfertilization, the embryos will be counted and checked for viability. Metrics for the study will include spawning success, clutch size, and percent of viable embryos. This data will be important to researchers, husbandry staff, facility managers, and tank designers because it will allow them to make decisions about the most appropriate way to house pairs of zebrafish based on scientific data.

P196 Do Rat Moms Need a Break?

S Cloutier*, RC Newberry

Center for the Study of Animal Wellbeing, Washington State University, Pullman, WA

In their natural environment, nursing rats spend time away from the nest and the pups. In a standard laboratory cage the expression of this behavior is limited by the size and layout of the cage. We hypothesized that rat dams provided access to an area not easily accessible to the pups, would spend more time away from their pups as they progress towards weaning. We assessed the response of 12 Long–Evans rat dams (*Rattus norvegicus*; HsdBlu:LE; found free of internal/external parasites and disease via quarterly serology and parasitology, and yearly necropsy evaluations) and their litters housed in either a 2-level cage (with a shelf not easily accessible to the pups) or a standard cage (equipped with a small loft hanging from one side of the cage, easily accessible to the pups,). The location (shelf/loft compared with floor) and activity (active compared with inactive) of dams and pups was recorded every 5 min for 5 h throughout the light period on days 5, 10, 15, and 20 (± 1 to 2 d) after birth. Cage type did not affect the overall activity level of dams and pups. However, dams housed in the 2-level cage spent a greater proportion of their time on the shelf when the pups were 20 d old than when they were 5 ($P = 0.01$) and 10 d of age ($P = 0.005$) whereas dams housed in a standard cage spent the same proportion of time on the loft at all ages. These findings have implications for the dam's physical and psychologic welfare because providing an area not easily accessible to the pups gives her behavioral freedom to modulate her proximity to her pups.

P197 Mouse Mummies: Mouse Bandaging Techniques for Epicutaneous Inoculations

VM Bradford*

NIAID, NIH, Capitol Heights, MD

Mouse bandaging is a challenging technique that involves wrapping the mouse in adhesive bandaging so as to maintain skin exposure to an inoculant that is on a patch of sterile gauze. The process includes placing the mouse under anesthesia, shaving the area that will be manipulated, exposing the area to the inoculant by placing inoculant soaked gauze on the area, and finally bandaging the skin area to maintain the inoculant soaked gauze on the desired skin location. This procedure presents the challenge of keeping the bandage on the mouse for a desired amount of time while the animal is awake and fully functional in the cage. This poster will present the technique of inoculating and wrapping the mouse so that the bandage remains on the mouse while avoiding potential health problems such as breathing restriction and penile prolapse caused by bandaging that is wrapped too tight.

P198 Assessment of Intracage Complexity: A Form of Environmental Enrichment in Reducing Aggressive Behavior in Grouped Housed Male Mice

VM Bohrer*[2,1], LJ DeTolla[1,2], SP Atamas[2,1]

[1]Veterinary Resources, University of Maryland School of Medicine, Baltimore, MD; [2]Research, VA Medical Center, Baltimore, MD

The aggressive nature of some mouse strains may confound experimental outcomes due to uncontrolled fighting between male cage mates. As a result, the use of females is increasing for strains known for their aggression. Individual housing is recommended for highly aggressive strains of mice such as SJL. To assess the validity of a complex enriched environment, SJL mice from 2 vendors were acquired at 5 wk of age, to demonstrate the following: 1) cage environment of corncob, with only the addition of a cotton square, or structural shelter alone will not maintain stable social groups, and reduce aggression; 2) in contrast, a complex environment consisting of paper bedding, additional nesting material, and the use of a structural shelter, will reduce social tension and aggression. Twenty SJL male mice from each vendor were housed into the following treatment cage groups, consisting of 4 mice per cage and observed for 8 wk: 1) corncob bedding, 2) corncob bedding with a cotton square, 3) corncob bedding with a shelter, 4) corncob bedding with a cotton square and shelter, and 5) complex environment. Weekly observations included number of wounded animals, and the number of wounds per animal. At the end of the 8-wk observation period, vendor 1's SJL mouse groups maintained social stability for the duration of the experiment, observing only minor territorial agnostic fighting which quickly resolved in all groups. Vendor 2's SJL mouse groups in contrast exhibited severe aggression in the treatment groups, 1 to 4, in which animals had to be removed from the study due to the amount of severe aggression. However, at the end of the 8-wk study treatment group 5 remained as a group of 4, and reestablished a new dominance hierarchy, which supported our hypothesis: a complex environment can reduce aggression and maintain social stability and group dynamics. However, genetic variation between vendors of the same strain seems to be apparent in the study, and may explain the 2 different results.

P199 Retrospective Analysis of the Seasonal Breeding Performance of C57BL/6JNarl and BALB/cByJNarl Mice (2010-2012)

W Lin*, T Lin, S Sung, C' Liang

National Laboratory Animal Center, National Applied Research Laboratories, Tainan, Taiwan

Fluctuations in seasonal breeding performance have long been noted in the production of laboratory mice. These fluctuations affect colony size and supply and the marketing balance of laboratory mice. We retrospectively analyzed the production records of breeding colonies of SPF C57BL/6JNarl (B6) and BALB/cByJNarl (B/c) mice in our barrier facility (Temp = 22 ± 1°C, RH = 55% ± 5%, 12:12-h light:dark cycle, ACH = 10 to 15) from 2010 to 2012. The animals were housed in open cages and bred in a polygynous mating system (1 male × 2 female) with random mated breeding beginning at 8 wk of age and retired at 30 wk of age. Reproductive data including conception rate (percent pairs fertile), mean number of pups born per litter, pup survival rate, (survival to weaning) and production index were collected monthly. A mean of 1,180 B6 and 2,181 B/c mice breeder pairs were analyzed from 2010 to 2012. Statistical analysis was performed by one-way repeated measures ANOVA. The one-way ANOVA showed that there were no significant differences among months or seasons (spring: February to April, summer: May to July, autumn: August to October, winter: November to January) in any breeding performance variable, including the conception rate, mean number of pups born per litter, pup survival rate and production index ($P > 0.05$), for either mouse strain. Therefore, the reproductive performance of the C57BL/6JNarl and BALB/cByJNarl mice in our barrier facility was not influenced by month or season. In the future, we will further observe 2 inbred strains from 2013 to 2015 and analyze the effects of fluctuations in order to achieve a supply and marketing balance.

P200 Breeding at Older Ages Decreases the Infertility of CAnN.Cg-Foxn1nu/CrlNarl Mice

W Lin*, S Lee, C' Liang

National Laboratory Animal Center, National Applied Research Laboratories, Tainan, Taiwan

Our CAnN.Cg-Foxn1nu/CrlNarl (nude) mouse colony with a permanent homozygous-heterozygous mating system had a high infertility rate. In this study, we examined whether pairing the breeders at 8 wk of age instead of 3 wk would improve the fertility of the nude mice. The animals were kept in individually ventilated cages (IVC) units with standard macroenvironmental conditions (23 ± 1°C, 55% ± 5% RH, 12:12-h light:dark cycle, and 50 ACH). Monogamous mating pairs (nu/nu male × nu/+ female) were established either at 3 wk ($n = 313$) or 8 wk of age ($n = 151$). Infertility was recorded until the mice reached 16 wk of age. The reproductive-performance data from fertile colonies were analyzed at 52 wk of age, at which point the mice were retired. The results showed that a delayed pairing of the nude mice at 8 wk of age led to a significant decrease in infertility compared with pairing at 3 wk of age (19.2% compared with 28.4%, $P < 0.05$). Furthermore, the fertile colonies of the mice paired at 8 wk of age had a longer period between litters (60.2 d compared with 53.64 d, $P < 0.05$) and a smaller PI value (0.56 compared with 0.62, $P < 0.05$) than those paired at 3 wk of age. However, no significant difference was observed in the mean number of reproductive days per dam, the number of litters, the total number of pups born per dam, the total number of pups weaned per dam, or the number of pups born per litter between the 2 groups of fertile colonies. The decrease in infertility that resulted from breeding at an older age could be due to Lee–Boot and Whitten effects.

P201 Hand-Held Jugular Vein Phlebotomy Technique for Unanesthetized Hamsters

A Godbold*

Pharmacology and Discovery Services, WIL Research, Ashland, OH

A method was needed for repeat blood collections from hamsters for potential future client studies. Known bleeding methods for hamsters either require the use of anesthesia, are used as a terminal procedure, or are very difficult to perfect. After researching the different methods of blood collections from hamsters, it was found that the jugular vein collection is mainly used with anesthesia due to the difficulty of the restraint hold. Because the repeat use of anesthetics may have an effect on an animal's metabolism as well as being traumatic for the animal, we have learned how to perform a jugular vein blood collection on an unanesthetized hamster. A method development study was conducted to see if the unanesthetized jugular vein collection can be successfully used for repeat blood collections of approximately 0.25 mL. Fifteen hamsters were bled at 2 to 3 timepoints in a 24-h period on 2 separate occasions with at least 2 d in between. A clinical veterinarian examined all animals after the first day of collections and all 15 hamsters had no significant clinical observations. In conclusion, a properly trained technician can successfully perform an unanesthetized jugular vein blood collection from hamsters from 3 timepoints in a 24-h period, obtaining 0.25 mL.

P202 Can We Combine Efficacy and Safety?

A Rideout*, KA Adams

Astrazeneca, Waltham, MA

Reducing the number of animals used in studies is an important part of 3Rs. Measurement of toxicity endpoints in efficacy studies can reduce animal use and facilitate earlier decision making in drug development. This study evaluated heart rate, mean arterial pressure, core body temperature, and the central nervous system in an infection efficacy study. Two groups of animals were surgically prepared at another insitution. Group 1 animals were implanted with telemetry probes. Ten days later, all animals in groups 1 and 2 received dual canulas in the femoral and jugular veins for drug delivery and blood collection respectively then shipped to our institution in Waltham. Three days after arrival and 4 d before study, all animals were rendered neutropenic with cyclophosphomide, 120 mg/kg IP. On the day of study, all animals were infected with *E. coli* Arc4 using the dual thigh method. Two hours postinfection, 4 animals in group 2 were euthanized to establish a baseline level of infection. All other animals received either vehicle or AZxxxx, 1 h intravenously. Twenty-four hours after infection, all animals were euthanized. Dual thighs were collected to measure the levels of infection present and the effect of drug on bacteria growth. There were no differences in the measured levels of infection between groups 1 (telemetry) and 2 (no telemetry). In addition, group 1 animals provided more information regarding the safety of the administered drug. To this end there were no changes observed in MAP, HR, CBT or CNS activity. This study shows that measurement of safety endpoints during efficacy offers an opportunity to evaluate safety parameters and provides an opportunity to make decisions earlier and, more importantly, allows for designing better molecules.

P203 Renal Artery Nerve Distribution and Density in the Porcine Model: Biologic Implications for the Development of Radiofrequency Ablation Therapies

A Dardenne*[1], S Rousselle[2], A Peppas[1], C Gongora[1], J Wicks[2], W Grundy[2], PA Mount[1], LV Anglin[1], A Carter[1], M Morales[1], A Tellez[1], G Kaluza[1], J Granada[1]

[1]Skirball Center for Cardiovascular Research at the Cardiovascular Research Foundation, Orangeburg, NY; [2]Alizee Pathology, Thurmont, MD

Catheter-based renal artery denervation has been demonstrated to be effective in decreasing blood pressure among patients with refractory hypertension. The anatomic distribution of renal artery nerves may influence the safety and efficacy profile of this procedure. We aimed to describe the anatomic distribution and density of renal artery nerves in the porcine model. A total 10 porcine renal arteries were analyzed. A tissue block containing the renal arteries and perirenal tissue was extracted. Each artery was divided into 3 individual segments (proximal, mid, and distal) and stained for histologic analysis. Histologic sections were assessed for total number, size (0 to 50 µm, 50 to 100µm, 100 to 200 µm, 200 to 500 µm) and depth (1 to 6 mm from renal artery) of the nerves according to the location. Immunohistochemistry, targeting tyrosine hydroxylase (efferent nerve fibers [ENF]) and calcitonin gene related peptide (CGRP, afferent nerve fibers [ANF]) was performed in a midsection. Nerve counts were greatest proximally (57.2% of the total nerves) and decreased gradually in distal sections (23.5% in midsections and 19.3% in distal sections). The distribution in nerve size was similar across all 3 sections (approximately 40% of the nerves = 50 to 100 µm, approximately 30% of the nerves = 0 to 50 µm, approximately 20% of the nerves = 100 to 200 µm and approximately 10% of the nerves = 200 to 500 µm). In the proximal segments approximately 33% of the nerves were located within 2 mm from the arterial wall. In the mid (28%) and distal segments (42%) were located within 1 mm from the arterial wall. Sympathetic efferent fibers overwhelmingly outnumbered sensory afferent fibers. The afferent and efferent fibers were frequently intermixed within the nerve bundle. In the porcine model, renal artery nerves are more frequently seen in the proximal segment of the artery. Nerve size distribution appears to be homogeneous throughout the artery length. Nerve bundles progress closer to the arterial wall in the distal segments of the artery. This anatomic distribution may have implications for the future development of renal denervation therapies.

P204 Does Social Housing Alter Experimental Outcomes in a Mouse Stroke Model?

A Casper*, N Libal, Y Chen, E Schnell, NJ Alkayed, SJ Murphy

Anesthesiology and Perioperative Medicine, Oregon Health and Science University, Portland, OR

The *Guide* recommends that social animals like mice be housed in compatible groups. However, many stroke researchers will singly house mice due to concerns that social housing may alter study outcomes. In a small pilot study, we evaluated the effects of singly compared with pair housing in a mouse stroke model. Male C57BL/6J mice were singly or pair-housed for 7 d before and 7 d after undergoing 1 h of right middle cerebral artery occlusion (MCAO; n = 6 per group) or sham MCAO (n = 4 per group). Mortality rate and body weight were recorded and neurobehavioral tests were done during the recovery period to analyze general health (neuroscore), forelimb use (paw preference), spontaneous locomotor activity (open field), and cognitive function (novel object recognition). At the end of the study, brain infarct volume was determined by 2,3,5-triphenyltetrazolium chloride staining while neurogenesis was assessed in one brain from each group by immunohistochemistry. No deaths occurred in sham MCAO mice but mortality was 3 times higher in singly (50%) compared with pair- (17%) housed MCAO mice. Sham MCAO mice had no changes in body weight. Singly housed MCAO mice had reduced body weight only on day 3 after surgery while pair-housed MCAO mice had sustained decreases in body weight on days 3, 6, and 7 after surgery. General health (neuroscore) was worse (higher) in MCAO compared with sham MCAO mice regardless of housing group. No impaired forelimb use was observed in any of the groups on days 3 or 7 after surgery. However, singly housed MCAO mice were too impaired for forelimb use evaluation on day 7 after surgery. No differences in spontaneous locomotor activity were seen among groups at baseline or 6 d after surgery. There was no effect of surgery or housing on cognitive function, and no differences in infarct volume in singly compared with pair-housed MCAO mice. Subventricular zone neurogenesis was more evident in MCAO compared with sham MCAO mice but comparable in singly compared with pair-housed mice in MCAO and sham MCAO groups. Our initial findings suggest that social housing may reduce mortality rate and delay recovery of baseline body weight after MCAO. However, social housing may not affect poststroke general health, forelimb use, spontaneous locomotor activity, cognitive function, infarct volume, and neurogenesis.

P205 Effect of Progesterone Contraception on Transmission of Viruses to Contact Sentinel Mice

AE Kwiatkowski*[1], J Carlson Scholz[1], PC Smith[1], K Lencioni[2], SR Compton[1]

[1]Section of Comparative Medicine, Yale University, New Haven, CT; [2]Office of Laboratory Animal Resources, California Institute of Technology, Pasadena, CA

Rodent quarantine health surveillance is essential for preventing the introduction of infectious agents into laboratory animal facilities. Contact sentinels are commonly used for this purpose due to more efficient transmission of infectious disease and, therefore, greater detection sensitivity when compared with soiled-bedding–transfer sentinels. Female contact sentinels are preferred since males have a higher likelihood of fighting and may impregnate the imported females. The duration of exposure to contact sentinels is generally longer than the mouse gestation period; therefore, standard quarantine practices produce large numbers of litters, which are euthanized. In the interest of reducing animal numbers, administration of progesterone to sentinel mice has previously been shown to successfully prevent pregnancy, but studies are needed to evaluate whether administration of progesterone interferes with transmission and detection of common murine pathogens. In this study, male and female CD1 mice (Crl:CD1(ICR)) of peak breeding age were used to investigate the effects of progesterone on transmission and detection of murine norovirus (MNV) and murine parvovirus (MPV). In each group, 32 males were inoculated with MNV or MPV and 3 d later were housed with 2 females, one of which received a contraceptive dose of medroxyprogesterone acetate, and the other saline. After

25 d of direct contact, seroconversion rate, pregnancy rate, and litter size were assessed in the contact sentinels. We found no difference in seroconversion to MNV or MPV between females given progesterone and those given saline. Contrary to what we expected, we also found that progesterone did not reliably prevent pregnancy in contact sentinels; however, it did delay the onset of pregnancy. In conclusion, administration of long-acting progesterone does not appear to have an effect on the humoral immune response to MNV or MPV in CD1 mice. Further studies evaluating progesterone dosing regimens are being considered.

P206 Development of an Indirect ELISA for the Detection of *Burkholderia pseudomallei*

A Leon*[1], G Khara[1], K Brittingham[2]

[1]BioReliance Corporation, Rockville, MD; [2]Batelle, Columbus, OH

Burkholderia pseudomallei causes melioidosis, a severe life-threatening bacterial infectious disease that affects both humans and animals. *B. pseudomallei* is a gram-negative, aerobic bacterium found in the soil throughout Southeast Asia and northern Australia. Screening nonhuman primates that are imported from Southeast Asia for *B. pseudomallei* exposure has become critical to ensuring naïve animals are used in *B. pseudomallei* research. This will minimize the potential impact of preexisting antibodies on the survival of *B. pseudomallei* challenged animals while minimizing results' variability and reducing the number of animals required for developing melioidosis therapeutics. Currently, there are no commercial assays for *B. pseudomallei* exposure detection. We developed an indirect ELISA assay to detect the presence of serum IgG antibodies reactive to *B. pseudomallei* for screening of nonhuman primates. The assay was developed using detergent lysed *B. pseudomallei* bacteria. Before use in the assay, detergents were removed from the lysate and inactivation of bacteria was verified before removal from a BSL3 laboratory. Uninoculated growth media was used as control antigen. Positive samples were obtained from nonhuman primates that were experimentally infected with *B. pseudomallei*. Naïve samples were obtained from animals raised in China and the US. The appropriate concentration of the components was determined by cross-titration of antigen and control antigen using a positive control antibody produced in vaccinated goats. Comparisons between the results obtained from positive and naïve samples indicated that the optimal coating antigen concentration was 100 ng/mL. Proof of principle was demonstrated by coating plates with 100 ng/mL of *B. pseudomallei* antigen and control antigen, and testing 19 positive and 40 naïve samples. The assay distinguished between positive and negative samples at a preliminary cut-off value of OD405 = 0.6. The assay was qualified by testing 19 positive and 40 naïve US born nonhuman primate samples in triplicate. The assay is 100% sensitive and 93% specific and has a confirmed cut-off value of OD405 = 0.6. To date, this assay has been used to screen rhesus macaques imported from vendors in Southeast China resulting in rejection of 19.3% of the animals from being used in *B. pseudomallei* research.

P207 Developing an Assay to Measure B Cell Production in Horses with Common Variable Immunodeficiency

B Reddyjarugu*[1], MB Felippe[2], RL Tallmadge[2]

[1]Center for Animal Resources and Education, [2]Department of Clinical Sciences, College of Veterinary Medicine, Cornell University, Ithaca, NY

Common variable immunodeficiency (CVID) is characterized by late-onset marked B cell lymphopenia with hypo- or agammaglobulinemia, and susceptibility to bacterial infections. Recent work in our laboratory suggests that the loss of B lymphocytes is due to a block in their production in the bone marrow (BM). One measure of B cell production is the presence of recombination of immunoglobulin (Ig) gene variable and constant regions, which creates signal and coding joints. We hypothesized that CVID-affected horses have relatively low number of signal joints in peripheral blood lymphocytes (PBL) compared with healthy horses. To assess the B cell production from the BM, we developed PCR assays to detect Igλ light chain signal joints. Conventional PCR was performed on PBL genomic DNA of CVID-affected (n = 7) and healthy horses (n = 7); amplicons were visualized by gel electrophoresis and sequenced. Quantitative PCR was performed for a reference gene (FLT3LG) in CVID-affected (n = 22) and healthy (n = 23) horses and Igλ signal joint (n = 5 each). Robust signal joint amplicons were observed in healthy horses, and sequencing of these products confirmed primer specificity. Unexpectedly, conventional PCR on PBL from a subset of CVID-affected horses indicated the presence of Igλ signal joints. FLT3LG reference gene quantification has shown equivalent genome numbers between healthy and CVID-affected horses. However, preliminary quantitative PCR assays using Igλ signal joint primers on PBL (n = 5 each) showed a statistically significant difference (Wilcoxon–Mann–Whitney rank sum test, $P < 0.01$) between healthy and CVID-affected horses. This preliminary work suggests that CVID-affected horses have low number of Ig signal joints compared with healthy horses, and is currently being substantiated with additional samples. We believe that quantifying Ig signal joints in CVID-affected horses will provide an insight into the stage of B cell differentiation achieved in the BM, and potentially a unique diagnostic tool.

P208 Nonsurgical Uterine Transfer Technique for Mouse Embryos after Cryopreservation, In Vitro Fertilization, ES-Cell Injection, and Sperm during Artificial Insemination

B Stone*

ParaTechs, Lexington, KY

Many procedures used for assisted reproductive techniques can be damaging to the integrity of embryos. Therefore, we chose to determine if embryos could be successfully transferred with nonsurgical methods after cryopreservation, in vitro fertilization (IVF), or embryonic stem (ES) cell injection as an alternative to traditional surgical embryo transfer. The nonsurgical embryo transfer or NSET technique requires the use of a device that has a tapered polytetrafluoroethylene catheter capable of precise liquid delivery. Once embryos are loaded into the device, the catheter passes through the vagina and traverses the cervix to deposit embryos into the uterine horn of a recipient mouse. The NSET technique was used to transfer cryopreserved B6C3F2 1-cell embryos cultured to blastocysts to female CD-1 recipients. These transfers resulted in a birth rate of 39.4% (n = 16). IVF of fresh oocytes from B6C3F1 female mice and cryopreserved sperm from B6C3F1 males was performed. Fertilized embryos were cultured to blastocyst stage and transferred to CD1 recipients. Transfer of IVF-derived blastocysts resulted in a birth rate of 43.3% (n = 20). C57BL/6 blastocysts were injected with JM8A3.N1 ES cells and transferred to CByB6F1/J recipients. The birth rate from NSET transfers of ES cell-injected blastocysts was 30.5% (n = 25). These results indicate that the nonsurgical transfer of blastocysts is effective for transfer after embryo manipulations and cell culture. We also hypothesized that the nonsurgical transfer technique could be used for delivery of sperm for artificial insemination. Fresh sperm from B6C3F1 males was transferred to the uterine horn of hormone-induced CD1 females, resulting in live births and a 36% pregnancy rate (n = 19). The nonsurgical transfer technique for embryos or sperm is fast, does not require anesthesia or analgesia, and postprocedure recovery is not necessary. These results provide proof that the technique successfully produces live pups after transfer of 1) embryos after cryopreservation, IVF, or ES-cell injection and 2) sperm during artificial insemination.

P209 A Novel Approach to Automated Glucose Monitoring in Awake and Freely Moving Animals

B Gien*[1], C Rohde Johnson[1], S Peters[1], J Squires[1], P Petillo[2], D Aillon[2]

[1]Discovery Center, BASi, West Lafayette, IN; [2]Pinnacle Technology, Lawrence, KS

With the staggering increase in the incidence of diabetes in the United States, it is becoming ever more important to have useful tools for exploring the disease and associated therapies. One key endpoint in diabetic research is the monitoring of glucose levels, and current techniques cause significant stress to the animals, potentially causing unreliable results. The ability to record glucose concentration data from undisturbed animals would be a valuable tool in diabetes research. In pursuit of this goal, we adapted an implantable neural glucose sensor to the flow path of an automated blood sampling system. This combination allowed the measurement of glucose concentrations without causing

disruption or stress to the animal and has the potential to provide accurate and reliable data for diabetes research. In order to adapt the implantable neural glucose sensor to the sampling system, a sensor interface was created within the blood flow path. This interface made it possible to draw samples of blood through the sensor and provide instant glucose concentration levels. Minor adjustments were made to the glucose sensor and interface over the course of several studies. With this configuration, it was possible to either continue collecting the blood into a refrigerated vial for pharmacokinetic drug concentration analysis or to return the whole blood sample to the animal with no net blood loss. To verify the method, 20 male Sprague–Dawley rats received an oral administration of glucose. Blood samples were simultaneously compared with data from commercially available external glucose monitors and demonstrated the flow-through glucose sensor to be reliable and accurate. This method has the potential to both reduce animal numbers and to improve the quality of data when conducting diabetes research. We also believe that the combined approach used in this study has additional applications in therapeutic areas other than diabetes.

P210 Murine Norovirus-4 Infection Does Not Alter *Helicobacter*-Induced Inflammatory Bowel Disease in *Il10*$^{-/-}$ Mice

C Hsu*, J Paik, PM Treuting, A Seamons, SM Meeker, TL Brabb, L Maggio-Price

Department of Comparative Medicine, University of Washington, Seattle, WA

Murine norovirus (MNV) infection in laboratory mice is widely prevalent. MNV has been previously shown to alter a mouse model of bacterially induced inflammatory bowel disease (IBD), the *Helicobacter bilis*-triggered IBD model in Mdr1a$^{-/-}$ mice (FVB.129P2-Abcb1atm1Bor). To further characterize the effects of MNV infection on IBD, we used the *H. bilis*-triggered IBD model in Il10$^{-/-}$ mice (B6.129P2-Il10tm1Cgn/J). Two independent experiments were performed using female, 6- to 8-wk-old, Il10$^{-/-}$ mice orally gavaged with *H. bilis* to synchronously initiate and accelerate colitis. The amount and extent of inflammation was scored histologically to compare mice singly infected with *H. bilis* compared with mice coinfected with *H. bilis* and MNV-4; uninfected mice and those infected with MNV-4 alone served as controls. In experiment one (n = 20 per group), when comparing mice singly infected with *H. bilis* compared with mice coinfected with *H. bilis* and MNV-4, no differences were found in IBD scores (14.8 ± 4.6 compared with 24.9 ± 4.7, mean ± SEM, P = 0.07), incidence of IBD (IBD score > 0) (45% compared with 75%, P = 0.11), or frequency of severe IBD (IBD score > 30) (25% compared with 45%, P = 0.32). Likewise, in experiment two (n = 35/group), no differences were found in IBD scores (21.7 ± 3.5 compared with 23.6 ± 3.5, P = 0.88), incidence of IBD (77% compared with 69%, P = 0.59), or frequency of severe IBD (40% compared with 46%, P = 0.81). MNV-4 infection alone resulted in no appreciable IBD and was comparable to uninfected mice. In vitro MNV-4 infection of Il10$^{-/-}$ bone marrow-derived macrophages cocultured with *Helicobacter bilis* antigens increased the gene expression of IL1β, IL6, and TNFα. Overall, our findings suggest that, unlike in Mdr1a$^{-/-}$ mice, the presence MNV-4 in Il10$^{-/-}$ mice does not alter IBD induced by *H. bilis* infection.

P211 Efficacy and Safety of 4 Anesthetic Agents as Compared with MS222 in the Adult Zebrafish

C Collymore*[1,2], S Rasmussen[1], C Lieggi[2], R Tolwani[1]

[1]Comparative Bioscience Center, The Rockefeller University, New York, NY; [2]Center of Comparative Medicine and Pathology, Weill Cornell Medical College and Memorial Sloan-Kettering Cancer Center, New York, NY

The efficacy and safety of different anesthetic agents, other than MS222, are not well characterized in adult zebrafish. We compared 4 less commonly used anesthetic agents: isoflurane (0.5 mL/L), lidocaine hydrochloride (300, 325, 350, 400, 500 mg/L), metomidate hydrochloride (2, 4, 6, 8, 10 mg/L), and gradual cooling to MS222 (150 mg/L). Ten fish per anesthetic dose were evaluated and compared with an unanesthetized control group. The efficacy and safety of each anesthetic agent was evaluated by observing loss of equilibrium, slowing of opercular movement, response to tail fin pinch, recovery time, and anesthesia-associated mortality rates. Fifteen minutes after recovery from anesthesia, fish were behaviorally screened using a standard novel tank test to assess whether exposure to individual anesthetic agents influenced subsequent anxiety-like behavior. Behavioral parameters measured included latency to enter the upper half of the tank, number of transitions to the upper half of the tank, number of erratic movements, and number of freezing bouts. Efficacy and safety amongst agents varied; however, postanesthesia behavior was not significantly altered. The highest doses of metomidate hydrochloride and lidocaine hydrochloride induced a rapid loss of equilibrium. Opercular movement decreased more rapidly with all agents except for isoflurane and the lowest dose of lidocaine hydrochloride when compared with MS222. All fish anesthetized with metomidate hydrochloride responded to a tail fin pinch. Recovery time varied for each agent. Mortality was greater than 30% with isoflurane and the highest doses of lidocaine hydrochloride. Metomidate hydrochloride may be used for sedation or long nonpainful procedures. Gradual cooling may be acceptable for certain procedures where the anesthetic agent may interfere with research results. Lidocaine hydrochloride and isoflurane are not suitable as sole anesthetic agents for adult zebrafish. MS222 remains the best agent for reliable and consistent surgical anesthesia.

P212 Environmental Enrichment Decreases Anxiety-Like Behaviors in Singly Housed Zebrafish

C Collymore*[1,2], S Rasmussen[1], R Tolwani[1]

[1]Comparative Bioscience Center, The Rockefeller University, New York, NY; [2]Center of Comparative Medicine and Pathology, Weill Cornell Medical College and Memorial Sloan-Kettering Cancer Center, New York, NY

The objective of environmental enrichment is to provide laboratory-housed species with opportunities to express natural behaviors and self-regulate their home environment, thereby minimizing stress. We hypothesized that environmental enrichment would reduce anxiety-like behavior in adult mixed-sex zebrafish and that the behavioral effect would be greater when fish were singly housed. For a period of 3 wk adult zebrafish were housed singly or in groups of 5 in either enriched 2.5-L tanks containing one 5-in. plastic plant, or in unenriched 2.5-L tanks. Individual fish were selected from each of the four housing environments (singly housed enriched, singly housed unenriched, group-housed enriched, group-housed unenriched) for behavioral testing in novel tank (n = 10), light/dark (n = 10), and place preference (n = 20) tests. The place preference test provided fish with the option of spending time in proximity to either 3 mixed-sex conspecifics or the 5-in. plastic plant used as environmental enrichment in the 2.5-L tanks. The addition of environmental enrichment did not affect the behavior of group-housed fish when tested using novel tank and light/dark behavioral tests. All group-housed fish demonstrated a strong preference for conspecifics. Singly housed fish maintained in unenriched 2.5-L tanks demonstrated a stronger preference for conspecifics and had a higher number of freezing bouts in the place preference test than singly housed fish that had been maintained in enriched 2.5-L tanks. Singly housed fish maintained in enriched 2.5-L tanks spent equal time investigating environmental enrichment compared with associating with conspecifics. In summary, when housing with conspecifics is not a viable option for scientific reasons, environmental enrichment in the form of plastic plants may enhance the welfare of singly housed adult zebrafish. Group-housed adult zebrafish did not demonstrate a preference for environmental enrichment when provided with the opportunity to associate with conspecifics. This suggests that housing with conspecifics without additional enrichment may be optimal for the welfare of adult zebrafish.

P213 *Helicobacter bilis* Induces Gastrointestinal Lymphoid Hyperplasia of B220+ B Cells in Gnotobiotic Swiss Webster Mice

C Wang*[1], Y Feng[1], Z Shen[1], Z Ge[1], S Muthupalani[1], BH Horwitz[2], MT Whary[1], JG Fox[1]

[1]Division of Comparative Medicine, Massachusetts Institute of Technology, Cambridge, MA; [2]Department of Pathology, Brigham and Women's Hospital, Boston, MA

Helicobacter bilis is an enterohepatic *Helicobacter* species (EHS) reported to cause hepatitis in inbred and outbred mice and typhlocolitis in immunodeficient mice. *H. bilis* also has undefined immunomodulatory properties in wildtype mice including the induction of immune responses to commensal microbiota and attenuation of *H. pylori* gastritis in C57BL/6 mice. We have previously shown that *H. bilis* may be unique among EHS in causing significant hyperplasia of gastrointestinal associated lymphoid tissue (GALT) in gnotobiotic Swiss Webster mice in the absence of overt inflammatory lesions. To investigate this further, twelve 8-wk-old male germfree and SPF Swiss Webster mice were gavaged with the Missouri strain of *H. bilis* or were controls. Compared with *H. bilis* infected SPF mice, mice monoassociated with *H. bilis* for 4 weeks developed hyperplastic isolated lymphoid follicles (ILFs) in the cecum and colon. Although CD4+ T cells, including T follicular helper cells, were increased, the major cell subsets in ILFs as shown by immunohistochemistry and flow cytometry were B220+ B cells. While high levels of *H. bilis* colonization were evident in the GI lumen using quantitative PCR, *H. bilis* was not observable within ILFs by fluorescence in situ hybridization (FISH). Consistent with a predominant B cell response to *H. bilis*, mRNA levels of B cell chemotactic CXCL13 were significantly higher in *H. bilis* infected mice. IFNγ and TNFα mRNA levels were also elevated despite none to only mild typhlocolitis. These observations are consistent with the hypothesis that while inducing minimal clinical disease following infection of SPF mice, *H. bilis* infection of germfree mice causes marked B lymphoid hyperplasia in the colon that is associated with high levels of CXCL13 but not bacterial invasion.

P214 Novel Method for Quantitation of Cells in Bone Marrow Tissue Sections

C Lyons*, JL Dorsey, KA Metcalf Pate, JL Mankowski

Molecular and Comparative Pathobiology, Johns Hopkins University, Baltimore, MD

Quantitation of cell types within the bone marrow presents many challenges and qualitative analysis of the relative proportion of red and white blood cell precursors remains the most common method of bone marrow analysis. Previously used quantitative methods have required samples in suspension for flow cytometry or radioactive labeling of tissue sections. We needed a method that would allow the quantitation of individual cells in paraffin-embedded bone marrow tissue. Unbiased stereology uses a motorized stage and random sampling to quantitate cell types in neurology and other disciplines. We hypothesized that we could adapt unbiased stereology to quantify cells within the bone marrow. We chose megakaryocytes, large cells with multilobulated nuclei that produce platelets, as the initial cell type to quantitate. Samples of fixed paraffin-embedded bone marrow were cut into 5-µm slices and stained with hematoxylin and eosin. Slides were visualized at 20× magnification, and at least 3 separate sections totaling 3.35 mm^2 gated for analysis. A motorized stage was used to facilitate the counting of all megakaryocytes with visible nuclei at 200× magnification; this number was then normalized to the area of bone marrow to determine megakaryocyte density (megakaryocytes /mm^2). Bone marrow sections from 37 animals were evaluated in a blinded fashion; the median megakaryocyte density was 11 megakaryocytes/mm^2 (maximum of 29 and minimum of 3). This method was highly repeatable, and when multiple sections from individual animals were evaluated in a blinded fashion, the standard deviation between the original and repeated count was less than one cell (median 0.85 cell/mm^2). We used this method to define megakaryocyte density longitudinally throughout infection in the SIV-infected pigtailed macaque model (days 0, 10, 35, 42, and 84 postinnoculation), and found no change in megakaryocyte density during infection. This method can be used in the future to quantify other cell types within bone marrow sections.

P215 Development of a Cerebrospinal Fluid Lateral Reservoir Model in Rhesus Macaques

C Lester McCully*[1], J Bacher[2], R Pung MacAllister[4], E Steffen-Smith[1], K Saleem[3], M Thomas[2], R Cruz[4], K Warren[1]

[1]Pediatric Neuro-Oncology, National Cancer Institute, Bethesda, MD; [2]Office of the Director, [3]National Institues of Mental Health, National Institutes of Health, Bethesda, MD; [4]Office of the Director, National Cancer Institute, Bethesda, MD

The rapid, serial, and humane collection of cerebrospinal fluid (CSF) in nonhuman primates is a desired element of many studies. Currently this is accomplished via 2 different CNS models. The Ommaya reservoir is a fourth ventricular catheter system with a silastic dome permitting CSF circulating flow yielding an unbiased sample. The second model is a lateral ventricular catheter and port system permitting static access to CSF. The Ommaya reservoir is associated with ease of use, but also with loss of catheter patency during an intensive, prolonged recovery. The lateral ventricle port model is not associated with these obstacles, but also does not permit CSF circulating flow for unbiased sample collection and is more arduous and restrictive for sample collection. A CSF lateral reservoir model was developed taking advantage of the features, but avoiding limitations of the 2 previous models by permitting circulating CSF flow for unbiased sampling, increasing patency, and reducing postoperative care and recovery. Six adult male rhesus monkeys were used. Presurgical MRI was performed to determine the coordinates of the lateral ventricle, the angle of surgical approach, and location of the choroid plexus. Two surgical approaches were determined to avoid the choroid plexus and major blood vessels. The ventricular catheters were surgically implanted at the predetermined coordinates and attached to a CSF reservoir implanted subcutaneously. Postsurgical MRI validated placement. Both surgical approaches were equally successful. The predetermined coordinates were 100% accurate. The lateral CSF reservoir system functioned successfully, 0.5 1.0 mL of CSF withdrawn without contamination of blood, implanted 4 to 7 mo, remaining patent, and without neurologic sequela, in 83% of the animals. There was a 50% reduction in postoperative treatment and animal recovery time. The development of the lateral reservoir model was successful in rhesus macaques and is an appropriate replacement for the fourth ventricular reservoir and lateral ventricular port models.

P216 What Is That Smell? Investigating the Potential Impact of Shared Behavior Space in a Rat and Mouse Facility

CG Alvarado*[1,2], JD Saeger[3], M Hankins[2], EC Bryda[2], CE Hagan[2]

[1]Comparative Medicine Program, [2]Veterinary Pathobiology, [3]University of Missouri, Columbia, MO

Mice and rats are both common in biomedical research; however, abundant facility space and unlimited resources are not common. While numerous studies have investigated the effects of cohousing rats and mice, there are few studies exploring how sharing procedure rooms or equipment between these 2 species may impact their behavior. Our study was designed to provide insight regarding the extent to which behavior testing of rats influences mouse behavior if performed in the same room using the same equipment. Eight female C57BL/6 mice were allowed to freely explore a 3-chamber social apparatus that had previously only been used for testing of mice. After this initial control exposure, a rat was placed in one chamber of the apparatus for 10 min immediately prior to retesting of each mouse. Preliminary data demonstrate that while mice did not avoid the chamber that had held the rat, they did show increased freezing behavior in that chamber. Studies are ongoing to determine whether this freezing behavior is specific to rat odors or whether it is simply a generalized response to a novel odorant. We are also investigating whether these effects are sex or strain-specific. A better understanding of the effect that lingering odorants have on behavior will help shape facility management practices and minimize concerns among investigators who may use different species but must also share procedure rooms and equipment.

P217 An Overall Picture of the Seroprevalence of Murine Norovirus in Brazil

DM Rodrigues*, JC Jo, CY Issey, SC SAntos, LG Andrade, R Gilioli

Animal Health Laboratory, University of Campinas,UNICAMP, Campinas, Brazil

Murine norovirus (MNV), a member of a Caliciviridae family, is one of the most prevalent viral agents identified in laboratory animal facilities

worldwide. The effect of the disease on the research models and even in the experimental results is not fully understood. Several reports show the prevalence of MNV in North America, Japan, and Korea. Until recently, there was no available data about the seroprevalence of MNV infection in Brazilian animal facilities. Furthermore, the demand to perform health monitoring for viral agents has increased over the years. Our main objective was focus on obtaining an estimate of the prevalence of specific antibodies to MNV in mouse colonies by the establishment of an indirect immunofluorescence assay (IFA). Serological screening using the antigen MNV isolated from feces of a naturally infected mouse was performed in 347 serum samples collected from mouse of both sexes, different genetic background and ages ranging from 21 d to 1 y. All the animals were obtained from 15 animal facilities, including 2 facilities from other Latin America countries and usually came from breeding and experimental units and one commercial vendor. Of the total of 347 sera analyzed, 100 (28.8%) were positive for MNV specific antibodies. In the same way, 8 outbred mice were experimentally infected and tested for MNV antibodies. From these total, 100% of the mice orally inoculated with MNV isolate were identified as positive in the IFA and used as a positive control in the reactions. As demonstrated by other literature reports, these data support the idea that MNV is widely spread among the mouse colonies in Brazil, although clinical signs are not observed in infected animals. Finally, the IFA using this MNV isolate provides a sensitive tool to help in the detection of MNV infection in Brazil.

P218 Effect of Red or Yellow Caging on the Cardiovascular Physiology of Male Sprague–Dawley Rats

D Hickman*, MP Swan

Laboratory Animal Resource Center, Indiana University School of Medicine, Indianapolis, IN

Because rats do not see the colors red and yellow, provision of a tinted plastic intracage shelter allows them to perceive sheltered darkness without interfering with daily assessment. This study evaluated the effect of using red- or yellow-tinted caging. It was hypothesized that tinting the caging would improve the rat's wellbeing by providing them with a larger usable space without compromising the circadian rhythm and staff ability to observe the rats. Six male Sprague–Dawley rats were implanted with a radiotelemetry transmitter. They rotated for 2 wk each in the following housing conditions under a 12:12-h light:dark cycle: 1) clear caging, 2) yellow-tinted caging, and 3) red-tinted caging. No more than 2 rats were in each condition at a time, and all were in each housing condition at least twice. Additionally, all rats were housed in 24 h of dark for 2 wk. Physiologic data was collected continuously during the second week of housing. Heart rate and activity data from 0800 to 1000 (light) and 2000 to 2200 (dark) was averaged by rat by day. These averages were compared between treatment groups using an ANOVA. The heart rate of rats housed in the clear caging was significantly increased ($P = 0.0103$), and rats housed in the red caging had increased activity ($P = 0.0074$). Rats housed in 24 h dark demonstrated a loss of circadian rhythm in the second week of housing, but this was not seen in the other groups. The staff reported that although it was easier to observe the rats in the yellow caging, it was still possible with the red caging. The use of tinted caging resulted in a reduction of the heart rate, but did not interfere with the circadian rhythm or the staff ability to perform daily assessments. This study demonstrated that tinted caging may provide improvements in rat wellbeing as measured by physiology.

P219 Multilevel Caging Enhances the Welfare of Rats as Assessed by a Spatial Cognitive Bias Assay

R Wheeler, MP Swan, D Hickman*

Laboratory Animal Resource Center, Indiana University School of Medicine, Indianapolis, IN

To evaluate welfare of rats housed in a commercially available multilevel caging system, 36 male Sprague–Dawley rats were randomly assigned as follows: 1) bottom level only, no change; 2) access all levels, no change; 3) bottom level only, then access all levels; or 4) access all levels, then bottom level only. They were trained to perform a spatial discrimination cognitive bias task; the housing environment was changed (per treatment group); and they were subsequently tested with 3 ambiguous "probe" locations (reward plus, neutral, or nonreward plus). The latency times to approach locations were compared between groups (ANOVA). Findings were consistent across locations, but only average latency data for the neutral location is presented here. Group 3 average latency time was significantly decreased (15.51 ± 5.73 s, $P = 0.0375$), suggesting positive cognitive bias associated with enhanced caging access. Group 4 average latency time was significantly increased (37.03 ± 5.65 s, $P = 0.0252$), suggesting negative cognitive bias when enhanced caging access was removed. There were no differences in the average latency times in groups 1 and 2, where there were no changes in their environment (22.03 ± 5.65 s and 29.11 ± 5.73 s, respectively, $P = 0.5202$), suggesting habituation. Increases in neutrophil:lymphocyte ratio have been associated with chronic distress. A significant decrease in this ratio was seen in group 2 as compared with the other groups ($P = 0.0426$), suggesting improved welfare. These findings suggest the welfare of these rats was enhanced through access to both levels of multilevel caging.

P220 Measuring Acute Stress in Laboratory Rats and Mice Using the Lymphocyte Coping Capacity Assay

MP Swan, R Wheeler, D Hickman*

Laboratory Animal Resource Center, Indiana University School of Medicine, Indianapolis, IN

Measurement of acute stress often requires complicated laboratory modalities, such as measurement of corticosterone assays via radio immunoassays. Identification of an alternative method to evaluate acute stress could facilitate studies that are evaluating animal wellbeing. The lymphocyte coping capacity (LCC) is an assay that has been used to measure acute stress in humans, nonhuman primates, and wild rodents. The assay relies on measurement of bioluminesnce and can be performed using relatively inexpensive, portable device. This study was designed to characterize the LCC as a measurement of acute stress in rats and mice. Twenty-four male and female C57BL/6 mice were randomly assigned to a stressed or a nonstressed experimental group. The stressed mice were restrained for 60 s, consistent with the published wild rodent studies, then anesthetized with isoflurane before a terminal blood collection. The nonstressed mice were anesthetized, but not restrained. The blood was used to measure the LCC and corticosterone. The study was repeated with 24 Sprague–Dawley rats. There were significant differences in the serum corticosteroid levels of the stressed mice ($P = 0.0477$) and rats ($P = 0.0301$), but there were no significant differences in the LCC values of the stressed mice ($P = 0.6960$) or rats ($P = 0.6557$). We hypothesized that the lack of difference in LCC values was because the laboratory rodents are free of pathogens, which is not the case in humans, nonhuman primates, and wild rodents. To test this hypothesis, we repeated the study with 20 male and female mice obtained from a retail pet store. In these mice, there was a significant increase in the LCC ($P = 0.0007$) and corticosterone ($P = 0.0487$) values of the stressed mice as compared with the nonstressed mice. This study suggests that although the LCC has been reported as a simplified measure of acute stress, this method is not sensitive enough to determine acute distress in laboratory rats and mice.

P221 Use of the Neutrophil to Lymphocyte Ratio to Evaluate Moderate Stress in Laboratory Rats and Mice

MP Swan, D Hickman*

Laboratory Animal Resource Center, Indiana University School of Medicine, Indianapolis, IN

Measurement of moderate stress often requires complicated laboratory modalities, such as measurement of glucocorticoids via radio immunoassays. Additionally, interpretation of moderate stress using glucocorticoids is problematic because they can be influenced by multiple other factors, including circadian rhythm. Identification of an alternative method to evaluate stress could facilitate studies that are evaluating animal wellbeing. Evaluation of the neutrophil to lymphocyte (NE:LY) ratio is an assay that has been used to measure moderate and chronic stress in humans, nonhuman primates, and wild rodents. The assay can easily be performed by running a complete blood count. This study was designed to characterize the NE:LY ratio as a measure-

ment of acute stress in rats and mice. Twenty-four male C57BL/6 mice were randomly assigned to a stressed or a nonstressed experimental group. The stressed mice were exposed to mild stressors, such as a wet cage, for less than 4 h/d for 1 wk; the nonstressed mice were housed in standard caging (control). Both groups were housed separately. At the end of the week, a blood sample was collected to measure the NE:LY ratio. The study was repeated with 24 male Sprague–Dawley rats. There were no significant differences in the NE:LY ratio of the mice ($P = 0.1611$), but it was significantly increased in the stressed rats ($P = 0.0464$). This study suggests that the NE:LY ratio can be used as a simple measurement of moderate stress in rats, but additional investigation is required to determine its value for laboratory mice.

P222 Differential Cardiovascular Physiology and Pathology in Selected Lineages of Miniature Swine and Comparison to Human

A Stricker-Krongrad[2], T Madsen[2], BC Hanks[2,1], D Brocksmith[*1], J Liu[2], LD Brown[1,2], GF Bouchard[1,2]

[1]Sinclair BioResources, Auxvasse, MO; [2]Research, Sinclair Research Center, Auxvasse, MO

In support of the role of the miniature swine as a valid alternative to canine and nonhuman primates in regulatory toxicity, we investigated the cardiovascular physiology and pathology of the Yucatan, Hanford, and Sinclair miniature swine. Anatomic parameters were obtained at necropsy. Blood vessels diameter, velocity, and flow were obtained by Doppler ultrasonography. Cardiac electrophysiology was obtained using clinical electrocardiogram (ECG) and surgical monitor units. Macroscopic lesions and histopathology assessments were conducted on heart and kidneys. Data were compared with published measurements of adult human illustrating similarities or differences (for practicality, male data are reported here). Across the 3 lineages, heart-to-body weights ratio ranged from 0.41 to 0.50 and were higher than human (0.42). The geometric corrections for heart rate adjustment to body size ranged from 215 to 297 and were comparable to human (241), indicating that heart volume and function were well-adjusted to the reduction in body size. The miniswine hearts showed a coronary artery distribution comparable to human. The right coronary internal diameters ranged from 1.44 to 1.79 mm and were comparable to human (3.9 mm) when adjusted to body surface area (weight range: 10 to 30 kg). External femoral blood flows at rest averaged 93 mL/min and were slightly lower than human (260 mL/min) when adjusted to body size. Electrophysiological heart segments duration (for example, RR ranged from 360 to 662 ms) and their ratio (QT/RR) were proportional to human and well-adjusted to body size. Macroscopic lesions were nonexistent. Histopathology findings were rare and limited to sublevel myocardial inflammation with low incidence in the Hanford lineage. The similarities between the cardiovascular systems make these three lineages of miniature swine suitable animals to model the human counterpart. In addition, the differences will aid investigators select a relevant lineage of miniature swine if specific cardiovascular parameters are required.

P223 A Novel Murine Model of Cardiac Hypertrophy Caused by Dysregulated AMPK/mTOR Pathway

DK Hirenallur-S.[*1], J Ramirez[1], NL Reyes[1], E Minami[3], M Kim[2], N Bojjireddy[2], R Tian[2], BM Iritani[1]

[1]Comparative Medicine, [2]Anesthesiology and Pain Medicine, Bioengineering, [3]Division of Cardiology, University of Washington, Seattle, WA

Cardiac hypertrophy-induced heart failure is one of the leading causes of human morbidity and mortality in the United States. However, the molecular pathways involved in the development of cardiac hypertrophy remain incompletely defined, limiting therapeutic options. Metabolic diseases such as diabetes and obesity increase the risk of developing cardiovascular diseases. Understanding energy homeostasis mechanisms in cardiomyocytes is therefore essential to elucidate the pathogenesis of heart failure and to identify novel therapeutic targets. Recent reports suggest that dysregulation of AMP Kinase (AMPK), a central cellular energy sensor activated under conditions of low energy and/or cellular stress, and the anabolic mTOR pathway, are directly involved in the development of cardiac hypertrophy. Our laboratory recently reported that Folliculin interacting protein-1 (Fnip1) plays an important role in suppressing mTOR signaling during early B cell development. In this study, we tested the hypothesis that loss of Fnip1 alters AMPK-dependent regulation of mTOR signaling and leads to the development of cardiac hypertrophy in Fnip1-deficient mice. We found that the ratio of heart to brain weight was significantly elevated in Fnip1-/- relative to wildtype (WT) hearts. Cardiac ultrasound revealed that interventricular septum and left ventricular wall thickness are significantly higher in Fnip1 deficient hearts compared with controls, which correlated with increased percent fractional shortening and decreased heart rate. 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 deficient cardiomyocytes compared with WT controls. Biochemical studies suggested that AMPK and mTORC1 pathways are concurrently activated in Fnip1 null cardiac tissue, suggesting that AMPK-mediated suppression of mTORC1 is impaired in Fnip1 null mice. Our findings collectively suggest that Fnip1 normally regulates cardiac morphology and function, perhaps by permitting AMPK-dependent suppression of mTOR signaling in response to energy stress.

P224 Implementation of Ultrafiltration Method in Production of Viral Antigens for Serological Applications

E Prikhodko[*], G Khara, C Quinones, E Andrus, H DeFelice

Animal Health Services, BioReliance, Sigma-Aldrich, Rockville, MD

Purity of the antigens is a critical component of high quality serological assays. Very often antigen preparation is labor intensive, time consuming, and expensive. For instance, a standard procedure of the antigen purification is a multistep process, which may include concentration of the large volume of the infected cell culture, centrifugation and density gradient ultracentrifugation followed by extensive dialysis. Here, we report the results of the development of a simplified method for the production of several rodent antigens including TMEV strain GDVII, MAd-Fl and MAd-K87, MHV strains JHM and A59, and MNV. This method is based on an ultrafiltration technique with a commercially available centrifugal device that reduces the antigen purification to a 1- or 2-step process. Purified antigens obtained by ultrafiltration were compared with those prepared by the standard procedure. Purity of the prepared antigens was assessed by Western and SDS-PAGE with coomassie staining. Antigen immunoreactivity was analyzed by ELISA for analytical sensitivity, analytical specificity, diagnostic sensitivity and diagnostic specificity. For example, for the TMEV antigens the ELISA analytical sensitivity was on average 2-fold higher for the centrifugal device prep in comparison to the antigen prepared with a standard method. Assessment of the ELISA data for the analytical specificity demonstrated high comparability of two tested preps. Less than 1% variability in the ELISA diagnostic specificity and diagnostic sensitivity between 2 preps was also observed. Thus, the TMEV antigen prepared by ultrafiltration was similar in ELISA application to the TMEV antigen prepared by the standard method. However, due to a significant decrease in preparation steps and time, the overall cost of the ultrafiltrated antigen was substantially lower. In conclusion, using the ultrafiltration method based on the centrifugal device for antigen preparation results in the production of high quality viral antigen and substantially reduces the preparation cost.

P225 Comparison of the Whole Inactivated Viruses and the Recombinant Proteins of the Same Specificity as Antigens for Serological Application

E Prikhodko[*], E Andrus, H DeFelice, C Quinones, G Khara

Animal Health Services, BioReliance, Sigma-Aldrich, Rockville, MD

A replacement of wildtype inactivated viruses by the corresponding recombinant proteins as antigens has been broadly used for serological applications. The use of recombinant proteins reduces the difficulties associated with amplification of the wildtype virus in cell culture. Although the implementation of the recombinant proteins can be very beneficial, using a single protein as the major antigenic determinant instead of the whole virus can present some limitations and decrease

the sensitivity or specificity when used in serological assays. Here, we compare several wildtype viruses and the recombinant proteins of the same specificity used as antigens in indirect ELISA. Three groups of the recombinant proteins were evaluated: 1) capsid proteins self-assembled in VLPs (mouse minute virus and norovirus), 2) nucleoproteins (LCMV and Hantavirus), and 3) a matrix protein (Marburg virus VP40). The ELISA was assessed for analytical sensitivity and analytical specificity with an absorbance cutoff value of 0.17 at 405 nm wavelength. For instance, analytical sensitivity of the ELISA with the MMV VP2 recombinant protein as an antigen was 4- to 8-fold higher than that of the ELISA with the whole inactivated MMV antigen. In addition, MMV analytical specificity tested against a panel of heterologous sera was improved with the recombinant protein. While the VP2 recombinant strongly interacted only with the MMV specific serum, wildtype MMV antigen not only recognized the MMV antibodies, but also nonspecifically reacted with MNV and REO3 sera. No significant difference was observed in diagnostic sensitivity. The VP2 recombinant, however, resulted in fewer false positive samples and a higher diagnostic specificity. Less than 1% false positive samples were identified by the recombinant protein ELISA, at the same time 3% to 15% of all tested samples were cross-reactive in ELISA with whole virus as an antigen. Similar studies were performed for all groups. Although, the results slightly varied for all tested viruses and the corresponding recombinants, overall data confirmed that in many instances a recombinant protein can successfully substitute the purified wildtype virus as an antigen in a serological assay.

P226 HIV-1 Transgenic Mouse with Mixed Lineage Leukemia/Lymphoma

EN Ateh*, H Davis, J Bryant, Y Tagaya

Institute of Human Virology, University of Maryland, Baltimore, MD

The HIV-1 T26 transgenic mice which bears a gag-pol deleted HIV-1 genome, develops a mixed lineage B and T cell leukemia/lymphoma. HIV infection is associated with a much higher risk for the development of nonHodgkin lymphoma (AIDS-NHL). The principal causes of lymphomagenesis in HIV-infected individuals are thought to be the loss of immune function and co infection. T-cell lymphoma is associated with HIV infection, albeit less frequent than B-cell malignancies. In an attempt to establish in vitro and in vivo model to further study lymphoma development associated with HIV infection, we have established immortalized cell clones from this HIV-1 transgenic mice. We have confirmed that all these cell clones develop into a fatal lymphoma in immunocompromised SCID/NOG (NOD +gc knockout) mice. In the B clones, we have observed the expression of an unreported splice variant of Pax5, a critical factor that dictates B cell commitment which is implicated in select B cell malignancies. This new variant may code for a novel N-terminus sequence that is different from one of the reported normal Pax5. These B cell clones show evidences that they have undergone the rearrangement of TCR γ locus. Pax 5 is known to convert T or monocyte lineage cells into B cells when expressed in these nonB cells. Thus we hypothesize that some of malignant B cells associated with HIV infection might have been converted from other lineages. We believe this concept may shed new light on the nature of HIV-associated lymphoma that could lead us to develop new strategies to control AIDS-related lymphoma.

P227 Evaluation of Cardiac Phenotype in TO2 Hamsters Using Ultrasound Biomicroscopy

FB Wright*[1], AR Olzinski[2], RE Bernard[2], B Hoang[3], CG Schnackenberg[2], RW Coatney[1]

[1]Laboratory Animal Sciences, R&D, PTS, UM, [2]HF DPU, R&D, PTS, UM, [3]Discovery Core Technologies, R&D, PTS, UM, GlaxoSmithKline, King of Prussia, PA

Published reports of echocardiographic phenotyping of TO2 hamsters are limited to conventional M-mode imaging. Higher resolution ultrasound biomicroscopy (UBM) may provide enhanced evaluation of left ventricular (LV) structure and function using 2D greyscale (2D) and strain (STE) imaging techniques. We evaluated TO2 hamster cardiac phenotype using UBM. F1B control strain and TO2 hamsters (male, 12 wk old) were anesthetized using isoflurane. Midventricle short axis and parasternal long axis images were obtained (40 MHz transducer). Image quality (border resolution) was evaluated. 2D, M-mode and STE were analyzed. End diastolic and systolic volume (EDV, ESV), stroke volume (SV), cardiac output (CO), LV chamber dimensions (LVIDd, LVIDs), wall thickness, ejection fraction (EF), fractional shortening (FS), and radial and circumferential strain and strain rate (SR, SC, SrR, SrC) were obtained. TO2 values were compared with F1B. Histology was performed on LV myocardium. 2D endocardial border resolution was less than 80% due to narrow acoustic window and rib shadow; however, M-mode endocardial borders were well defined and used to evaluate cardiac structure and function. Hyperechoic midmyocardial bands were visualized on 2D and M-mode images and consistent with midmyocardial wall fibrosis histologically. TO2 had lower body weight (BW, 16%). TO2 LVIDd, SV, and CO were lower (11%, 27%, and 27%, respectively). Normalized to BW, LVIDd, and CO were not different from F1B. EF% and FS% were not different. TO2 systolic SR was lower (26%). In summary, hamsters have narrow acoustic window that limited resolution of 2D images. UBM detected midmyocardial fibrous bands which have not been reported using conventional echocardiography. TO2 hamsters (12 wk) have normal cardiac function when parameters are normalized to BW. Decreased systolic SR suggests early LV dysfunction. Previous studies using conventional echocardiography report decreased M-mode functional parameters. The difference may be related to the improved resolution of UBM.

P228 Complete Genome Sequencing of Rodent Pneumonic CAR Bacillus

F Ike*[1], A Kajita[1], A Yoshiki[1], M Okubo[2], T Murata[2], K Oshima[3], M Hattori[3], T Kokubo[4]

[1]Experimental Animal Division, [2]Gene Engineering Division, RIKEN BioResource Center, Tsukuba, Japan; [3]Graduate School of Frontier Sciences, The University of Tokyo, Kashiwa, Japan; [4]Fundamental Technology Center, National Institute of Radiologic Sciences, Chiba, Japan

The cilia-associated respiratory bacillus (CARB), an unclassified, extracellular, gram-negative filamentous bacterium, colonizes the ciliated respiratory epithelium of rodents and causes persistent respiratory diseases. CARB was reported to grow in mammalian cell culture. Recently, we developed support cell free, CARB single culture system by using Vero E6 culture supernatants. Using this single culture system, we extracted genomic DNA and determined its full genome sequences. SMR strain of CARB isolated from rat was cultured in Vero E6 cell culture supernatants in non-adhesive plate. Growth of CARB was monitored using phase contrast microscopy. Grown CARB was confirmed by immunofluorescence assay and PCR. *Mycoplasma* spp. and other bacterial contamination were not detected. Collected CARB by centrifugation was treated by proteinase K and RNAse, and genomic DNA was extracted to construct pair-end sequencing library. Draft sequencing was performed using a new long-read sequencing kit. Then, gap closing and resequencing of low quality regions were carried out. CARB genome sequence was about 1.44 Mbp long from 5 scaffolds contig and it did not possess plasmids. Open reading frames were extracted by metagene program analysis. BLAST search was carried out against nr databases. We found 34 tRNAs, one rRNA set and about 1,200 coding sequences (CDS). Then, we did further PCR sequencing and determined full genome sequences. CARB is known to belong to cytophaga-flavobacteria-bacteroides phylum by 16S rRNA gene sequences analysis. However, almost all of the CDS obtained from this study showed low homologies against reported genes indicating that CARB had quite unique genomes among bacteria whose genomes were analyzed to date. Thus, the detailed analysis will be very useful to find new mechanisms in CARB infection.

P229 The Effect of Diet-Induced Diabetes on Circadian Physiology, Acclimation, and Postsurgical Recover Time in Mouse

G DeMarco*

Comparative Medicine, Pfizer, Cambridge, MA

The mammalian circadian timing system is governed by a central "master" clock, the suprachiasmatic nucleus (SCN) of the hypothalamus

and clocks in most peripheral tissues. The SCN directly or indirectly through peripheral clocks synchronizes behavior and physiology to an organism's photoperiod. Disrupted circadian rhythms may play a role in the pathophysiology of metabolic diseases including diabetes and metabolic syndrome. In 2 experiments, we tested the hypothesis that circadian physiology and gene expression would be altered in a mouse model of diet-induced of diabetes. Male C57BL/6J mice were fed a high-fat diet to induce and maintain diabetes (DIO) and control mice (CTL) fed standard lab chow. Body temperature and locomotor activity were recorded using implanted telemeters. Animals were singly housed in environmental chambers and randomized into Zeitgeber time (ZT) groups. After entrainment, brain and peripheral tissues were harvested at ZT times 0, 6, 12, and 18 for circadian gene expression analysis. In a follow-up study body weight changes in DIO and CTL mice postarrival and postsurgery were closely monitored. DIO mice demonstrated prolonged acclimation and postsurgical recover time and delayed time to entrainment when phase advanced. Locomotor activity and body temperature in DIO mice exhibited a diurnal shift and less robust circadian rhythms than controls. Per 2 gene expression in the SCN of DIO mice was flattened and appeared to have a phase advance. The delayed phase advance suggests altered photic responsiveness of the SCN and the diurnal shift suggests modulation of effectors downstream from the SCN in DIO mouse. Changes in Per 2 expression in the SCN parallel changes in DIO mouse circadian physiology. These data indicate the diet-induced diabetic state has a profound effect on the circadian timing system and suggest DIO mouse is particularly sensitive to environmental changes.

P230 Development of a Novel Ophthalmic Airflow Chamber for the Induction of Keratoconjunctivitis Sicca in Rodents

G Gum[*1], S Tolpen[2], SL Pritt[1]

[1]Absorption Systems, San Diego, CA; [2]San Diego State University, San Diego, CA

Keratoconjunctivitis sicca (KCS) is an eye condition caused by inflammation of the cornea and conjunctiva resulting from the lack of tear production. KCS manifests in different ways, but a primary influence for disease development is the surrounding climate. Current therapies are ineffective or are not viable for long term use. The rodent dry eye model is often used to test new KCS therapies. In order to reduce cost and set up time of current environmental chambers and systems, we developed a novel method of KCS in rodents using an airflow chamber under controlled environmental conditions. The novel airflow chamber incorporates the surrounding environment's air conditioned system and floor dehumidifiers to ensure a maintained relative humidity of 20%, while a dry bulb temperature is fixed at 23 °C. The device houses mounted 80 × 80 mm fans to increase the velocity of air circulating through the rodent's cage. After theoretically testing a device model through flow simulation using an analysis software, hotwire tests were performed to obtain actual air velocities. Flow simulation and hotwire tests helped visualize air flow patterns throughout the cage and mathematically analyze the air velocity at specific areas of interest. A study was undertaken that tested the chamber using a total of 12 laboratory mice (CD1mice) through fluorescein staining and Schirmer tear tests. Of the 12, 5 were tested in the simulated environment without the air chamber, 5 with the air chamber, and 2 as control group (standard room condition without the air chamber). Mice housed in the airflow chamber showed a decreased tear brake up time and Schirmer tear test values when compared with those mice without the air chamber and the control group. This device can be used to enhance a dry eye model in rodents allowing researchers a simple cost effective assembly and experimental set up.

P231 Genotyping DNA Isolated Using Cross-Linked Iminodiacetate Styrene Divinylbenzene Copolymer Beads

GP Boivin[*1,3], V Otano-Rivera[2], A Boakye[2], N Grobe[2], M Di Fulvio[2]

[1]Laboratory Animal Resources, Wright State Univ-EES, Dayton, OH; [2]Pharmacology and Toxicology, Wright State University, Dayton, OH; [3]Veterans Affairs Medical Center, Cincinnati, OH

Genotyping mice rely on isolation of DNA from tissues typically involving painful procedures such as tail snipping, digit removal, or ear punch and the use of expensive kits. Although harvesting of hair has been proposed in the past as a source for genomic DNA, there has been a perceived complication because of low DNA yields and fear of contamination. We hypothesized that DNA isolation from hair follicles will be as effective as more painful procedures for mice genotyping. We tested an adapted, simplified, fast, and cheap version of a common forensic method used for fingerprinting human samples and compared the results against tissue-based enzymatic commercial kits. As source of DNA for genotyping, we used 10 to 20 hair follicles plucked from 3 different groups of genetically engineered mice. Briefly, over 100 samples of hair follicles with appropriate positive and negative controls were directly placed in 10% cross-linked iminodiacetate styrene divinylbenzene copolymer beads resin (Beads) and incubated 20 min at 100 °C with gentle swirling. An aliquot of the supernatant corresponding to 1% to 2 % of the total volume containing follicle's DNA released by the action of the resin was directly used as template for hot-start polymerase chain reactions (PCR) coupled to custom-designed gene-specific oligonucleotide primers. The results demonstrate that our procedure provides specific and accurate genotyping results in less than 4 h at a cost per PCR of a tenth of commercially available kits. We recommend that in circumstances where haired mice can be analyzed, the use of Beads-digested hair follicles is an excellent source of DNA for PCR genotyping of mice.

P232 Comparison of the Rabbit, Diabetic Miniature Swine, and Nonhuman Primate to Evaluate the Clinical Biopotency of Insulin Products

A Stricker-Krongrad[1], LD Brown[2,1], E Blair[1], T Madsen[1], BC Hanks[1], J Liu[1], GF Bouchard[*1,2]

[1]Research, Sinclair Research Center, Auxvasse, MO; [2]Sinclair BioResources, Auxvasse, MO

The potency of human insulin has classically been evaluated in rabbits following the US Pharmacopeia (USP) guideline. However, since insulin analogues are intentionally different there is a need for a bioassay to assess clinical specific activity (U) in different species. To this extend, we compared the biopotency (U) of different insulin products in rabbits, in type 1 diabetic miniature swine and in normal nonhuman primates, and used human and pork insulin as reference standards. New Zealand White rabbits were fasted and injected subcutaneously at a dose level of 0.5 U/kg. Yucatan miniature swine (*Sus scrofa*) were made diabetic by intravenous administration of alloxan and insulin products were injected subcutaneously at dose level of 0.1 U/kg in overnight fasted animals (no feed or insulin for 18 h). Normal insulin suppression tests (nIST) were conducted in fasted male cynomolgus primates (*Macaca fascicularis*) receiving a bolus intravenous infusion of glucose (ivGTT; 0.25 g/kg) and treated with somatostatin. Insulin products were given at dose level of 0.05 U/kg. Glucose levels were recorded using handheld glucometer devices. The blood glucose kinetic (BGPK) and the blood glucose area under the curve (BGAUC) were used to assess biopotency in the rabbit and in the diabetic miniature swine. The slope of the blood glucose clearance (kG) was used to assess biopotency during the nIST. Our data indicate that the biopotency of insulin products can be assessed using BGAUC and kG, but that only the type 1 diabetic miniature swine can discriminate between differences in biopotency for all aspects of BGPK. In conclusion, the BGPK for short-acting human and pork insulin were similar in the rabbit assay but their respective BGAUCs were different (ratio of 1.2). Accordingly pork insulin was more potent during nIST in the nonhuman primate (slope of −0.012 compared with −0.010; pork and human, respectively).

P233 Constitutive Expression of CYP3A mRNA in Bama Miniature Pig Tissues

H Shang[1], K Guo[1], Y Liu[1], J Yang[2], H Wei[*1]

[1]Department of Laboratory Animal Science, College of Basic Medical Sciences, Third Military Medical University, Chongqing, China; [2]Department of Biochemistry and Molecular Biology, Medical School, Jinggangshan University, Ji An, China

The pig, particularly the miniature pig (minipig), is becoming an important animal model due to its physiologic and anatomic similarities to humans. Bama minipigs (*Sus scrofa domestica*), a Chinese natural minipig breed, are a promising animal model. The pig is a useful model for drug metabolism and pharmacologic studies due to the similar properties of cytochrome P450 (CYP)3A between pigs and humans. However, a detailed investigation regarding the abundance and expression of CYP3A in porcine tissues, particularly in minipig tissues, has not been performed. The present study investigated constitutive expression of CYP3A mRNA in Bama minipig tissues using real-time reverse transcriptase-polymerase chain reaction (RT-PCR). We found that the expression of CYP3A mRNAs relative to the endogenous control, β-actin (ACTB), was lower than when compared with the expression of the endogenous control, TATA-binding protein (TBP), except for the expression of CYP3A29 mRNA in the spleen, adrenal gland, testis, and epididymis, and CYP3A46 in the spleen. Expression levels of all 3 CYP3As were highest in the liver amongst all the tissues tested, and the order of relative mRNA expression level of the 3 CYP3As was different between other tissues. We also analyzed the relative expression of the 3 CYP3A mRNAs in each tissue. CYP3A46 had the highest expression in all extrahepatic tissues, whereas CYP3A22 had the highest expression in the liver, and CYP3A29 had the lowest expression in all tissues except in the duodenum, where it had higher expression than CYP3A22. Because CYP3A22 and CYP3A46 were the most highly expressed isoforms, it could follow that they are probably important functional CYP3A genes for Bama miniature pig. Our present work will broaden the understanding of the physiologic functions of CYP3As in the Bama minipig and promote its application in drug metabolism and pharmacologic studies. Our results also indicate that the breed, age, and castration status of the pig should be considered when using the pig as an animal model in pharmacologic applications.

P234 Comparison of In Vivo Receptor Occupancy Studies Using a Radioligand-Based Approach or a Liquid Chromatography/Mass Spectrometry-Based Approach

H Cao*[1], S Champagne[1], M Orsini[2], J Johnston[1], M Goulet[2], R Lew[2], M Quinton[2]

[1]Biomedical Research Models, Worcester, MA; [2]Sunovion Pharmaceuticals, Marlborough, MA

In vivo receptor occupancy (RO) is a valuable tool used in preclinical drug development to confirm target engagement and facilitate selection of compounds for further development. We have been using radioligand-based RO as means of measuring target engagement in the rat brain. In this approach, compounds of interest are peripherally injected prior to intravenous injection of a selective radioligand. If the compounds of interest occupy the target, binding sites are no longer available for the radioligand. Therefore, binding of the radioligand, which is evaluated by imaging brain sections, is inversely correlated to target occupancy. This approach is very sensitive, has a fast turnaround (2 to 3 d) and provides the ability to analyze multiple brain areas independently of their size. However, many targets have no validated radioligand available, and the cost of radioligand-based RO can be prohibitive. These limitations prompted us to evaluate liquid chromatography/mass spectrometry (LCMS)-based RO as an alternative method. In this method, levels of the cold ligand are measured in brain homogenates. LCMS-based RO is an inexpensive and rapid method for determining RO of compounds in small animals as well as screening for potential new ligands. In order to compare radioligand-based RO with LCMS-based RO, 2 different ligands were chosen: raclopride for D2 RO and MDL100907 for 5-HT2A RO. Dose-response curves were generated using both methods for haloperidol and quinpirole (D2 RO), and olanzapine (5-HT2A RO). We found an excellent correlation between the RO50 (in terms of dose and plasma levels) measured using radioligands and cold ligands. Binding of MDL100907 was not affected by citalopram and baclofen (negative controls) which substantiate the selectivity of the tracer and validate the LCMS detection method. In conclusion, LCMS-based RO is a good alternative method compared with radioligand-based RO and will allow us to screen potential ligands for new projects.

P235 Development of a Noninvasive Murine Pregnancy Test Using Urine or Fecal Samples

VR Pauley[2], IM Washington*[1,2]

[1]Office of Animal Care, Seattle Children's Research Institute, Seattle, WA; [2]Comparative Medicine, University of Washington, Seattle, WA

Current methods for determining pregnancy in mice during early gestation are unreliable, or require invasive techniques or anesthesia and sophisticated imaging. We hypothesized that a reliable and noninvasive murine pregnancy detection method could be developed using urine or feces that would ultimately reduce unnecessary euthanasia of nonpregnant laboratory mice. Urine and fecal samples were collected from B6 and CD1 mice at gestational days 0.5 to 18.5 and compared with samples from nonpregnant controls. Urine samples were assayed for protein to creatinine ratios, to correct for urine concentration, and subsequently analyzed using SDS-PAGE gels stained with Coomassie blue. Band densities were calculated for samples from mice at different stages of pregnancy and compared with controls. Bands were excised and proteins identified by mass spectrometry (MS). Fecal samples from nonpregnant mice were used to optimize a progesterone enzyme immunoassay (EIA) and ensure antibodies recognize murine fecal metabolites. Fecal samples from mice at random estrous cycle stages were lyophilized for >48 h, pulse-vortexed in 70% ethanol, and stored at −20 °C before measuring progesterone metabolites. Our SDS-PAGE image analysis results indicated no statistical difference between density of bands in urine samples from nonpregnant mice and mice at different stages of pregnancy. This is consistent with the identification of major urinary proteins (MUP1, 2, 3), centrosomal protein CEP57L1, and keratin 5, but the absence of pregnancy-specific proteins, in the excised bands using MS. Preliminary EIA results demonstrate detectable progesterone levels (54 to 408 ng/g feces) at random stages of the estrous cycle, using as few as 1 to 2 fecal pellets per mouse. Thus, we are optimistic that a fecal progesterone assay may provide a viable noninvasive method to detect pregnancy in mice during early gestation.

P236 Understanding Excipient Limitations in Rodent Pharmacokinetic Studies

J Spear*, M Damzal, G Geraci, S Ferreira

Novartis Institutes for BioMedical Research, Cambridge, MA

Many new chemical entities (NCEs) have very poor solubility, placing an emphasis on the use of cosolvents and excipients to produce an intravenous formulation for evaluation of initial pharmacokinetic parameters. Additionally, in order to circumvent poor solubility, increasing dose volumes were being exploited on study. These studies resulted in an increased exposure to vehicle excipients, coincident with an increase in observable adverse reactions to dose administration in vivo. While these studies were in compliance with IACUC guidelines, the volumes requested were near or at the maximum recommended volumes. Due to these adverse reactions ranging from lethargy to death, an evaluation of the excipients used in formulating solution dosing was undertaken in rats and mice. Initially, studies were conducted by preparing predetermined vehicle cocktails that were based on previous studies that showed adverse effects. Cocktails of the excipients (without compound) were dosed intravenously or intraperitoneally, 2 routes where adverse effects were commonly observed. Animals (n = 1 per group) were observed 4 h postdose for adverse effects. Attributing an adverse reaction to a specific excipient proved difficult as varying dose volumes also compounded the outcome. Therefore, the next phase used fixed dosage volumes to establish consistency and to prevent any serious adverse effects (death/early termination) from occurring. Again, vehicle excipients were dosed either intravenously or intraperitoneally, and animals (n = 1 per experimental excipient) were observed 4 h postdose. It was concluded that for rat (5 mL/kg IP), vitamin ETPGS should not be used and should not exceed 3% when dosing a rat at 1 mL/kg IV. For mouse (at 5 mL/kg IV) NMP and DMA, if used, should not exceed 5%, PG concentrations should not exceed 10%, sodium acetate buffer should be ≤10 mM, sodium citrate buffer should be ≤25 mM. Assessing vehicle compatibility for in vivo research improves the success rate for animal dosing and places emphasis on producing more soluble compounds.

P237 Ultrasound-Guided Cholecystocentesis as a Refined Method for Bile Collection in Support of Drug Disposition Studies

J MacGuire[*1], M Fox[1], E Janovitz[2]

[1]Veterinary Sciences, [2]Discovery Toxicology, Bristol-Myers Squibb, Princeton, NJ

Most pharmaceuticals are eliminated via hepatic metabolism and biliary excretion in mammalian species. Understanding the extent to which novel pharmaceuticals are eliminated in this manner is an important step in drug discovery. Obtaining bile for this purpose is a challenge due to the anatomy of the hepatobiliary tract. The bile duct cannulated (BDC) dog is the enduring large animal model for investigating bile elimination of new drug candidates. However, this invasive model carries multiple shortcomings, notably, the initiation of physiologic changes that may affect drug pharmacokinetics. We developed and validated a novel, minimally invasive procedure to collect bile from large animals termed ultrasound-guided cholecystocentesis (USG-CC). In this procedure, ultrasound is used to guide a needle transcutaneously through the liver and into the gall bladder, allowing for precise bile sample collection. With this refined method, we can now safely and efficiently obtain bile samples from dogs and monkeys in support of drug disposition studies.

P238 Clinical Pathology of Mice Exposed to Various Paper Enrichment Devices

J Monk[1], J Villano[*1,2]

[1]University of Texas Medical Branch, Galveston, TX; [2]University of Michigan, Ann Arbor, MI

An enrichment program is an integral component of the animal care and use program and has a primary aim of enhancing animal wellbeing. However, enrichment devices may also serve as a confounding variable in animal research studies and may pose detrimental health effects to the animals. In this regard, we investigate the hematologic and clinical chemistry effects of various paper products used as enrichment devices for mice. Mice were exposed for 3 mo to paper tubes, paper towels, paper fibers, or delicate task wipes. All groups of mice had mostly normal clinical pathology as referenced against data provided by the animal vendor and textbook references. Hematology for all groups were normal but the delicate task wipes' group had significantly increased white blood cell, lymphocyte, monocyte, and eosinophil counts; the paper fiber group had significantly increased monocyte and eosinophil counts; and mice without enrichment (control) had significantly increased lymphocyte count. Mice given paper tubes, paper towels, and paper fibers had increased alanine aminotransferase (ALT) levels at the end of the study. In the absence of clinical signs, other hepatic enzyme tests, and gross liver lesions, the ALT increases may be insignificant. Taken collectively, we conclude that the four paper enrichment devices can be used for mice without any adverse clinical pathology effects. With recent economic challenges plaguing the biomedical industry, institutions face a dilemma of meeting requirements with limited budget and paper substrates provide an economical and viable alternative for enhancing animal welfare.

P239 Development of an Epaxial Intramuscular Injection Technique in Juvenile Rats

J Callahan[*1], L Croft[2], G Baxter[2]

[1]Training and Compliance, [2]Safety Assessment, Huntingdon Life Sciences, East Millstone, NJ

In order to effectively test compounds in the juvenile rat, the FDA requires that certain test articles be administered to an animal from the age of 7 d onwards. This equates in the clinical environment to the human infant (newborn to approximately 2 y old). Since certain compounds may be administered to a child in that age range, it is imperative to have a reproducible, effective method of dose administration, such as intramuscular injections. A literature search yielded little information on this dose route in the juvenile animal. A specific study required the intramuscular injection route of administration due to the clinical indication of the compound. Intramuscular injections are typically administered into the gluteal muscle in adult rats, with care taken to avoid depositing test material on or near the sciatic nerve, which may result in subsequent limb paralysis. Challenges faced included the limited amount of muscle mass in rats of this age compounded by the fact that the test material had the potential to cause muscle paralysis. Damage to the sciatic nerve or surrounding muscle and subsequent limb paralysis could lead to the possibility of the dam rejecting the pups if they were not viable due to paralysis. Because of these factors, a method for epaxial intramuscular injections was developed. In order to accurately assess the location of the intended muscle mass, as well as train personnel in the proper handling and restraint techniques, adult mice were used initially, followed by rat pups beginning on postnatal day (PND) 7 and older. Care had to be taken with handling and restraint, and administration of material, as well as the volume injected, in order to avoid any damage to the muscle and surrounding areas. Success of the technique was evaluated by visual assessment of the injection site externally and internally, initially using a colored dye, (Figure 1) and subsequently by assessing the site after approximately 1 wk for signs of edema, trauma (bleeding), or changes in gait or locomotion.

P240 Laboratory Evaluation of Inexpensive Temperature Indicators for Potential Use in Rodent Airline Transportation

JC Smith[*1], D Mussmacher[2]

[1]Veterinary Bioscience Institute, Winston Salem, NC; [2]Quality Assurance, Taconic Farms, Oxnard, CA

The impact of airline transport on rodent physiology is not well documented. Inexpensive temperature indicator strips have been suggested for use in rodent shipments to provide an accurate, nonreversible record of heat exposure over the passage of time. This study provided laboratory validation on time to first indicator change of these strips prior to their use in air shipments. We hypothesized that these strips would perform to package indications within the quality control laboratory. To test this hypothesis we obtained four different types of nonreversible, color indicator, adhesive backed temperature strips, rated for 25, 30, and 31° C. Ten of each type were evaluated in each group: group 1 (26 °C 8-h run time), group 2 (26 °C 7-d run time), group 3 (30 °C 8-h run time), and group 4 (31 °C 7-d run time). All strips were preconditioned prior to use as per manufacturer instructions, and were placed onto into a previously validated incubator at either 31 °C (groups 1 and 2) or 36 °C (groups 3 and 4). Groups 1 and 3 were evaluated for indicator first change every 5 min for the first hour and then every 15 min for up to 8 h. Groups 2 and 4 were similarly evaluated for the first 8 h and then 2 times daily. Groups 1 and 2 had first indicator changes at 22 (SD 4.22) and 23(SD 4.83) min, respectively. Groups 3 and 4 had first indicator changes at 61.5 (SD 7.4) and 62 (SD 7.15) min, respectively. A *t* test between sample means shows no significant difference between groups 1 and 2 ($P = 0.236$) or between groups 3 and 4 ($P = 0.332$). These data show that all tested strips performed as per the manufacturers claims, in terms of indicator first change time. In conclusion, these strips tested could be used in rodent air shipments to obtain wellbeing information relating to the temperature exposure over a period of time.

P241 Enrichment Strategies for Mice: An Evaluation of a Variety of Commercially Available Options Measuring Use, Effect on Handling, and Chronic Stress Markers

JL Peveler[*], MP Swan, R Wheeler, C Boehm, D Hickman

Laboratory Animal Resource Center, Indiana University School of Medicine, Indianapolis, IN

Environmental enrichment provides coping mechanisms for laboratory animals. We set forth to examine what enrichment strategies might be most beneficial and preferred by mice to provide guidance to institutions planning enrichment strategies. In this 12-wk study, we provided 197 mice with 11 different environmental enrichment strategies that encouraged natural behaviors such as foraging, exercise, exploration, and socialization. Every other week, mice were scored on observed interactions with enrichment and agreeableness of handling by the staff.

Before disturbing the cage, scorers rated the rodents' use of enrichment as: 0 = no indication of use, 1= shows sign of use, 2= currently using enrichment. The cage was opened, and the animal was weighed. Next, a handling score was assigned where 1 = not agreeable to handling (aggressive, elusive, etc.), 2 = tolerates handling as expected, and 3 = seems content/friendlier than usual. At the end of the study, a necropsy was performed to determine organ weights. The mice showed evidence of use of all offered, and no mice displayed significant decreases in ability to handle. Mice in both red and disposable shelters showed a significant increase in adrenal weight (P = 0.0465 and P = 0.0425, respectively). Overall body weight was increased for our nesting enrichments (P = 0.0448) and companioned mice (P = 0.0600), supporting recent findings of the importance of thermoregulation on metabolism. We found that feeding 5 sunflower seeds every day did not increase overall body weight for mice as compared with the other groups (P = 0.4587). We also found that the running wheel resulted in significant body weight decrease (P = 0.0002) with an accompanying significant increase in heart weight (P = 0.0002). The findings of this study can provide guidance to research personnel who are planning and evaluating enrichment strategies for mice and their possible effects on research projects.

P242 Rabbit Gastric Ulcer Models: Comparison and Evaluation of Acetic Acid-Induced Ulcer and Mucosectomy-Induced Ulcer

J Maeng*, E Lee, D Lee, S Yang

Utah-Inha DDS and Advanced Therapeutics Research Center, Incheon, Republic of Korea

Gastrointestinal (GI) ulcer remains a major human disease with a high incidence rate. GI ulcers are defined as a degenerative and necrotic breakdown of gastrointestinal mucosa. Most gastric ulcer-related studies such as screening of antiulcer drugs and studying ulcer pathology rely on laboratory gastric ulcer animal models. Most experiments use rodents for laboratory ulcer models, preferring rats to mice. Rabbits have a predominant population of chief cells on the base of the gastric wall. Thus, rabbits shares more structural similarities with humans. We examined 2 types of rabbit gastric ulcer models: acetic acid-induced ulcers (AAU) and mucosectomy-induced ulcers (MRU). Clinical relevancy of those models was compared with rat models. The stomach of the rabbit was exposed by median laparotomy. The exposed stomach was cut open in line with the large curvature. For AAU, the mucosa was treated with bottled acetic acid for 15 s. For MRU, normal saline was injected in the submucosal layer. The swollen part of the mucosa was resected with scissors. Progressive healing was observed for 7 d after recovery in the whole stomach. Both models showed well-defined ulcer areas with very clear ulcer margins, which were not observed in rat models. Histology also revealed progressive ulcer healing in both models. In particular, MRU showed more precipitated ulcer healing. In conclusion, rabbit gastric ulcer models are more relevant, reliable and simple laboratory ulcer models than small rodent models for estimation of the ulcer healing process and screening of antiulcer drugs. MRU also served as a good model for gastric bleeding, which is nowadays regarded as the most concerning complication of endoscopic mucosectomy.

P243 Determining Circulating Fentanyl Levels in Ovine Blood to Identify Optimum Preoperative Drug Loading of Transdermal Fentanyl Patches before Surgery

JW Rawlinson*, RA Oliver, GJ Mitchell, WR Walsh

Surgical and Orthopaedic Research Laboratory, University of New South Wales, Sydney, NSW, Australia

Preoperative sedation and preemptive pain relief are integral components in the ethical management of sheep used in scientific and medical research. Debate and conjecture arose amongst researchers, veterinarian, and institutional ethics committee members regarding timing for application of transdermal fentanyl patches (TFP) to achieve optimal preemptive analgesia prior to surgery. This study quantified the absorption and elimination curves of TFP using a strict application method. Eight healthy 2-y-old crossbred wethers (average weight 86 kg) were enrolled in the study. The left antebrachum of each sheep was clipped and skin defatted for optimum delivery (dose release of 2 µg/kg/h) from the transdermal patch. The study comprised 2 groups (n = 4). At 24 h (group 1) and 72 h (group 2) prior to a theoretical surgical procedure TFPs were applied. Blood samples were drawn at 0, 3, 6, 12, 24, 36, 48, 72 h after application. A second patch was applied to group1 at 24 h and group 2 at 72 h, respectively, and the blood sampling regimen repeated. Blood was allowed to clot normally, spun at 3000 rpm for 10 min and serum extracted. Fentanyl levels were measured using a BQ Fentanyl ELISA kit. Animal behavior was graded by trained observers. Three hours after patch application, all sheep were noticeable calmer with little resistance to restraint and manual handling, which corresponded to initial fentanyl levels detected in serum. By 6 h, sheep were interacting with handlers and standing unrestrained for sampling. This behavior continued through to 48 h and beyond for most of the sheep. Fentanyl levels declined by 72 h and all sheep were noticeably less compliant to restraint although still cooperative. Fentanyl concentrations peaked at 12 h and remained constant after application of the second patch at 24 h. This study has demonstrated that TFP provides an optimum drug loading regimen between 12 and 24 h after application providing effective sedation without excessive manual handling and restraint.

P244 A Reliable, Fast, and Affordable Noncontact Infrared Thermometer for Measuring Murine Body Temperature

J Hershey*, D Aler, BA Scharf, S Miller

UMDNJ, Piscataway, NJ

There is a significant need for body temperature measurement in mice used for research, including as an overall marker of animal health, a prognostic indicator, and as an experimental endpoint. Temperature is traditionally measured either by placing a thermometer into the rectum of the test subject or implanting telemetry equipment. Rectal temperature can be difficult to perform in an awake mouse as it can be stressful to the animal and there is inherent risk of rectal damage. Telemetry is extremely invasive and has a high risk of side effects and complications. In this study we have shown that surface body temperature, measured at the xyphoid process of the sternum, highly correlates to core body temperature. Other sites such as the ears and base of tail were avoided as these regions tend to have more variability in temperature. Twenty adult mice (1:1 male:female, various strains) were anesthetized with isoflurane gas (2% in pure oxygen) and measurements were taken both traditionally (rectal temperature with a wire thermistor) and using a handheld, noncontact infrared recording device. The infrared thermometer was capable of reading a 1-mm region in 150 ms (± 0.75 °C) from a distance of 62 mm. The reading region of 1 mm is significantly smaller than other infrared temperature recording devices and is ideally suited to small rodents. Recordings were taken at 10-min intervals for 30 min. Additionally, animals were placed either on a warm (37 °C) or room temperature (20 to 22 °C) table to quantify the effect of exogenous warming on mouse body temperature during anesthesia and determine the robustness of the IR measurements. Surface temperature correlated >99% to core temperature in mice both on a heated and unheated table. These data demonstrate that surface temperature is highly predictive of core temperature in mice and is a superior alternative to traditional methods of temperature recording.

P245 Twelve-Week Bone Implantation Study in Sheep to Assess Local Tissue Reaction and Mechanical Strength

J Bartrom*, L Stevenson

NAMSA, Northwood, OH

The objective of this study was to evaluate the local tissue reaction at the bone implantation sites and mechanical bone strength following implantation of an experimental bone void filler (test article) and a marketed bone void filler (comparative control article) in femoral defects created in sheep. No in vitro model can reproduce the conditions encountered in clinical application, which is why an in vivo study was conducted in sheep. Due to the size of the test and control articles, an animal model with bone structures of similar size to humans was needed. For these reasons, young adult sheep were used to evaluate the potential effects and local tissue reaction at the defect sites. One metaphyseal defect was created in each femur of 16 animals. The defect

sites were implanted with either the test or control article. Immediately after implantation, one animal was euthanized to serve as a baseline for mechanical bone strength testing. To ensure appropriate placement of the articles, lateral and anterior/posterior radiographs of each femur were taken immediately following implantation and at study conclusion. Animals were observed twice daily for general health and body weights were collected throughout the study (all animals gained body weight throughout the study and were considered healthy). At 12 wk, the animals were euthanized and the sites were harvested. The femurs of 10 animals were processed and examined microscopically. Pushout testing was conducted on the femurs from the 5 remaining animals. The pushout data indicated that the test and control article defect sites were not statistically significantly different when comparing average maximum pushout force, average maximum shear stress, and average energy to failure. New bone formation occurred in all defect sites implanted with the test and control articles. At 12 wk after implantation the observed tissue response and bone formation was similar between the test and control articles.

P246 Evaluating Gastric Conditions in Beagles

J Roberts*, J Jona, M Peterson, K Salyers, K Stocking, M Wells, Z Zhao

Amgen, Thousand Oaks, CA

Gastric pH in fasting canines has been reported to be higher and more variable than humans. For ionizable molecules, pH has an impact on solubility and this may affect determinations of oral bioavailability in the canine model. The purpose of this study was to assess the gastric pH conditions in beagles and to evaluate the effectiveness of an orally administered buffer (0.1N HCl, 2 mL/kg) to adjust gastric pH. Using radiotelemetry capsules, the gastric conditions were assessed in 3 different conditions: 1) after standard daily feeding, 2) fasted overnight, and 3) fasted overnight with oral pretreatment 15 min before pH recording. For each group, the total gastric retention time (GRT), the high and low pH during the GRT, and the portion of GRT where pH was below 4 were determined. The mean GRT in the fed condition was 779 ± 336 min, in which a mean of 616 ± 341 min was below pH 4. The mean GRT for the fasting condition was 169 ± 64 min and some beagles did not achieve gastric pH values below 4. With pretreatment the GRT was 168 ± 50 min and all dogs tested achieved pH below 4, although there was variability in the duration of low pH. This study confirmed high intersubject variability in gastric pH of fasting canines and validated the use of an oral pretreatment to lower gastric pH without affecting GRT.

P247 Seromonitoring of Viral Agents in Rat Colonies of Brazil: A 17-Year Study

JC Jo*, CY Issey, DM Rodrigues, SC SAntos, LG Andrade, R Gilioli

Animal Health Laboratory, University of Campinas, UNICAMP, Campinas, Brazil

The health monitoring routine of laboratory animals in both breeding and experimental facilities is essential and a great concern to maintain high-quality animals to be used in biomedical research. However, most of the facilities do not have a periodic monitoring program established due to several factors. In the recent years the demand to perform the health monitoring has increased. The present study was conducted to determine the occurrence of viral agents in rat colonies from 49 Brazilian institutions with different sanitary standards. Serological techniques as indirect immunofluorescence assay (IFA), hemagglutination inhibition assay (HAI), and microagglutination assay (MA) were used to detect specific antibodies against 16 antigens for rats. Between the years 1995 to 2012, 1,646 serum samples of rats, mainly the Wistar strain, were analyzed. A higher prevalence of rat coronavirus (RCV/SDAV) (17%), *Mycoplasma pulmonis* (13%), rat Theilovirus (RTV) (9%) and rat minute virus (RMV) (8%) was found. By using the IHA, Kilhan rat virus (KRV) and Toolan H-1 showed positivity of 4% and 2%, respectively. On the other hand, by the microagglutiantion assay *Corynebacterium kutscheri* showed 4% of positivity and *Bordetella bronchiseptica* showed 3%. Although it is not a comprehensive survey, this data showed that the incidence of parvovirus has had a significant growth as have RCV/SDAV, *Mycoplasma pulmonis*, and RTV. These 17-y monitoring data provide an overall picture of the health status for viral agents of rats maintained in the Brazilian facilities. This panel also showed the importance of regular monitoring and good management practices when dealing with laboratory animals.

P248 Effects of Genistein and Daidzein on the Gonadal Function in Wistar Rats

JC Illera*[1], S Caceres[1], L Martinez-Fernandez[1], A Martin-Ruiz[1], M Illera[1], P Millan[1], A Gonzalez-Gil[1], L Peña[3], L Diez[3], CC Perez-Garcia[2], I Diez[2], G Silvan[1]

[1]Animal Fisiology, Fac Veterinaria, University Complutense of Madrid, Madrid, Spain; [2]Medicina, Cirugia y Anatomia Veterinaria, Fac Veterinaria, Universidad de Leon, Leon, Spain; [3]Medicina y Cirugia Animal, Fac Veterinaria, Universidad Complutense de Madrid, Madrid, Spain

In the recent years the interest on the beneficial effects of isoflavones in protection against some hormone-dependent types of cancer and on the immune system, among others has been studied. However, it is still not clear whether isoflavones have effects on the reproductive system under normal dietary intake and overdose. Our aim was to determined how genistein and daidzein, affect the gonadal function on male prepubertal rats by analyzing the testosterone (T) and dihydrotestosterone (DHT) levels in serum with an EIA, which has been previously validated for this species. One-hundred and twenty-five male prepuberal Wistar rats divided in 7 groups: 1 control group and 6 experimental groups were orally administered a daily high and low dose (HD and LD, respectively) of genistein, daidzein, and a mixture of both for 5 wk. Significantly lower T and DHT levels ($P < 0.05$) in all experimental groups compared with the control were detected. In the control group, there was a peak of T and DHT levels (5.71 ng/mL) associated to the onset of puberty at the third week. However, in LD groups, the same peak was found at the fourth week of the experiment (genistein = 2.12 ng/mL; daidzein = 5.4 ng/mL; mixture = 6.2 ng/mL), indicating a delay in the onset of puberty. The histology results revealed that at second week of experiment, the control group started to have a significant quantity of spermatozoa, whereas the isoflavones-treated groups started at the third week. This difference in the time of observation of spermatozoa can also be related to the delay of puberty in isoflavones-treated groups. Moreover, HD groups did not show this T and DHT peak, making undetectable the onset of puberty. We can conclude that administration of isoflavones in male rats affect to the gonadal functions retarding the secretion of both testosterone and dihydrotestosterone, and causing the delay or loss of the onset of puberty.

P249 Effect of Ketamine–Fentanyl on Serum Glucocorticoid Concentrations in NZW Rabbits

A Gonzalez Gil[1], G Silván[1], A Martín-Ruiz[1], S Cáceres[1], L Martínez Fernández[1], L Camacho[1], I Díez Prieto[2], C Pérez García[2], J Illera*[1]

[1]Animal Physiology, Veterinary Medicine School, Complutense University of Madrid, Madrid, Spain; [2]Animal Pathology, Veterinary Medicine School, University of León, León, Spain

Anesthetics can affect several physiologic parameters in laboratory animals. This study was performed in order to characterize the effects of the anesthetic combination ketamine–fentanyl on the serum glucocorticoid concentrations in New Zealand white rabbits (*Oryctolagus cuniculis*). Ten rabbits received 2 treatments with a minimum interexperiment interval of 10 d and were allocated to 2 groups: control group (1-mL saline solution) and treatment group (ketamine, 25 mg/kg IM and fentanyl, 0.02 mg/kg IM). A 24-gauge intravenous catheter was placed in the marginal ear vein under local anesthesia with EMLA cream. Samples (2 mL each) of blood were drawn from the intravenous catheter at 6 time points: just before drug administration and at 10, 30, 60, 120 min and 24 h after injection. Serum glucocorticoid concentrations were measured by competitive enzyme inmmunoassay. The depth of anesthesia was monitored by using the pedal withdrawal, ear pinch, and righting reflexes. Heart and respiratory rates were significantly decreased at 10 to 120 min when compared with control and baseline levels (time 0). The administration of ketamine–fentanyl induced a significant increase in serum cortisol from 10 to 60 min, and corticosterone from 10 to 30 min after injection when compared with control and baseline levels. Based on the literature,

the increment of glucocorticoid concentrations was probably due to the ketamine component of the mixture and a possible suppression of serum glucocorticoid by fentanyl was not observed and seems not to counteract the stimulatory effect by ketamine. Therefore, corticoadrenal function was increased after ketamine–fentanyl administration.

P250 Acute Phase Proteins as Markers of Mouse Transport Stress

J Zaias*[1,2], M Mineau[1], Y Rivas[2], C Cray[2]

[1]Division of Veterinary Resources, [2]Pathology, University of Miami, Miami, FL

Transport of mice has been documented to result in physiologic changes. Many studies in various species have demonstrated changes in adrenal cortical hormones (for example, corticosterone), loss of body weight, and diminished immunologic function in response to transport in land or air-based vehicles. Acute phase proteins are blood proteins that contribute to restoring homeostasis and have been shown to be elevated due to infection, inflammation, trauma, or stress, including specifically transport stress. We measured acute phase proteins (for example, C-reactive protein and haptoglobin) in addition to body weight, corticosterone, and white blood cell count in CD1 and BALB/C mice after transport from the vendor (day 0 = arrival day; days 1, 2, 3, 7, 10, and 14). CD1 mice from day 0 to 2 after arrival showed a nonsignificant loss of weight as compared with age matched acclimated control mice. Thereafter, the mice showed significant weight gain as expected. There was a mild but nonsignificant increase in CRP 1 to 2 d after arrival as compared with controls. There were no significant changes in serum corticosterone, WBC, or other parameters after transport as compared with controls. BALB/c mice weighed significantly less on arrival (day 0) than age matched control mice (17.87 g compared with 19.20 g; Tukey q = 6.1, $P < 0.05$). By 2 d after transport, mice regained weight and continued to increase body weight as expected thereafter. Preliminary evaluation of CRP levels show increases beginning 1 d after transport but were not significantly higher until day 7 after transport. CRP remained elevated relative to control mice through the study (to day 14). Similarly, serum corticosterone began to increase at day 7, peaked at day 10, and then reduced by day 14 after transport. Initial results indicate mild alterations in acute phase proteins; however, there appear to be strain differences. Evaluation of the changes in acute phase proteins and the times for return to baseline provide important acclimatization information for researchers studying inflammatory, infectious, and other disease processes.

P251 Risk for Mosquito-Vectorless Transmission of Dengue Virus between Nonhuman Primates in a Research Setting

J Stephens-DeValle*, J Putnak

NMRC, Silver Spring, MD

Dengue is a mosquito vector-transmitted viral disease caused by 4 virus serotypes, dengue virus (DENV) DENV-1, 2, 3, and 4, which cause acute, febrile diseases of varying severity affecting more than 100 million people in the tropics and subtropics each year. Although several dengue vaccines are in clinical trials, none are yet licensed for human use. Nonhuman primates (NHPs), especially rhesus macaques, are the animal model of choice for preclinical evaluation of candidate dengue vaccines. After DENV infection rhesus macaques may develop circulating virus (viremia) for up to 12 d, but without disease. As highly social animals NHPs have additional requirements that must be considered when these animals are used for research, and pair/group housing to enrich animal welfare is desirable whenever possible. Our study is the first to attempt to model the risk for unplanned dengue transmission between pair- or group-housed animals from a bite, scratch, or other incidental contact involving the exchange of body fluids. An experiment was performed in which 2 rhesus macaques were initially infected with a high-titered, laboratory-propagated challenge virus stock. A small amount of sera (0.05 mL) collected during the viremic period from each infected animal was then transferred to 2 dengue naïve "virtual cage-mates" by subcutaneous inoculation to simulate incidental contact resulting in the transfer of body fluids. The testing of sequentially collected blood samples for dengue viremia demonstrated that both contacts became infected, demonstrating that DENV can in fact be efficiently transmitted between animals by incidental blood-blood contact. Therefore, pair/group housing in studies involving DENV challenge may be contraindicated, at least during the viremic period when the animals are potentially infectious.

P252 Established Method to Assess Cardiorespiratory Parameters in Inhalation Safety Pharmacology Studies in Conscious Beagle Dogs

J Sentz*

Huntingdon Life Sciences, East Millstone, NJ

A critical aspect of inhalation cardiorespiratory safety pharmacology studies is the collection of good quality cardiovascular and respiratory data immediately prior to dose, during dose and immediately after dose to be able to recognize any acute effects of the test article. This is especially challenging when the test article is administered via inhalation. Here, we describe the method for achieving a successful outcome in these types of studies, including the factors that must be considered in the design and execution. Effects of proper habituation to equipment and carefully scheduled study activities were assessed based on the overall character of cardiorespiratory response from 3 different studies. Prior to the first data collection, all animals were surgically implanted with telemetry transmitters and habituated to the exposure/data collection system. Cardiovascular and respiratory parameters were collected using a respiratory inductance plethysmography (RIP) and an acquisition and analysis system. Telemetry data were collected continuously for at least 2 h while animals were in their homecage, after transfer to the exposure suite for an additional 30 min prior to either air or the vehicle, during the 1-h exposure period, 30 min after exposure, and for 24 h postdose in 3 different studies. Each study used a different vehicle and an air (sham) control was administered to assess the vehicle effect. Administration of the vehicle produced no effects on blood pressure, heart rate, body temperature, respiratory rate, tidal volume, and minute volume during predose, exposure period, and for the 24-h postdose recording period when compared with air (sham) control in all 3 studies. Very similar patterns in blood pressure and heart rate were noted in all 3 studies; however, each study has a specific pattern for body temperature and respiratory parameters, which was probably due to individual variation in these parameters. Cardiorespiratory data collected from 3 different inhalation safety pharmacology studies demonstrated that we have successfully developed a consistent and reliable method of collecting cardiorespiratory data from conscious beagle dogs prior to, during and after inhalation exposure in our testing facility environment.

P253 Electrocardiogram Changes Presage Gait Disturbances in SJL/J Mouse Model of Experimental Autoimmune Encephalomyelitis

K Harbert*[1], J Flanagan[1], TG Hampton[2]

[1]Biomedical Research Models, Worcester, MA; [2]Mouse Specifics, Quincy, MA

Multiple sclerosis (MS) is a debilitating immune-mediated disease of the central nervous system. Clinical symptoms may range from numbness and tingling to blindness and paralysis. Autonomic changes in humans have also been described. Much of our current knowledge about MS has been gained from inducing experimental autoimmune encephalomyelitis (EAE) in mice. SJL/J mice induced for EAE exhibit demyelination and clinical symptoms (for example, paralysis) similar to the human condition. The EAE mouse model is widely used to study prevention, improvement, or reversal of the motor disturbances that are routinely scored visually. This study evaluates 20 SJL/J mice induced with EAE using the myelin proteolipid protein peptide (PLP139-151) and pertussis toxin model. Animals had gait and autonomic alterations assessed, which can be used as more quantitative and objective predictive physiologic biomarkers of disease. Progression of locomotor deficit was monitored using ventral plane treadmill videography. Autonomic changes were assessed by changes in heart rate (HR) and HR variability (HRV) monitored via a noninvasive platform for electrocardiogram (ECG) monitoring in awake animals. The videography reported numerous postural and kinematic changes that occurred prior to the onset of overt clinical observations. Significant increases in HR and decreases in HRV were manifest in EAE mice in advance of any motor changes.

This study demonstrates that autonomic changes in the EAE model are profound and in fact presage motor changes. Moreover, automated treadmill gait analysis provides more objective and quantitative assessment of the motor changes in EAE.

P254 Time Course Study on the Effect of Enalapril on Diabetic Nephropathy in the ZSF1 Rat

K Lincoln*, H Chen, P Harrison, E McFarlane, H Clifford, K Boucheva, H Qian, C Boustany

Cardiometabolic, Boehringer-Ingelheim Pharmaceuticals, Ridgefield, CT

Diabetic nephropathy (DN) is the most common cause of end-stage renal disease (ESRD) in Western societies accounting for approximately 600,000 cases in the US. Medicare expenditures in 2012 for ESRD rose 8% to US$32.9 billion. Angiotensinogen converting enzyme inhibitors (ACEi) and angiotensin receptor blockers (ARB) have been shown to slow the decline in patients with DN. The ZSF1 obese rat exhibits the early signs of diabetic nephropathy including hypertension, proteinuria, and hyperglycemia. A 15-wk time course study was conducted to assess the effect of enalapril (ACEi) initiated at different times on proteinuria, fibrosis, and blood pressure. Male ZSF1 obese rats (n = 9 per group), instrumented with telemetry devices for continuous blood pressure recording, were randomized into 6 groups. Enalapril treatment (3 mg/kg in the drinking water; clinically relevant dose) was initiated on study weeks 1, 3, 5, 8, and 10. Enalapril treatment duration was 5, 7, 10, 12, and 15 wk. Vehicle treated ZSF1 obese and lean controls were used as comparators. Weekly urine, body weight, water consumption, food consumption, and clinical observations were performed. Blood pressure was continuously recorded. Blood collections for creatinine and pharmacokinetic determinations were performed on relevant weeks. Kidney histology was conducted at termination (week 15) for incidence of glomerulosclerosis and interstitial fibrosis. Enalapril administered in a prophylactic and therapeutic manner significantly decreases mean arterial pressure (10 to 14 mm Hg for animals treated for 15, 12, and 7 wk) and urinary protein to creatinine ratio (0.8 to 6.5 mg/d for all durations of enalapril treatment) compared with corresponding vehicles (4.5 to 7.5 mm Hg and 5 to 14 mg/d). Glomerular and interstitial lesions were modestly reduced but did not reach statistical significance. In summary, shortening the duration of the treatment with enalapril from 15 to 10 wk still allows for significant effects on proteinuria and blood pressure, while reducing the time spent in metabolism cages and associated distress.

P255 Long-Term Microbiota Stability Associated with *Trichuris muris* Infection

K Scott*[1,2], L Cook[2], J Urban[2], A Ericsson[2], G Turner[2], CL Franklin[2]

[1]Oklahoma State University, Stillwater, OK; [2]Veterinary Pathobiology, University of Missouri, Columbia, MO

Inflammatory bowel diseases (IBDs) affect approximately 1.4 million Americans. Current therapies primarily focus on immune suppression and efficacy greatly varies. Anecdotal evidence and preliminary clinical trials suggest that inoculation with *Trichuris suis* can reduce clinical signs of IBD. Mechanistically, it has been demonstrated that the presence of *Trichuris* spp. in pigs and nonhuman primates modulates mucosal inflammatory responses, alters intestinal microbiota or has both effects. Our laboratory uses a *Helicobacter hepaticus*-triggered typhlitis in female A/J mice as a model for IBD. We have recently shown that infection with *Trichuris muris* exacerbates disease severity in this model and that this change in severity is associated with modulation of the immune response. However, it remains unknown whether *T. muris* infections can also alter microbiota. Thus, we sought to assess microbiota changes immediately following *T. muris* infection and at a later time point when helminths had been cleared. To this end, 2 groups of 8 mice were inoculated concurrently with *H. hepaticus* and *T. muris* at weaning and 2 groups of 8 control mice were inoculated with *H. hepaticus* only. To assess acute fecal microbiota changes, mice from one experimental and one control group were necropsied 4 d after inoculation. To assess chronic changes in microbiota, the remaining groups were necropsied at 90 d after inoculation, a time when disease was ongoing and *T. muris* had been cleared. To identify changes in microbiota, automated ribosomal intergenic spacer analysis (ARISA) was used. ARISA banding patterns, which reflect microbiota diversity and complexity, were highly variable in both 4-d postinoculation groups and no differences between groups were seen. This variability is not uncommon among weanling animals whose microbiota have yet to stabilize. ARISA banding patterns from 90-d postinoculation groups were analyzed blindly for pattern clusters. Again no differences were found between groups. These data suggest that the modulation of disease in this model by helminths is not associated with a long-term shift in fecal microbiota populations.

P256 Nonsurgical Embryo Transfer in Mice Is an Easy, Effective, and Ethical Replacement for Surgery

K Steele*, B Stone, J Hester, A Fath-Goodin

ParaTechs Corporation, Lexington, KY

Surgical embryo transfer (ET) is an effective method to deposit embryos into the uterine horn of mice. However, surgery is expensive, time-consuming, and requires technical expertise. Surgery is also a stressful procedure for the mouse, which has to be anesthetized and treated with an analgesic. We have developed a simple, brief procedure for ET using a nonsurgical device, and our hypothesis is that this nonsurgical procedure is less stressful for the mouse, as effective as surgical ET, and the procedure can be repeated on the same mouse. In order to compare the effectiveness between the nonsurgical and surgical procedures, we performed side-by-side comparisons with 20 mice per method and repeated the experiment using 4 different strains. Pregnancy rate is higher in mice that have undergone nonsurgical ET than mice subjected to surgery, and litter size and birth rate from the 2 procedures are similar. We then performed the nonsurgical method up to 2 more times on individual CD1 mice, each time allowing the mouse to recover for at least 20 d postpartum before becoming pseudopregnant again. The data demonstrate that the nonsurgical ET procedure can be used multiple times on a mouse, but there is a reduced pregnancy rate. Since nonsurgical ET does not require sedation, opening of the inner body cavity, or use of an analgesic, we hypothesized that this procedure is less stressful for the mouse than surgery. We used electrocardiography (n = 11) and fecal corticosterone ELISA (n = 15) to monitor pseudopregnant mice that underwent anesthesia only, nonsurgical ET with and without anesthesia, or surgery. Our results show that the nonsurgical procedure without anesthesia does not affect heart rate or alter the levels of the stress biomarker fecal corticosterone, whereas surgery and anesthesia alone lower heart rate for at least 1 h after administration and increase levels of fecal corticosterone. Responsible animal scientists are required to follow Russell and Burch's 3Rs of animal research: to replace, reduce, and refine. The nonsurgical procedure refines the ET method by minimizing animal stress, and can reduce the number of mice needed for experiments, offering an advantageous alternative to surgical transfer.

P257 Potential Inflammatory Effects of Depilatory Creams in Lewis Rats

L Ratcliffe*, JB Finlay, S Vonderfecht, R Ermel, TW Adamson

Division of Comparative Medicine, City of Hope, Duarte, CA

Chemical depilatory creams are frequently used in rats used in biomedical research to remove fur prior to a surgical or nonsurgical procedure. Although their use is ubiquitous, there is little information available on their proper usage or what effects they may have on research outcomes. It is widely known that when depilatory creams are used on humans, they can have side effects such as skin inflammation and local irritation. In turn, inflammation is known to affect healing, tumor growth, and the immune response. Therefore, it is of utmost importance to examine the potential consequences of using depilatory creams on rats. The aim of this study was to determine the minimum application time necessary for fur removal and to evaluate any potential tissue inflammation at the site of the depilatory cream application. Twelve male Lewis rats each had depilatory cream applied to one flank for either 30 s or 3 min, and the opposite flank was shaved with mechanical clippers. The rats were euthanized at 24 h, 72 h, or 7 d postapplication and tissues were examined grossly. Skin samples from both flanks, in addition to skin that had no experimental manipulation, were collected postmortem and analyzed histologically. Microscopic changes were limited to skin

treated with a chemical depilatory cream and occurred after as little as 30 s of treatment. Changes were characterized by epidermal hyperplasia, hyperkeratosis, parakeratosis, exudate composed primarily of neutrophils on the epidermal surface, and occasional focal ulceration. These lesions were initially seen 24 h after treatment, peaked in incidence and severity at 72 h, and were partially resolved at 1 wk. These data suggest that although mild, the local tissue inflammation seen histologically with depilatory cream application may have an effect on certain studies.

P258 *Uncv* Mice Display Tylosis Esophageal Cancer-Like Phenotype

L Wenlong*[1], W Dongping[1], Y Leilei[2], L Bing[1], Z Lin[1]

[1]Laboratory Animal Center, Academy of the Military Medical Sciences, Beijing, China; [2]Beijing Institute of Radiation Medicine, Beijing, China

Tylosis esophageal cancer (TOC) in humans is characterized by palmoplantar keratoderma and esophageal cancer susceptibility. Due to the lack of animal models, the mechenism of TOC is not yet clear. In a BALB/c genetic background, homozygous *Uncv* mice present a hairless phenotype. A previous study demonstrated that the *Uncv* mouse hair abnormalities were linked to single autosomal gene mutations and were incomplete dominant inheritance. Using genetic analysis, the *Uncv* locus was mapped to an interval between markers D11mit338 and D11mit337 on mouse chromosome 11. In this study, we found that *Uncv* mutant mice present a hypertrophic phenotype in the esophageal epithelium by histologic analysis at postnatal day 120. The mutant esophageal epithelium revealed significantly increased proliferation by Ki67 and keratin 14 staining compared with wildetype littermates (n = 8 per genotype). Furthermore, the expression of epithelial differentiation marker was also found to be increased in the mutant esophagus. Dorsal epidermis of *Uncv* mutant mice is thickened as shown by H&E staining compared with wildetype littermates (n = 12 per genotype). The expression of keratin 14 and markers of epidermal differentiation were increased in dorsal epidermis of Uncv mice at postnatal day 9. In summary, this study identifies that the *Uncv* mutant mice develop TOC-like phenotype with hyperproliferative and hyperkeratotic epidermis and esophageal epithelium. The *Uncv* mice could serve as a murine model of tylosis esophageal cancer.

P259 Challenges in Performing Preclinical Imaging in a Large Cohort Therapeutic Efficacy Study of Murine Cancer Models

L Ileva*[1], M Bernardo[2], NL Patel[1], L Riffle[1], C Graff-Cherry[3], C Robinson[3], S Difilippantonio[3], JD Kalen[1]

[1]Small Animal Imaging Program/Laboratory Animal Sciences Program, [2]Molecular Imaging Program/Laboratory Animal Sciences Program, [3]Laboratory Animal Sciences Program, SAIC-Frederick, Frederick National Laboratory for Cancer Research, Frederick, MD

Preclinical noninvasive in vivo imaging is used to assess therapeutic efficacy in cancer murine models and requires a large number of mice to produce statistically significant data. Institutional biosafety stipulates that handling of mice within 10 d of dosing necessitates additional personnel protection equipment and can impose additional challenges for handling and imaging of animals. Our goals were to: 1) understand the challenges of implementing minimally invasive animal handling techniques and imaging of large number of tumor bearing animals during a daily chemotherapy study, 2) protect the imager from potential chemical hazards, and 3) monitor the tumor therapeutic response by using fast and cost-effective in vivo imaging methods. Animal studies were performed according to ACUC guidelines on a clinical MRI 3.0T scanner using specially designed 2-mouse whole body imaging coils. To reduce contamination from the excreted drug, all animal manipulations were performed in a biosafety cabinet (BSC) or vented hood containing an anesthesia induction chamber. Animals were placed into a clean cage prior to transport to the imaging suite. Special imaging beds were manufactured to provide for anesthesia, ventilation to the laboratory exhaust, output from the pulmonary pad for monitoring, and an outer plastic tube to isolate the animal from the surrounding environment and protect the animal handler. Each imaging bed is inserted into an individual imaging coil and the 2 coil system chamber is air heated to maintain the animal's internal temperature. During the imaging session the next set of animals were prepared in a similar manner resulting in high throughput. Mice underwent two nongated T2W imaging sessions: baseline and 2-wk post treatment. Changing to clean cages, microisolation techniques, and an isolation imaging bed allowed the imager to wear standard personal protective equipment (PPE) for animal handling procedures. The specially designed 2-mouse coil array provided for high throughput and permits a small animal imaging facility to easily image large cohorts of mice (48) in one working day.

P260 Anesthetic Management of a Porcine Stroke Model Using Hypothermia

LM Denning*[1,2], KL Siroen[2,1], TK Mattingly[1,2], PL Lopez-Ojeda[1,2], SP Lownie[1,2]

[1]Clinical Neurologic Sciences, Western University, London, ON, Canada; [2]Clinical Neurologic Sciences, Lawson Health Research Institute, London, ON, Canada

Swine are excellent translational models for human conditions and treatments. Stroke models and endovascular device evaluation are both available in pigs. We developed a stroke model in swine to study endovascular selective hypothermia. The combination of focal cerebral ischemia, endovascular device introduction, selective brain hypothermia, and cardiopulmonary bypass presents unique anesthetic challenges. Twenty-eight domestic swine (50 to 55 kg) were anesthetized using tiletamine–zolazepam–xylazine, intubated, and mechanically ventilated with nitrous oxide–isoflurane. Following placement of femoral venous and arterial sheaths, a frontoorbital craniotomy was performed. A single middle cerebral artery was temporarily clipped for 3 h of ischemia, followed by 3 h of reperfusion. During reperfusion, normothermia was maintained in half the animals, and the other half underwent selective hypothermia using a unique catheter consisting of a 14 °F aortic catheter and a 9.5 °F coaxial carotid balloon catheter. A cardiopulmonary perfusion unit removes blood at body temperature from the aorta, and reinfuses into the carotid at desired temperature. This system was continued until 3 h of reperfusion had occurred. The animals were euthanized with pentobarbital, and the brain perfusion fixated and removed for evaluation of stroke volume. Two animals were unusable due to brain contusion during craniotomy. One animal died from complications of pulmonary stenosis. Ipsilateral nasopharyngeal temperatures dropped to 26.5 °C, while core temperature only dropped to 34.0 °C ($P < 0.001$). Blood pressure, heart rate, hemoglobin, glucose, and oxygenation levels did not differ between normothermic and hypothermic cohorts. Mild acidosis developed in the hypothermia cohort ($P = 0.001$). Phenylephrine or ephedrine was required in 2 normothermic animals but 8 hypothermic animals. Equal numbers of animals required glucose infusions. This model of focal cerebral ischemia in domestic swine evaluating selective hypothermia has unique physiology. Selective hypothermia via an endovascular approach is not overtly destabilizing but attention to metabolic and physiologic changes is mandatory.

P261 Impact of Ventilated Caging on Water Intake and Loss in Four Strains of Laboratory Mouse

M Nicolaus*, L Joseph, VK Bergdall, I Davis, J Hickman-Davis

The Ohio State University, Columbus, OH

Water consumption by mice is affected by availability of food, temperature, humidity, strain and caging. The goal of this study was to understand water turnover in common strains of mice housed in ventilated or static caging. 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. Male and female SCID, SKH, C57BL/6 (C57), and FVB mice were housed 5 to a cage in static or individually ventilated cages (IVC). Hydration levels in all strains were assessed every 48 h by measuring body weight, TEWL, urine osmolality, and water consumption. Intracage temperature and humidity were measured every 48 h for comparison to the macroenvironment. Static cages were monitored for 7 d and IVC for 14 d before cage change out. Females drank less water than males in all strains. With the exception of C57 strain, IVC housed mice of all strains and sexes drank less water than those housed in static cages. Water consumption in IVC ranged from

3.32 ± 0.4 to 4.8 ± 0.6 mL/d. TEWL levels for SCID, SKH and C57 mice paralleled water consumption for all housing conditions. TEWL for IVC housed mice ranged from 7.7 ± 2 to 25.9 ± 19 g/m^2/h compared with 8.8 ± 5 to 10.6 ± 6 g/m^2/h for static cages. Temperature and humidity within the cage was significantly higher than the macroenvironment for all housing conditions, mouse strains and sexes. Temperatures within IVC ranged from 76 ± 2 °F to 82 ± 2 °F compared with 69 ± 2 °F in the room. Humidity within IVC ranged from 68% ± 18% to 84% ± 13% compared with 28% ± 8% within the room. These data indicate that macroenvironment measurements do not reflect the intracage environment and demonstrate the impact of caging type on TEWL.

P262 Evaluation of the Impact of Injection Techniques on Mice by Telemetric Measurement

N Reitz[2], G Wexel[2], K Reifenberg[1], M Beisele[*3,1]

[1]DKFZ, Heidelberg, Germany; [2]University of Mainz, Mainz, Germany; [3]Novartis Vaccines and Diagnostics, Marburg, Germany

Pain and distress can influence the physiology of laboratory animals and significantly affect research results. Thus, it is important to evaluate the impact on animals by experimental procedures objectively. Heart rate (HF), body temperature (T), and activity (A) of laboratory animals represent suitable parameters for assessment of pain and distress. However, these parameters are per se dramatically influenced in human presence. This disadvantage can be overcome by the use of implantable telemetry. The aim of our study was to objectify the potential pain and distress induced by routine injection procedures (subcutaneous, intravenous, intraperitoneal, and footpad) and to detect the potential role of genetic factors. Therefore, we implanted telemetry transmitters in 16 female C57BL6/J, BABL/c, and NMRI mice, respectively. The control groups were restrained, but not injected. To minimize interindividual variation, control and injection groups were rotated. Injection volume and needle size were chosen according to guidelines of the Committee of Animal Welfare Officers of the GV-SOLAS. The parameters HF, T, A were recorded from 3 d before (baseline), to 3 d after manipulation. Mice were manipulated during the light and dark cycle, respectively. All values were statistically analyzed by repeated measure ANOVA. During the light cycle, mere handling of the mice increased HF (Δ approximately 120 bpm), T (Δ approximately 1°C), and A (Δ approximately 20 AU, arbitrary units) significantly, as compared with the baseline. In contrast, only slight physiologic reactions to handling could be observed during the dark cycle (HF: Δ approximately 20 bpm), T: Δ approximately 0.2 °C, A: Δ approximately 2 AU), indicating that the significant increase of HF, T, and A observed in the light cycle was predominantly induced by the disruption of the inactivity phase of the animals. Consequently, handling cannot be interpreted as a major stress factor. No significant differences of HF, T, or A reactions could be observed between the injection and control mice, neither in the light nor in the dark cycle. Thus, our data shows that the mentioned injection techniques do not have a significant influence on HF, T, and A, and most probably do not represent a relevant stress factor. This trend could be observed in all strains investigated, suggesting that genetic factors are of minor importance.

P263 Lumbar Intrathecal Catheterization in the Nonhuman Primate

M Batchelder[*1], J Ehrmann[2], WL West[2], W Johnson[3], S Aborn[1]

[1]Veterinary Sciences, [2]Veterinary Sciences, [3]Veterinary Sciences, Bristol-Myers Squibb, Pennington, NJ

Alzheimer disease (AD) is a progressive neurodegenerative disorder characterized by an excessive production of extracellular amyloid plaques and intracellular neurofibrillary tangles in the brain. It is estimated to affect 5.3 million people and is the sixth leading cause of death in the Unites States. In an effort to support the investigation of specialized cerebrospinal fluid (CSF) biomarkers, a reliable and reproducible chronic system was developed to collect lumbar CSF from conscious primates. Lumbar intrathecal catheters were surgically placed in 6 male cynomolgus macaques and connected with subcutaneous vascular access ports. Sequential samples of CSF can be collected from the ports, using sterile technique, while the animals are restrained without sedation in a standard primate chair. All 6 animals were successfully catheterized, and all have ports which are still functioning after an average of 496 d. This model simulates the procedures used in humans for studying specialized CSF biomarkers such as Aβ and p-tau. It permits our scientists to gain insights into the central pharmacokinetics and pharmacodynamics of compounds in plasma and lumbar CSF in the study of AD biomarker modification. The complication rates and longevity of the intrathecal catheters compare favorably with those in a similar canine model.

P264 Withdrawn

P265 Design and Conduction of an In Vivo Study Using Homeopathic Drugs: The Effect of Mercuric Nitrate in Mice

ME Monterde-Coronel[2], ME Aguilar[*1], J Asbun-Bojalil[2], JL Aguilar-Faisal[2]

[1]Animal Facility, [2]Postgrado e Investigación, Escuela Superior de Medicina IPN, Distrito Federal, Mexico

Homeopathic medicine is a recognized but scarcely evidence-based alternative for human diseases. Moreover, no animal-based studies supporting safety or effectiveness are commonly performed. The rationale behind is the very low concentration of substances used in clinical practice. Nonetheless, standardization of studies that support homeopathic medicine is needed. Commonly prescribed drugs in homeopathy include substances derived from plants, animals, or metals. In order to determine the effects of a homeopathic substance derived from mercury (mercuric nitrate, used in the treatment of human periodontal disease), we conducted a dose-finding study in CD1 mice. Protocol was approved by our IACUC. Starting from the clinical used concentration of 12C (centesimal, equivalent to 5 × 10 to 21 ppm), 5 groups (n = 5 per group) with different dilutions of mercuric nitrate (placebo, 1:100, 1:10, 1:1, 1:0.1) were treated orally (50 µL) once a day for 7 d. Maximum dose (1:0.1 dilution) represents more than 450 times the usual human dose. Animals were daily observed for behavioral changes, euthanized, and necropsied by blinded investigators. ANOVA with Dunnet post hoc analysis was used, and $P < 0.05$ was considered statistically significant. We found no evident clinical or behavioral changes in tested mice. Endpoint biochemistry revealed no significant differences for WBC, RBC, Hb, platelets, creatinine, AST, ALT, ALK, or BUN among groups. At H&E no tissue alterations were detected. We conclude that mercuric nitrate was safe at tested dilutions. Classic clinical homeopathy uses mental symptoms, present as comorbidities for the disease, to choose the adequate dose/substance for treatment. Therefore, resistance to test homeopathic drugs using preclinical toxicology can be observed in clinical settings. This is the very first study of a homeopathic drug in our institution using animal modeling.

P266 Development of a Thermal Injury Model in Mice

M Camara[*1], S Oldham[2], B McConnell[3], DL Goldsteen[2], K Stover[1], A DiGiandomenico[1]

[1]Department of Infectious Disease, [2]Laboratory Animal Resources, [3]Facilities Contractor, WW Welding, MedImmune, Gaithersburg, MD

Pseudomonas aeruginosa is a versatile gram-negative bacterium that causes life-threatening infections in immunosuppressed and critically ill patients. In particular, *P. aeruginosa* is a leading cause of infections in burn patients. Despite aggressive antibiotic treatment, *P. aeruginosa* infections persist, which is attributable, in part, to this bacterium's innate resistance factors and its ability to adapt to immune pressures. We sought to develop an infection model that mimics the clinical manifestation of infections in burn patients for the analysis of *P. aeruginosa*-specific antibodies. In collaboration with Facilities and Laboratory Animal Resources Departments, we developed a device that reduces the variability of the *P. aeruginosa* thermal injury model. After confirming that our thermal injury device induced a nonlethal third degree burn, we found that mortality in *P. aeruginosa*-infected mice was directly proportional to the percent of the injured total body surface area. We found that a burn corresponding to 12% of the body surface area was optimal for lethality at challenge doses that ranged from 1e2 to 1e6 CFU. We next analyzed our lead anti*P. aeruginosa* bispecific antibody that was

previously shown to be protective in mouse models of acute pneumonia and bacteremia. Several independent studies using both prophylactic and treatment modalities revealed that our antibody reduced lethality in infected mice when compared with a control antibody. In conclusions, our results indicate that we have successfully developed a model that will allow us to evaluate the protective activity of antibodies against bacteria in an animal burn model of infection.

P267 Successful Derivation of Rat Embryonic Stem Cell Lines from Parthenogenetically Developing Blastocysts

M Hirabayashi*[1], C Tamura[1], M Sanbo[1], T Goto[1,2], M Kato-Itoh[3], H Sato[3], T Kobayashi[4], H Nakauchi[3,5], S Hochi[6]

[1]Section of Mammalian Transgenesis, National Institute for Physiologic Sciences, Okazaki, Japan; [2]Nagoya University, Nagoya, Japan; [3]ERATO, Nakauchi Stem Cell and Organ Regeneration Project, Tokyo, Japan; [4]University of Cambridge, Cambridge, United Kingdom; [5]The University of Tokyo, Tokyo, Japan; [6]Shinshu University, Ueda, Japan

Parthenote-derived embryonic stem cells (pESCs) with germline competency have been reported only in mice. The present study was undertaken to establish the pESC lines from parthenogenetically developing rat blastocysts. Ten parthenogenetic blastocysts were prepared by chemical activation of mature oocytes recovered from homozygous CAG/Venus transgenic females with 5 mM ionomycin for 7 min and 10 µg/mL cycloheximide + 2 mM 6-dimethylaminopurine for 4 h at 37 °C in 5% CO_2 in air, and the subsequent in vivo culture. Four pESC lines were established using 2i system (PD0325901 and CHIR99021) combined with rat LIF and forskolin. In 3 out of the 4 lines, the expression of stem cell marker genes (Oct-4, Nanog, Fgf-4, Rex-1) was examined by RT-PCR and the degree of methylation at the DMR locus of 5 imprinted genes (H19, Meg3 IG, Igf2r, Peg5, Peg10) was analyzed by COBRA. Chimeric rats were produced by blastocyst injection, and the G1 offspring were analyzed for Venus expression. Three rat pESC lines were positive for alkaline phosphatase at passage 7, and expressed the stem cell marker genes. The DMRs in the pESCs remained to be demethylated as in the control ES cell lines established from normal blastocysts. Thirty-four chimeric rats (14 female and 20 male) were produced from 124 microinjected blastocysts, and 9 females (3 each from 3 lines) were selected for G1 analysis. One chimeric female gave birth to 38 G1 offspring, and 3 offspring among them were Venus-positive. In conclusion, a germline-competent rat ES cell line was successfully established from a parthenogenetic blastocyst. The pES cell line with uniparental disomy has unique advantages for model systems of transplantation therapies because their derivative cells can be transplanted into both homozygous and heterozygous recipients without immune rejection. This is the first report on successful establishment of pESCs in rats.

P268 Evaluation of the Wellbeing of Rats Housed in Commercially Available Multilevel Caging with Variable Use of Red-Tinted Polycarbonate

MP Swan*, D Hickman

Laboratory Animal Resource Center, Indiana University School of Medicine, Indianapolis, IN

In the laboratory setting, rats are commonly housed in clear caging in brightly lit rooms. As their natural history suggests a preference for low-light conditions, this practice is likely stressful for rats. The retinal anatomy of rats suggests that they have limited visibility to see the red spectrum of light, so red-tinted caging would replicate a darkened condition for the rodent. We examined the welfare of rodents housed in red-tinted caging using a multilevel caging type that allowed the rat to select their microenvironment. Forty-eight Sprague–Dawley rats were divided into 6 treatment groups: entirely clear, entirely red, red top/clear bottom, clear top/red bottom, clear with access restricted to top shelf, and entirely clear with a red intra cage shelter. Rodents were allowed to acclimate to their housing treatment for 5 wk before being tested in the elevated plus maze and open field tests. Video recordings were collected for each rat during the peak of the light cycle and the peak of the dark cycle to determine its use of the given environment and the role of color in selection of microenvironment. Results from the video recordings indicated that rats actively sought the red environment when it was provided ($P < 0.01$). Rats within the all red caging showed decreased anxiety within the elevated plus maze ($P < 0.04$), while those in a clear cage with a red intracage shelter exhibited increased anxiety behavior in the elevated plus maze ($P < 0.03$). These findings suggest that an all red cage could be beneficial to the rodent's wellbeing while intracage shelters may be detrimental.

P269 Efficiency of Generating JM8A3 ES-Cell Chimeric Mice Using BALB/cAnNTac Donor Mice with or without Superovulation

M Esmail*[1], P Qi[1], A Burds[2], A Garcia[1], JG Fox[1]

[1]Division of Comparative Medicine, [2]Koch Institute for Integrative Cancer Research, Massachusetts Institute of Technology, Cambridge, MA

BALB/cAnNTac (BALB/c) mice are routinely used as embryo donors for microinjection of embryonic stem (ES-) cells to generate chimeric mice. Previous studies using BALB/c mice suggested that superovulation efficiency varies relative to the substrain and age of donor mice used. In addition, recent studies have shown much higher chimerism in microinjected 8-cell donor embryos compared with blastocyst embryos. In the current study, we evaluated and compared efficiencies when using BALB/c embryo donors in three protocols: superovulation and breeding at 4 or 6 wk of age or breeding at >10 wk of age without superovulation. The latter group's data was obtained through retrospective analysis of unreported data. Three efficiency parameters measured include average number of injectable embryos per donor, percentage of live pups delivered of total embryos transferred to recipient, and the percentage of live chimeric pups. Donor 8-cell and blastocyst embryos were obtained from flushed mouse oviducts and uteri, respectively, after euthanasia. Donor embryos were obtained from both 4- and 6-wk-old mice at 2.5 and 3.5 d postcoitus (dpc) to obtain 8-cell and blastocyst embryos, respectively, and were injected with unmanipulated JM8A3 ES-cells (derived from C57BL/6N mice), on the same day. Donor blastocyst embryos obtained from >10-wk-old mice were microinjected with manipulated JM8A3 ES-cells on the same day. Injected blastocyst embryos were surgically transferred to Crl:CD1(ICR) pseudopregnant recipient mice. Average numbers of injectable embryos per 4-, 6-, and >10-wk-old mouse ($n = 18, 9, 27$) were 6.9, 1.2, and 2.4, respectively. Live pup success of total injected 4-, 6-, and >10-wk-old mice donor injected embryos transferred to recipient mice ($n = 6, 3, 12$) were 15.6%, 6.3%, and 42.0%, respectively. Chimeric pup success of all live pups ($n = 13, 2, 55$) from 4-, 6-, and >10-wk-old mice donor embryos were 29%, 0%, and 47.3%. No chimeric pups were generated from ES-cell injection of 8-cell embryos from any donors. This study suggests that superovulation leads to increased numbers of injectable embryos in 4-wk-old BALB/c mice; however, live pup and chimeric efficiencies are higher when breeding >10-wk-old BALB/c mice.

P270 Prevalence of *Batrachochytrium dendrobatis* in 120 Archived Specimens of *Rana catesbeiana* (American Bullfrog) Collected in California, 1924 to 2007

M Huss*[1], L Huntley[2], J Johns[1], V Vredenburg[3], SL Green[1]

[1]Department of Comparative Medicine, [2]Department of Electrical Engineering, Stanford University, Stanford, CA; [3]Department of Biology, San Francisco State University, San Francisco, CA

The chytrid fungus, *Batrachochytrium dendrobatis* (Bd), has been identified as a major cause of the current worldwide amphibian decline. Approximately 60 native amphibian populations in North America alone are under threat or have succumbed to Bd infection. The American bullfrog (*Rana catesbeiana*) has been reported as tolerant carrier of Bd. In this report, we used qPCR to test 122 archived American bullfrog specimens collected between 1924 and 2007 in California and in Baja, Mexico. The overall prevalence of Bd infection from this archived population of *R. catesbeiana* was 18.9%. The earliest positive specimen was collected in Santa Clara County in 1929 and is to date the earliest positive archived Bd specimen reported anywhere. This data documents the presence of Bd-infected wild *R. catesbeiana* in California for most of the past century and supports the prevailing hypothesis that the American bullfrog played significant early role in the spread of Bd.

P271 Diagnostic Utility of Cardiac Biomarkers for Cardiomyopathy in Squirrel Monkeys (*Saimiri* spp.)

M Huss*[1], F Ikeno[2], C Buckmaster[3], J Johns[1], R Moorhead[1], MA Albertelli[1]

[1]Comparative Medicine, [2]Med/Cardiovascular Medicine, [3]Psychiatry and Behavioral Sciences, Stanford University, Stanford, CA

Cardiomyopathy is a major cause of mortality in aging squirrel monkeys (*Saimiri* spp.); however, characterization and clinical presentation are not well understood. The diagnosis and management of squirrel monkey heart disease would greatly benefit from the identification of a sensitive biomarker of cardiomyopathy. In this study, we evaluated the diagnostic utility of 3 cardiac biomarkers used commonly in human and veterinary medicine: cardiac troponin I (cTnI), N-terminal prohormone of brain natriuretic peptide (NT-proBNP) and C-reactive protein (CRP). Physical examination, echocardiography (echo) and electrocardiography (ECG) were performed on a colony of 63 clinically healthy squirrel monkeys ranging from 3 to 19 y of age. Parameters evaluated included heart rate, P-wave interval and amplitude, PR, QRS and QT intervals, and R wave amplitude. 2D and M-mode echo was performed on unsedated animals to determine left ventricular internal diameter-systole (LVIDs) and -diastole (LVIDd), and ejection fraction (EF). Initial physical examination, ECG, and echo indicated normal cardiac function for all colony animals. The average EF across all ages was 74.5% (range: 50.3% to 90.6%). Three of 63 animals had an EF < 56.0% (50.3%, 52.5%, and 55.5%). Venipuncture was used to collect samples for the evaluation of cTnI, NT-proBNP, and CRP levels. Additionally, stored serum samples were run on 2 postmortem confirmed cases of cardiomyopathy. CRP and cTnI did not prove to be informative for cardiovascular disease. In postmortem confirmed cardiomyopathy cases and live animals with EF < 56%, the mean NT-proBNP value (6370 pg/mL) was approximately twice the mean value for clinically healthy animals (3130 pg/mL). More extensive studies are needed to establish reference intervals for squirrel monkeys. We conclude that NT-proBNP is a useful diagnostic biomarker and shows potential to be used as a screening test for cardiomyopathy in squirrel monkeys.

P272 Age-Related Changes in Heart Rate Variability in Nonhuman Primate

N Ageyama*[1], H Koie[2], H Kawashima[2], S Okabayashi[1], Y Ito[1,2], K Kanayama[2], T Sankai[1], Y Yasutomi[1]

[1]Tsukuba Primate Research Center, National Institute of Biomedical Innovation, Tsukuba, Japan; [2]College of Bioresource Science, Nihon University, Fujisawa, Japan

Studies of heart rate variability (HRV), which represents a useful marker of autonomic nerve activity, have yielded valuable information concerning the prognosis of heart failure and myocardial infarction. However, age-related changes in HRV associated with cardiovascular disease have yet to be fully elucidated. Little is known about age-related changes in autonomic function because very few studies have included older individuals. Therefore, we investigated HRV in nonhuman primate to determine age-related changes in autonomic nerve activity. We obtained 24-h Holter electrocardiogram in 5 young (mean age, 7.8 ± 1.9 y) and 6 aged (mean age, 19.8 ± 2.3 y) cynomolgus monkeys (*Macaca fascicularis*) bred at our institution. High (HF, 0.15 to 0. 40 Hz) and low (LF, 0.04 to 0.15 Hz) frequency components, as well as the LF/HF ratio were analyzed as HRV reflecting autonomic nerve activity by using power spectral analysis. We also simultaneously evaluated cardiac function and histopathologic findings in all monkeys. The HF components that reflect parasympathetic nerve activity were significantly lower, and the LF/HF ratio that reflects sympathetic nerve activity was higher in the aged than in the young monkeys. Histologic findings revealed significantly larger areas of interstitial fibrosis in the aged than in the young monkeys. Cardiac function indices did not significantly differ between the 2 groups. These results indicate that parasympathetic nerve activity decreases, whereas both sympathetic nerve activity and myocardial damage increase with age in cynomolgus monkeys, rendering them useful as models of age-related changes in the human heart and autonomic nervous system.

P273 In Vitro Fertilization Using Frozen-Thawed Genetically Engineered Mouse Sperm Derived from Cold-Transported Epididymides

N Nakagata*, K Fukumoto, Y Haruguchi, T Kondo, Y Takeshita, Y Nakamuta, H Matsunaga, T Umeno, M Nishimura, M Iwamoto, F Takahashi, E Kohagura, S Tsuchiyama, T Takeo

Center for Animal Resources and Development, Kumamoto University, Kumamoto, Japan

Transport of mouse epididymides is a useful technology to exchange genetically engineered mice between research facilities. We have applied the system to transport genetically engineered mice from our customers to our center and performed in vitro fertilization (IVF) for embryo production or sperm cryopreservation for archiving the genetic resource. However, the fertility of the cryopreserved cold-stored sperm is not clear. In this study, we examined the fertilization ability of the cryopreserved cold-transported sperm of genetically engineered mice by IVF. Epididymides were transported from our customers by our developed kit and then we performed sperm cryopreservation and IVF using these sperm in our center. Sperm preincubation and fertilization media were used. All specimens safely arrived at our center within 48 h (n = 69). The cryopreserved cold-stored sperm indicated high fertilization rates (87% ± 19%). In conclusion, in the future, the transport system of mouse epididymides at refrigerated temperatures will become an extremely powerful tool for exchanging a large number of genetically engineered mouse strains.

P274 A Novel AMPK Interacting Protein in Skeletal Muscle Metabolism

NL Reyes*[1], G Banks[2], J Ramirez[1], D Hirenallur[1], M Tsang[1], D Margineantu[3], D Hockenbery[3], J Chamberlain[2], H Liggitt[1], BM Iritani[1]

[1]Department of Comparative Medicine, University of Washington, Lynnwood, WA; [2]Department of Medicine, University of Washington, Seattle, WA; [3]Clinical Division, Fred Hutchinson Cancer Research Center, Seattle, WA

Skeletal muscle is characterized by the presence of 2 distinct categories of muscle fibers (type I slow twitch and type II fast twitch), which display marked differences in contractibility, metabolism, and susceptibility to fatigue. Slow twitch fibers are rich in mitochondria, use predominantly oxidative phosphorylation for energy production, and are resistant to fatigue. In contrast, fast twitch fibers (IIa, IIb, and IIx) 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 largely unknown. Using a chemical mutagenesis strategy in mice to discover novel immune function genes, we previously identified a novel pedigree that lacks B lymphocytes due to a 32-base-pair deletion in the folliculin interacting protein 1 (Fnip1) gene. Surprisingly, skeletal muscle from Fnip1-null mice also appeared deeper red coloration when compared with WT mice. Although the functions of Fnip1 protein are not known, it has been shown to interact with the master metabolic regulator AMP kinase, an enzyme which helps maintain energy balance. Biochemical and molecular analysis of Fnip1 null and wildetype skeletal muscle revealed that loss of Fnip1 results in increased myoglobin content, increased mitochondria number, and increased expression of mitochondrial genes and proteins. Cultured Fnip1 null muscle fibers also have higher oxidative capacity relative to wildetype fibers, and isolated Fnip1 null skeletal muscles sustain more prolonged contraction, and have more rapid post contraction recovery when compared with wildetype skeletal muscle. These results indicate that Fnip1 controls skeletal muscle fiber type specification, and suggest that inhibition of Fnip1 could be used as a potential therapeutic strategy to increase mitochondrial biogenesis and muscle function in patients with muscular dystrophy diseases, which are typified by defective mitochondrial function

P275 Open-Flow Microperfusion: A Novel Technique for Direct Sampling of Insulin in the Interstitial Fluid of Subcutaneous Adipose Tissue

N Steffensen*[1], TJ Alsted[1], C Höfferer[2], AR Sørensen[1], CL Brandt[1], CJ Bekker[1], JJ Fels[1], F Sinner[2,3], TR Pieber[2,3], C Fledelius[1]

[1]Diabetes Research Unit, Novo Nordisk A/S, Maaloev, Denmark; [2]Biomedical Technology and Monitoring, Joanneum Research, Graz, Austria; [3]Medical University of Graz, Graz, Austria

The biologic action of insulin is thought to be dependent on its appearance in the interstitial fluid (ISF) rather than in the plasma. Direct access to the ISF can be achieved by means of open-flow microperfusion (OFM), which uses a membrane-free sampling probe inserted in the tissue of interest. The aim of this study performed in rats, was to evaluate the application of OFM to: 1) compare pharmacokinetics (PK) of insulin in plasma and a peripheral tissue, 2) compare PK profiles in the 2 compartments with the pharmacodynamic (PD) effects of insulin during a hyperinsulinemic-euglycemic clamp. In a subset of animals, computer tomographic (CT) scanning was performed to explore subcutaneous adipose tissue (SAT) and verify the implantation of the OFM probes. Furthermore, in response to a subcutaneous insulin bolus, SAT was sampled and assayed for changes in insulin signalling. The OFM technique was performed in male Sprague–Dawley rats with permanent catheters for vascular access. Three OFM probes were inserted into the SAT under general anesthesia and a multichannel push-pull pump was used to perfuse the probes at a constant perfusion rate of 1 µL/min. Human insulin (HI) was infused at rates of 20 or 80 pmol/min/kg and the glucose infusion rate (GIR) was adjusted to maintain euglycemia (9 mmol/L). OFM and plasma samples were collected every 45 min. CT scanning data confirmed the correct placement of the OFM probes into the SAT, and insulin phosphorylation data confirmed that the SAT was responsive to insulin treatment. Steady-state (SS) concentrations of HI-80 were reached after 23 and 112 min in plasma and ISF, respectively, while SS GIR was reached after 105 min. Levels of free fatty acid and glycerol in the ISF decreased in response to insulin. It is concluded that OFM can be applied to measure PK and PD of insulin in a peripheral insulin-responsive tissue.

P276 In Vivo Fluorescence Imaging: Limitations for Whole Body Biodistribution Studies

NL Patel*[1], S Bhattacharyya[2], L Wei[2], L Riffle[1], G Hill[3], PM Jacobs[4], KR Zinn[5], E Rosenthal[5], JD Kalen[1]

[1]Small Animal Imaging Program/Laboratory Animal Sciences Program, [2]Advanced and Development Research Directorate, [3]Clinical Research Directorate/CMRP, SAIC-Frederick, Frederick National Laboratory for Cancer Research, Frederick, MD; [4]Cancer Imaging Program, Division of Cancer Treatment and Diagnosis/NCI/NIH, Bethesda, MD; [5]Department of Surgery, University of Alabama at Birmingham, Birmingham, AL

2D epifluorescence has been used to quantify drug distribution in organs (biodistribution), but due to the probe wavelength, only 2 to 3 mm depth is investigated. 3D transillumination fluorescence imaging has gained increasing popularity due to advancement in instrumentation, image reconstruction algorithms, and the availability of target specific near infrared (NIR) probes. X-ray CT is used to quantify photon attenuation in positron emission tomography (PET) and provide quantifiable biodistribution (% injected dose per gram of tissue), while 3D fluorescence reconstruction algorithms assume homogenous optical properties. Presented work highlights limitations in quantifying 3D transillumination biodistribution studies. Thirty female athymic nude mice were subcutaneously implanted with very low (BT-474), moderate (MDA-MB-231), and high (MDA-MB-468) HER1-protein expression levels tumors. Mice were intravenously injected via the tail vein with panitumumab labeled with [89Zr] (Pan-89Zr) or [IRDye800] (Pan-IR 800). Both compounds contained 100 µg of panitumumab. We compared in vivo kinetics and ex vivo biodistribution of the two compounds using the fluorescence FMT 2500 and the Inveon PET/CT scanners. 3D fluorescence resulted in higher (%ID/gm) estimation than PET/CT due to inability to quantify the injected dose, that is, MDA-MB-468 tumor at 24 h after injection (1.46 ± 0.92 and 0.18 ± 0.04) for 3D fluorescence and PET/CT, respectively. Even though absolute values did not agree, good correlation with PET/CT was observed for 2D ex vivo for lymph nodes (0.89), kidney (0.99), heart (0.73), lung (0.86), muscle (0.89), and brain (0.89) for all time points. Highly perfused organs resulted in lower fluorescence signal due to hemoglobin attenuation and therefore resulted in poor correlation for liver (0.14) and spleen (0.46). Preclinical 3D fluorescence offers a low cost and semiquantitative alternative to perform whole body biodistribution studies. However, caution must be taken as the results are dependent on probe wavelength and tissue optical properties and should not be used to estimate radiation dosimetry for the analogous radioactive probe.

P277 Comparative Evaluation on the Performance of Fluorescence Preclinical Optical Imagers

NL Patel*[1], LV Ileva[1], LA Riffle[1], PL Choyke[2], L Feigenbaum[3], P Grodinski[4], PM Jacobs[5], KL Komschlies[6], JL Tatum[5], JD Kalen[1]

[1]Small Animal Imaging Program/Laboratory Animal Sciences Program, SAIC-Frederick, Frederick National Laboratory for Cancer Research, Frederick, MD; [2]Molecular Imaging Program, Center for Cancer Research, National Cancer Institute, NIH, Bethesda, MD; [3]Laboratory Animal Sciences Program, SAIC-Frederick, Frederick National Laboratory for Cancer Research, Frederick, MD; [4]Center for Strategic Scientific Initiatives Office, [5]Division of Cancer Treatment and Diagnosis, Cancer Imaging Program, National Cancer Institute, NIH, Bethesda, MD; [6]Office of the Director, Frederick National Laboratory for Cancer Research, Frederick, MD

Fluorescence imaging is a cost effective method widely used in preclinical research. Selecting the correct scanner for in vivo small animal imaging depends on the biologic application, organ of interest, optical probe wavelength, ease of use, and budget. We investigated and compared the performance of 3 fluorescence imagers (henceforth referred to as: imager 1, imager 2, and imager 3). The primary objective of this study is to evaluate the performance and correlation between ex vivo and in vivo quantifications, within and between imagers. Five female athymic nude mice were injected into the dorsal lumber and thoracic regions with 1×10^7 HT29 (human colon adenocarcinoma) cells. Imaging commenced (per manufacturers' recommendation) 22 h after intravenous tail vein injection of 2 nmol/25 g of matrix metalloproteinase (MMP) activatable agent. Imaging sequence was imager 1 (Ex/Em 671-705/750-930 ± 2 nm), imager 3 (Ex/Em 750/780 nm) and then imager 2 (Ex/Em 745 ± 35/800-820-840 ± 20). Animals were then euthanized and ex vivo organs were 2D reflectance imaged using the prior scanner filter settings. Image analysis was performed according to the manufacturers' protocols and compared the total signal and concentration (total signal/ROI area) for the various scanners. Correlation between scanners resulted in $R^2 > 73\%$ when same size ROI and total signal was used and correlation of $R^2 < 50\%$ was observed when comparing concentrations for 2D and 3D datasets. Total signal within the ROI is less sensitive to placement and that concentration should be used when ROI's can be fitted (threshold). Unfortunately, all scanners do not have ROI threshold capability, and therefore, resulted in reduced correlation. Overall, the ability to unmix tissue autofluorescence from the fluorophore signal for nonNIR fluorophores, bright lamps/lasers to provide strong excitation signals, NIR capability, and the ability to threshold ROIs (segmentation) was found to provide scanner parameters that resulted in high correlations.

P278 Effect of Resveratrol Treatment on In Vitro Fertilizability of Mouse Epididymal Sperm

O Suzuki*

Laboratory of Animal Models for Human Diseases, National Institute of Biomedical Innovation, Ibaraki, Japan

Strain differences in in vitro fertilizability are problematic in mouse reproduction. The role of sirtuin in spermatogenesis has been reported. Therefore, the effect of long-term administration of the sirtuin activator resveratrol on the in vitro fertilizability of mouse epididymal sperm was assessed. Three doses (0.1, 0.5, and 1 mg) of time-release resveratrol pellets or corresponding placebo pellets (21-d release form) were

subcutaneously implanted in 9-wk-old 129X1 male mice. Three weeks later, in vitro fertilization was conducted using epididymal sperm from males and oocytes from superovulated ICR females. The numbers of 2-cell embryos and blastocysts were recorded at 24 and 96 h, respectively, after insemination. Testicular Sirt1 protein levels were measured by quantitative Western blotting using GAPDH as an internal control. Although no significant difference was found by weighted ANOVA with angular transformation in development to the 2-cell stage at any dose (mean ± SEM, $n = 3$; placebo compared with resveratrol, 0.1 mg: 65.0% ± 0.09% compared with 73.4% ± 0.13%; 0.5 mg: 65.2% ± 0.14% compared with 69.3% ± 0.08%; 1 mg: 65.7% ± 0.08% compared with 47.1% ± 0.03%), development to the 2-cell stage in the resveratrol groups at the 2 lower doses tended to be higher. At the highest dose (1 mg), resveratrol tended to suppress the 2-cell rate. The blastocyst formation rate showed the same tendency (0.1 mg: 65.0% ± 8.5% compared with 70.2% ± 1.3%; 0.5 mg: 61.1% ± 15.0% compared with 64.0 ± 7.1%; 1 mg: 64.6% ± 7.1% compared with 45.9 ± 2.2%). The Sirt1 protein level was not significantly different by ANOVA (0.1 mg: 0.86 ± 0.09 compared with 1.07 ± 0.07; 0.5 mg: 0.77 ± 0.11 compared with 0.99 ± 0.06; 1 mg: 0.71 ± 0.03 compared with 0.80 ± 0.07, $n = 3$), but tended to be higher in the resveratrol groups, especially at lower doses. In 129X1 male mice, low doses of resveratrol enhanced in vitro fertilizability of epididymal sperm, but the difference was not significant. Sirt1 is necessary for normal spermatogenesis, but its increased expression in male mice does not improve in vitro fertilizability of epididymal sperm if the mice have normal Sirt1 genes.

P279 Adventitious Agents Detected in Feral Mice over a 5-Year Period at a Cancer Research Institution

PL Gorelick*[1], W Hsieh[2], R Sriperumbudur[3], RM Werner[3], L Feigenbaum[4]

[1]SAIC – Frederick, LASP, Animal Health Diagnostic Laboratory, [2]SAIC – Frederick, LASP, Animal Molecular Diagnostic Laboratory, [3]SAIC – Frederick, LASP, Laboratory Animal Medicine, [4]SAIC – Frederick, Laboratory Animal Sciences Program, Office of the Director, Frederick National Laboratory for Cancer Research, Frederick, MD

Adventitious agents introduced from feral rodents present a significant challenge in the maintenance of biosecurity for animal facilities. We present data summarizing various agents detected in feral mice over a 5-y period of time in our laboratory. An intergraded pest management is employed consisting of the use of poison and live traps for locations outside of the animal facilities including utility rooms, loading docks, exterior doorways, etc., and live traps only for the inside the animal facilities to help secure the animal facilities. All traps are checked daily for the capture of feral mice. Between 2008 and 2013, a total of 143 feral mice were submitted to the laboratory and screened for adventitious viral and parasites agents. A high percentage of the captured mice were found positive for one or more adventitious agents that pose significant biosecurity threat to animal facilities. The agents detected include mouse hepatitis virus, mouse cytomegalovirus, mouse thymic virus, lymphocytic choromengitis virus, epizootic diarrhea of infant mice virus, mouse adenovirus, mouse parvovirus, *Clostrium piliformis*, *Helicobacter* spp., *Giardia* spp., *Spironucleus muris*, *Myobia musculi*, *Radfordia affinis*, and *Syphacia obvelata*. During this time period, the animal facilities experienced 3 separate disease outbreaks in 3 different buildings that we were able to trace back to the live trapped feral mice with the respective causative agents, 2 *Helicobacter* infections and one MCMV infection. In the case of the MCMV outbreak, a MCMV positive feral mouse was found amongst the clean cages being transported to animal rooms. In both *Helicobacter* spp. outbreaks, the filter covers of mouse cages were found penetrated by the feral mice on a suspended rack. We were able to live trap these mice and confirmed the mice were positive for *Helicobacter* spp. The outbreaks resulted in quarantine of animal facilities and caused significant interruptions in research.

P280 Development of Recombinant Fiber Knob Proteins as Antigens for Serological Detection of Mouse Adenovirus Type 1 and 2

RK Dhawan*, ML Wunderlich, B Bronson, E Pezzulo, L Campbell, B Acevedo

BioAssay Services, Charles River, Wilmington, MA

Recombinant 6X-His tagged fiber knob proteins of mouse adenovirus (MAV)-1 FL (28kDa) and MAV-2 K87 (32kDa) were expressed in insect cells using a baculovirus expression vector system. Expressed proteins were extracted from cell lysate and purified using nickel chelating chromatography. After analysis by SDS-PAGE and ELISA, the purified antigens were coupled to beads for use in the multiplexed fluorometric immunoassay (MFIA). Both MAV-1 and MAV-2 fiber knob proteins were highly sensitive and serospecific. Specificity of the both MAV-1 and MAV-2 antigens was further evaluated by screening known negative mouse and rat sera with 0 of 263 mouse and 0 of 152 rat samples showing positive scores by both antigens, that is, 100% specificity for both assays. Purity of the antigens was tested by screening heterologous sera positive for other infectious agents (for example, LCMV, MPV, MTLV, etc.). Results showed no cross-reactivity with nonMAV antibodies. Early detection of MAV-1 and MAV-2 antibodies by the recombinant antigen was compared with partially purified whole virus. Sera from mice and rats collected at various time points after experimental inoculations (day 0 to 35) with MAV-1 and MAV-2 were tested against both recombinant antigens in ELISA and MFIA assay format. The recombinant antigens detected seroconversion at the same time points as the whole virus antigen. MAV-1 and MAV-2 ELISA and MFIA assays demonstrated strong reactivity to serospecific positive sera only, that is, did not pick up cross reacting antibodies against other serotypes. The strain specificity of these 2 recombinant antigens allows for more precise diagnoses where whole virus antigens tend to cross react.

P281 Plasma Antibody Profiles in Nonhuman Primate Tuberculosis

RK Dhawan*[1], ML Wunderlich[1], I Khan[2], R Ravindran[2]

[1]BioAssay Services, Charles River, Wilmington, MA; [2]Center for Comparative Medicine, University of California, Davis, CA

Tuberculosis (TB) in nonhuman primates is highly contagious and often produces rapid disease. Therefore, identification of animals infected with *Mycobacterium tuberculosis* in a timely manner is critical. Animals in breeding colonies are periodically tested using the tuberculin skin test (TST) and/or a blood assay. However, these tests lack desirable sensitivity, specificity, efficiency, and throughput. We aimed to develop a blood-based immunoassay by exploiting the host immune response against *M. tuberculosis*-infected animals that contain plasma antibodies against *M. tuberculosis* antigens that can be used potentially for routine colony surveillance. Choice of antigen, however, is difficult because antibody responses against a given antigen are not detectable in all infected animals. We used a panel of 28 antigens in a multiplex immunoassay format where individually identifiable microbead sets from the blood assay were coated with each antigen and used in the simultaneous detection of antibodies against all antigens in a single sample (plasma or serum). Computer assisted multivariate analysis of the experimentally infected (*M. tuberculosis* strains: Erdman and H37RV) and 135 uninfected animals of 2 species (61 rhesus and 74 cynomolgus macaques) revealed diagnostically valuable antibody profiles against 8 antigens. All experimentally infected animals contained antibodies against at least one of these antigens. Importantly, plasma antibody profiles in rhesus macaques involved in a TB outbreak ($n = 15$) were studied in naturally acquired *M. tuberculosis* infection and disease. Animals with lung pathology consistent with TB ($n = 10$), contained antibodies to several additional antigens in a profile similar to that previously reported by us in human TB patients. The above results suggest that the multiplex panel of antigens reported here could be developed as a blood-based test for screening of *M. tuberculosis* in nonhuman primates.

P282 Precision and Accuracy of Biochemistry Assay Measurements in Multispecies

RK Dhawan*, WR Shek, P Cowley, AE Ellingwood

Charles River, Wilmington, MA

A biochemistry analyzer instrument was qualified for its utility in precise and accurate measurement of 8 urine analytes in rat (*Rattus norvegicus*), dog (*Canis lupus familiaris*), and nonhuman primates (NHP,

Macaca fascicularis) and 32 serum analytes in mouse (*Mus muscularis*), rat, rabbit (*Oryctolagus cuniculus*), dog, minipig (*Sus scrofa*), and NHPs. Urine and serum samples were tested for typical biochemistry assays including electrolytes. Intra-run (10 replicates) and Inter-run (5 runs) precision was calculated for all assays and dilutional linearity for most assays was also performed for each species. The average Intra-run coefficient of variation (%CV = SD/Mean × 100) for the urine chemistry assays was 1.4% and the average inter-run %CVs was 2.4%; intra- and inter-run %CV for all urine analytes were below 15% and 20%, respectively. The average intra- and inter-run %CV for the serum chemistry assays were 3.1% and 5.0%, respectively. Dilutional linearity (1:1 to 10-fold) was assessed by calculating the percent expected from undiluted samples and by linear regression analyses. The average recovery of the percent expected, slope, and R^2 values for urine chemistry assays of the diluted samples were 98.4%, 0.99 and 0.97, respectively. The average recovery of the percent expected for serum chemistries of diluted samples was 108.1%, with 86.6% of the recoveries within our acceptable range of 75% to 125%; the average recovery slope and R^2 value were 0.93 and 0.94, respectively with 81.9% falling in the acceptable range of 0.75 to 1.25 with 86.2% of R^2 values at or above our lower limit of 0.90. The qualification study demonstrated a high intra- and inter-run precision as well a good recovery of urine and serum analytes in diluted samples thus proving that the biochemistry analyzer can be used for routine measurement of these bioanalytical assays in common laboratory animal species.

P283 Measuring Wellbeing: Happy Rats Learn Faster

R Wheeler*, MP Swan, D Hickman

Laboratory Animal Resource Center, Indiana University School of Medicine, Indianapolis, IN

Measurement of emotional state can be a valuable tool for the assessment of animal wellbeing. This study used tickling to induce a positive emotional state in adolescent male Sprague–Dawley rats before measuring their performance in a spatial discrimination assay. Thirty rats were used on this study, with 11 tickled for 2 min daily and 19 handled without tickling. The testing apparatus consisted of one goal pot present in one of 2 locations per trial, either with an accessible treat (rewarded location) or with a nonaccessible treat (nonrewarded location). A week after arrival in the facility, the rats were acclimatized to the apparatus for 3 to 5 d, then trained every other day to discriminate between the rewarded and nonrewarded locations until they demonstrated a significant decrease in the latency time to approach the rewarded location as compared with the nonrewarded location for 2 consecutive training days or they failed to demonstrate a significant difference by day 27 of training. Each rat was assigned to one of 3 handlers who performed all tickling treatment and behavioral testing. The specific tickling technique varied among handlers, depending on individual experience. Data were analyzed using a single-sided ANOVA. The results for the latency to approach the nonrewarded pot showed no difference between the tickled and the nontickled rats ($P = 0.7245$). The latency to approach the rewarded location was significantly increased in the nontickled rats (58.0354 ± 6.128) as compared with the tickled rats (39.4396 ± 5.4267, $P = 0.0002$). There was a significant effect of handler for both the nonrewarded ($P = 0.0004$) and the rewarded locations ($P = 0.0006$). The decreased latency times for the rewarded location were suggestive of a positive emotional state in the rats that were tickled. The significant handler effect suggests that handler experience can influence this human-animal interaction, though all handlers were successful in inducing a positive emotional state. We concluded that the performance of a spatial discrimination task can be used to measure the inducement of a positive emotional state.

P284 Isolation and DNA Characterization of a Previously Undescribed Simian Retrovirus Isolate

R Rong*[1], C Shi[2], L Yang[2], S Chen[2], C Xie[1]

[1]Department of Biologic Sciences, Xi'an Jiaotong-Liverpool University, Suzhou, China; [2]VRL China, Suzhou, China

Asian monkeys are the natural hosts for simian type D retrovirus (SRV/D). At least 5 serotypes of SRV/D have been identified from Asian monkeys and their genomes have been fully sequenced. In addition to these 5 serotypes of SRV/D, new SRV/Ds have been continuously discovered in various Asian monkey species. In order to investigate the circulation of SRV/D infection in China, a study has been conducted to verify SRV/D serotypes from the positive monkeys in China breeding farms by amplification and comparison of env sequences with all reported SRV/Ds. During this study, we identified an undescribed SRV/D-like virus from a cohort of Cambodian origin cynomolgus monkeys. To verify this SRV/D-like virus, the full-length sequence of the new isolate was determined by sets of reverse transcription-polymerase chain reaction (RT-PCR) amplification of the viral genome and rapid amplification of cDNA ends (RACE), followed by direct sequencing of PCR products. Sequence comparisons and phylogenetic analysis indicated that the new isolate is closely related to the other known SRV/Ds but is distinct from the 5 molecularly characterized SRV/Ds. This result indicates that SRV/D viruses can evolve into various genetically different serotypes in their natural hosts, which may challenge SRV/D detection results.

P285 Feasibility of a MicroPET Imaging Protocol Using Paired $H_2{}^{15}O$/^{18}FDG Injections In Rats

SR Scherrer*[1], MA Cenci[3], Z Bimpisidis[3], S Peng[4], Y Ma[4], C Veith[1], S Agorastos[1], J Carrion[2], Y Choi[1], D Eidelberg[4], SL Dewey[1]

[1]Laboratory for Behavioral and Molecular NeuroImaging, Feinstein Institute for Medical Research, Manhasset, NY; [2]Hoffstra-NSLIJ School of Medicine, Hempstead, NY; [3]Department of Experimental Medical Science, Basal Ganglia Pathophysiology Unit, Lund University, Lund, Sweden; [4]Center for Neuroscience, Feinstein Institute for Medical Research, Manhasset, NY

MicroPET imaging protocols can effectively measure neurochemical changes in specific animal models of human disease. The feasibility of a dual-tracer $H_2{}^{15}O$/^{18}FDG microPET imaging protocol in the rat depends on physiologic conditions, drug treatments, surgical manipulations, anesthesia, availability of radiotracers, and physical characteristics of the tomograph. Thirty-seven female Sprague–Dawley rats (18 lesioned, 19 sham lesioned) received a subcutaneous injection of saline or LDOPA followed 20 mins later by ketamine (75 to 100 mg/kg IP) and xylazine (7.5 to 10 mg/kg IP) anesthesia. Three extended catheters were inserted: 25-gauge left intraperitoneally for anesthesia maintenance, 25-gauge right intraperitoneally to deliver ^{18}FDG, and a 24-gauge intravenous tail vein catheter to deliver $H_2{}^{15}O$. Once positioned in the microPET gantry, 1.6-2.1 mCi $H_2{}^{15}O$ was injected intravenously while obtaining a 2.5-min dynamic scan. Ten minutes later, an intraperitoneal injection of 1.8 to 2.0 mCi ^{18}FDG was given, followed by a 45-min uptake period and 10-min static scan. All images were analyzed using a novel brain network analysis. Of paired scans, 62% were successfully achieved. Pooling of scan types resulted in 70% to 80% success rate. Anesthesia was maintained for 1 h and 42 min with only 10% attrition. An 8% to 16% loss resulted due to failed injection of one or both tracers. Image quality was better for ^{18}FDG than for $H_2{}^{15}O$ in terms of signal to noise distributions. Analysis of ^{18}FDG data in lesioned-saline and sham-saline animals revealed a Parkinson disease (PD)-related metabolic brain network mimicking topography seen in PD patients. Network expression of this pattern was elevated in lesioned-saline animals but showed an opposite response in lesioned-LDOPA animals with ^{18}FDG scans compared with $H_2{}^{15}O$ scans relative to sham-LDOPA animals. MicroPET datasets can be successfully analyzed using analogous brain imaging methodology developed in PD patients. This imaging strategy produced a reliable method to generate paired images of cerebral blood flow ($H_2{}^{15}O$) and metabolism (^{18}FDG) in rats.

P286 Detection of *Mycobacterium liflandii* in *Xenopus* spp. Aquarium Sediment Detecting Unique DNA Sequence in the pMUM002 Plasmid with a qPCR Assay Using Fluorescent Probes

SH Feldman*[1], SE Pott[2]

[1]Center for Comparative Medicine, University of Virginia, Charlottesville, VA; [2]Department of Biology, North Carolina State University, Raleigh, NC

Mycobacterium liflandii is a recently recognized pathogen causing cutaneous ulceration, gastrointestinal and hepatic granuloma, coelomic effusion and subcutaneous hemorrhage in *Xenopus tropicalis* and *Xenopus laevis*. Specific identification of *M. liflandii* has been hampered by the bacteria's slow growth in culture and its genomic similarity to other mycolactone producing mycobacteria, notably *M. ulcerans* and *M. marinum*. Moreover, identification of *M. liflandii*-infected aquaria has required testing of clinically symptomatic frogs infected. Here, we describe the development and validation of a qPCR assay using fluorescent probes targeting unique DNA sequence in the pMUM plasmid and the ability to reliably detect as few as 10 copies of *M. liflandii* pMUM002 target in DNA isolated from infected frog tissues and the sediment found in aquaria containing assymptomatic infected *Xenopus* frogs.

P287 Canine Fetal Microchimerism

S Hansen*

University of Missouri, Columbia, MO

Fetal microchimerism has been thought to play contradictory roles in human health. These cells have been associated with lower risk for some cancers, have been identified in healing wounds and regions of cellular regeneration, and they have also been associated with risk of immune-mediated disease and some cancers. Microchimerism has been studied in mice, rats, and humans and has been identified in primates and cattle. Our hypothesis was that we would find evidence of fetal microchimerism in dogs. To elucidate the role of microchimerism in health and disease, dogs are the ideal animal model because they share our environment, develop spontaneous cancer and immune-mediated disease, have a well-annotated genome, and extensive lineages allow for tracking individuals within families. The most basic test for locating microchimeric cells is to identify the presence of Y-chromosomal material circulating within the bloodstream of females who have delivered male offspring. We obtained the DNA from 5 bitches within 2 mo postpartum after delivering at least one male puppy, as well as 90 samples of banked DNA from bitches having whelped a male puppy at some time previously (up to 96 mo prior). We developed a PCR assay based on Y-chromosome DNA sequences and analyzed products with gel electrophoresis. We found 100% of the immediate postpartum females tested positive for the presence of the Y-chromosome, as well as 35% of the banked samples, indicating true fetal microchimerism in the dog. These results support use of the dog a model to study the transmission and effects of microchimeric cells.

P288 Age and Seasonal Effects on Morning Urinary Cortisol in Young Chimpanzees

SD Breaux*[1], JJ Breaux[1], M Fontenot[1], SL Watson[2]

[1]Behavioral Sciences, University of Louisiana at Lafayette New Iberia Research Center, New Iberia, LA; [2]Department of Psychology, University of Southern Mississippi, Hattiesburg, MS

Urinary cortisol measures are a useful noninvasive means for examining hypothalamic-pituitary-adrenal axis activity in primates. A number of studies indicate that cortisol may be stable among individuals and perhaps reflective of future behavioral patterns. However, a number of physiologic and psychologic factors may influence cortisol levels. We sought to investigate the effects of age, sex, and season on morning cortisol levels in juvenile to adolescent chimpanzees ($n = 28$ chimpanzees (19 male, 9 female); aged 3.17 to 9.83 y (mean = 6.43 y)). Chimpanzees were trained via positive reinforcement technique to provide urine samples, which were collected weekly between 0800 to 1100 from March 2008 through May 2009. Urine samples were assayed using a commercially available ELISA kit. Cortisol was corrected for specific gravity, then residualized for time of collection and averaged for each subject for 5 seasons: spring 2008 (March to May), summer 2008 (June to August), fall 2008 (September to November), winter 2008 to 2009 (December to February), and spring 2009. Repeated measures analysis of covariance, covarying age, revealed a significant effect of season ($F(4, 104) = 7.91$, $P < .001$), indicating that cortisol was lowest in the spring compared with other seasons, and a season by age interaction ($F(4, 104) = 8.84$, $P < .001$). To analyze the interaction, age was categorized as juvenile, preadolescent, and adolescent. Univariate analyses indicated that cortisol levels in juvenile animals showed a significant decrease over time ($F(1, 25) = 8.33$, $P < 0.01$) and were significantly lower than adolescent levels in spring 2009 ($F(1, 25) = 5.09$, $P < 0.05$). Adolescent levels increased over time ($F(1, 25) = 8.72$, $P < 0.01$). There was no significant change over time among animals in the preadolescent category. Over time, cortisol levels vary between juvenile and adolescent chimpanzees and may reflect factors such as postweaning stress among juveniles and degree of reproductive maturation among adolescents.

P289 Complications of Total Bile Duct Ligation Mouse Model

S Luo*, Y Luo

Charles River, Raleigh, NC

Jaundice occurs in about 60% of all newborns. When severe jaundice goes untreated for too long, it can cause brain damage. Although, there are genetically jaundiced animal model available, surgically altered rats and mice are commonly used for jaundice research. The procedure involves total ligation of bile duct. Here, we evaluate outcomes resulting from the surgical procedure by examining clinical signs, liver function, and hepatic pathology. Twenty male Balb/c mice were randomly assigned in to 5 groups with 4 animals in each group. All mice underwent the bile duct ligation procedure. Animals were closely monitored once daily postsurgery for any clinical abnormalities. One group of mice was euthanized at postsurgical days of 3, 7, and 14. Serum and liver samples were collected from each animal for laboratory testing. Weight loss was the major clinical observation. By 14 d postsurgery, 95% animals lost 20% of their original weights. Total bilirubin and liver enzymes were significantly elevated from their reference ranges in all time points. All liver samples examined microscopically showed mild to severe hepatic necrosis. This study suggest the total bile duct ligation mouse model provides no more than 14 d research life when using 20% body weight loss limit as a terminal point. The data of liver function and hepatic pathologic findings suggest that total bile duct ligation in mice is not only a model to study jaundice but also a possible good model for investigation of acute and subacute hepatic injuries.

P290 Microbial Symbionts Accelerate Mammalian Wound Healing

T Poutahidis[1,2], S Kearney[1,3], T Levkovich[1], P Qi[1], B Varian[1], J Lakritz[1], Y Ibrahim[1], E Alm[3,4], SE Erdman*[1]

[1]Division of Compartive Medicine, MIT, Cambridge, MA; [2]Laboratory of Pathology, Aristotle University of Thessaloniki, Thessaloniki, Greece; [3]Biologic Engineering, Massachusetts Institute of Technology, Cambridge, MA; [4]Broad Institute, MIT and Harvard, Cambridge, MA

Wound healing capability is inextricably linked with diverse aspects of physical fitness ranging from recovery after minor injuries and surgery to autoimmune disorders and some types of cancer. Impact of the gut microbiome upon the mammalian wound healing process is poorly understood. Using a standardized skin biopsy assays in mouse models, we discover that enriching the gut microbiome with certain microbes accelerates the wound-healing process to occur in half the time required for matched control animals. Further, we find that microbial enhancement of wound-healing properties occurs through upregulation of the neuropeptide hormone oxytocin, a factor integral in social bonding and reproduction. We show that bacteria-triggered oxytocin serves to activate host CD4+CD25+ immune cells conveying transplantable wound healing capacity to naive Rag2-deficient animals. This study determined oxytocin to be a novel component of a multidirectional gut microbe-hypothalamic-immune axis, with wound-healing capability as a previously unrecognized output of this axis. We also provide experimental evidence to support long-standing medical traditions associating diet, social practices, and the immune system with efficient recovery after injury, sustained good health, and longevity.

P291 DNA Methylation Status of Imprinted Genes in Rat ICM Cells, Rat ES Cells, and Mouse ES Cells

T Goto*[1,2], C Tamura[1], M Sanbo[1], M Kato-Itoh[3], T Kobayashi[4], H Nakauchi[3,5], S Hochi[6]

[1]National Institute for Physiologic Sciences, Okazaki, Japan; [2]Nagoya University, Nagoya, Japan; [3]ERATO, Nakauchi Stem Cell and Organ Regeneration Project, Tokyo, Japan; [4]University of Cambridge, Cambridge, United Kingdom; [5]The University of Tokyo, Tokyo, Japan; [6]Shinshu University, Ueda, Japan

Regions of different DNA methylation status between maternal and paternal alleles (the so-called DMRs) exist in many imprinted genes of mammalian genome. The DMRs of imprinted genes in mouse embryonic stem (ES) cells are known to remain 'methylated' in one of the 2 alleles. The aim of the present study was to investigate the DNA methylation status of rat inner cell mass (ICM) cells and rat ES cells, as well as mouse ES cells. Mouse ES cell lines, served as control, were TT2 (CBA × C57BL/6), K3 (129Sv × C57BL/6), and BDF1 (C57BL/6 × DBA). Rat ICMs were immunosurgically isolated from e4.5 Crlj:WI blastocysts. Rat ES cell lines used were BLK2i-1, WIv3i-1, and WIv/v2iF-12. Genomic DNAs were extracted from 205 ICMs and 1 × 106 ES cells each, and treated with bisulfite. DNA methylation status of the DMRs in 3 imprinted genes (Peg5, Peg10, and Igf2r) was then analyzed by bisulfite sequencing. Despite different establishment profile of the ES lines, no line differences in DNA methylation status were found within the species. In mouse ES cells, Peg10 and Igf2r DMRs were methylated (47.2% to 80.3%) while Peg5 DMR was hypomethylated (7.6%). In rat ICM cells, Peg10 DMR was likely to be methylated (62.6%), but Peg5 DMR was demethylated (0%) and Igf2r DMR was hypomethylated (13.7%). On the other hand, DMRs in all the 3 imprinted genes were mostly demethylated in rat ES cells (0.5% to 2.1%). Considering that DNA methylation of DMRs was not maintained during the preimplantation embryonic development in the rat, the DNA methylation in imprinted gene DMRs may be established and maintained in a species-specific manner.

P292 Characterization of Rat Pinworm (*Syphacia muris*) Epidemiology as a Means to Increase Detection and Elimination

TM Meade*, J Watson

Molecular and Comparative Pathobiology, Johns Hopkins University, Baltimore, MD

Pinworm infestations persist in many research rodent colonies despite the availability of effective treatments. Furthermore, the introduction of a PCR assay for rodent pinworm diagnosis has increased detection rates indicating that traditional diagnostic methods such as tape tests are insensitive. Epidemiologic information is limited in availability for rat pinworms but could be useful for improving inhouse testing strategies. We, therefore, aimed to characterize the epidemiology of a rat pinworm (*Syphacia muris*) infestation in Sprague–Dawley rats (*Rattus Rattus*) with a goal of increasing the sensitivity of traditional diagnostic methods. Additionally, we attempted to determine the length of egg viability in the environment using an in vitro hatching protocol. Two litters with known *Syphacia* infestation were followed over several months and tape tests were collected weekly at varying times during the day. The cecum and colon of these animals were examined for adult pinworms under a dissecting microscope at timed endpoints. We found that the majority of eggs were shed in midafternoon as compared with other times of the day and that females consistently shed more eggs than males. Also, egg shedding showed a periodicity such that shedding decreased to zero at intervals of 2 to 3 wk. This periodicity frequently resulted in a false negative diagnosis of rat pinworms for cecal and colonic examination. Finally, eggs allowed to sit for 20 wk at room temperature hatched indicating continued viability. These results suggest that rat pinworm detection can be improved through judicious selection of animals and timing of sample collection. Furthermore, viable eggs can persist in the environment for at least 5 mo representing one potential source of reinfestation within colonies. Based on this research, we will offer specific recommendations to more accurately assess the status of *Syphacia muris* in rodent colonies.

P293 Interobserver Reliability Training Paradigm for Functional Observational Battery in Sprague–Dawley Rats

TA Pringle*, MR Bennett

Neurosciences, WIL Research, Ashland, OH

The functional observational battery (FOB) is a component of the neurotoxicity screening battery used to detect gross functional deficits in test animals. As per EPA and OCED guidelines, the FOB should be conducted by the same individual over the course of a study to avoid bias and observer variability; however, due to scheduling, staffing variables and neurotoxicity endpoints the same biologist is not always able to conduct all FOB testing on a study. The importance of obtaining consistent and reliable observations with reduced variability within/between biologists led to development of the FOB training and interobserver reliability program at our institution. The initial training and subsequent reliability assessment has been conducted with the use of positive control animals to appropriately train individuals in the FOB paradigm and minimize variability between biologists. This program consists of individuals attending multiple training sessions with a previously trained individual. First, individuals are introduced to the processes, procedures, and general techniques of the FOB. Then individuals attend training conducted in a group setting wherein individuals simultaneously observe animals dosed with compounds known to elicit functional deficits, allowing for open discussion and interpretation of findings. Lastly, individuals attend a session conducted in a group setting with simultaneous observation of animals treated with positive control agents but with no open discussion of the findings to test the consistency and the accuracy of the calls being made on a per individual basis. Commonly used compounds include 3'-3 iminodipropionitrile (IDPN), harmaline (HRM), amphetamine (AMPH), and chlorpromazine (CHL). In general, IDPN induces severe motor deficits, HRM induces tremors with associated motor deficits, AMPH increases activity/arousal, and CHL decreases activity/arousal. Following the completion of the assessment, biologists are scored based on their accuracy (in comparison with the trainer) and consistency while conducting the FOB. Individuals are graded by a panel of trained scientists that use multiple parameters to determine proficiency in the FOB. Grading consistency is one such parameter used, where graded observations should be within one grade of the trainer.

P294 Dermal Draize Reliability and Training Assessments in the New Zealand White Rabbit

TA Pringle*, ML Simons

Neurosciences, WIL Research, Ashland, OH

The Draize method is a widely accepted qualitative scoring procedure routinely used on guideline dermal toxicity studies conducted in the New Zealand white (NZW) rabbit. Since the Draize method relies on subjective assessments of key dermal endpoints (that is, erythema and edema), consistency in both intra- and interobserver dermal scoring is a primary concern at our institution. Due to a variety of factors involving study size and staffing availability, the same technicians may not conduct the dermal scoring at each interval in the study. Therefore, we developed a practical method for training and maintaining consistency among observers in the performance of the Draize method of dermal scoring. The general training procedures consisted of scoring the erythema and edema in male NZW rabbits (n = 5) dermal response to sodium lauryl sulfate (SLS) administered at 0%, 5%, 10%, and 20% in a random sequence to 1 of 4 selected dorsal skin patches (approximately 2.5-cm squares) per rabbit. The skin patches were wrapped/occluded. Following a 24-h exposure, each SLS treatment was removed, and dermal scoring was conducted by the technicians(s) and Draize trainer at 1, 24, and 48 h following the end of exposure. Technical staff were considered trained and consistency across observers was considered acceptable if each erythema and edema score was within ± 1 of scores recorded by the Draize trainer and when the primary dermal index differed by less than 0.5 from the Draize trainer.

P295 Jugular Phlebotomy in Juvenile Sprague–Dawley Rats

TA Pringle*, RM Moehle

Neurosciences, WIL Research, Ashland, OH

The FDA and EMEA require juvenile toxicity studies to be conducted prior to the start of clinical trials in the pediatric population. As part of this assessment in juvenile animals, toxicokinetic (TK) analysis is often

conducted to establish and confirm exposure levels, often at the initiation and completion of the treatment period. Due to the size of these juvenile offspring, particularly in rodents, the available blood volume required for TK analysis is a limiting factor. This often requires additional litters (including maternal animals) to be placed on study since methods and available information concerning blood collection is often limited. The purpose of this study was to develop a method for jugular vein sample collections in rats as young as postnatal day (PND) 4. This study was designed to determine the maximum blood volume for collection, compare serial compared with terminal blood collections and assess the effect of sample collection on body weight and body condition throughout the sampling period. Adult female Sprague–Dawley rats were bred inhouse and allowed to deliver offspring for use on study. Terminal blood samples were collected from the offspring via the jugular vein on PND 4, 7, 10, 14, and 21 to determine the maximum amount of blood that could be collected at each age. The average amount of blood collected from a terminal blood collection was generally similar to the total volume of blood collected over a 24-h period from serial samplings at each respective age and was approximately 0.15 mL on PND 4; 0.21 mL on PND 7; 0.33 mL on PND 10; 0.55 mL on PND 14; and 0.90 mL on PND 21. This study showed that serial jugular blood can be collected in rats as young as PND 4. However, due to blood volume and expected analytical limitations, serial jugular samples should only be collected on PND 10 and older, with terminal collections for animals younger than PND 10.

P296 Assessment of Short- and Long-Term Catheter Patency/Viability in Adult Male Rats Undergoing Self-Administration Testing

TA Pringle*, ML Hackman

Neurosciences, WIL Research, Ashland, OH

Proper catheter maintenance and viability are critical for the success of any infusion study. Extended catheter patency allows for more study activities to be conducted from a single animal, along with the generation of more robust, consistent, and valid data. Our objectives were to assess aseptic procedures used at our institution, and determine how these techniques impact the duration of sustained catheter patency. Fifty-two adult Sprague–Dawley rats had intravenous femoral vein catheters implanted with a scapular connection. Aseptic techniques were used during surgery, when changing connections on the infusion system and when handling associated infusion supplies (including only touching nonsterile parts of items, swabbing nonsterile connections with alcohol, capping open luer tips, and using sterile prefilled syringes). Following recovery from the surgery, the following catheter maintenance was conducted 6 d/wk: 1) removal of the heparin lock, 2) saline flush, and 3) catheter relocking (20 IU/mL heparin/saline). Catheter scoring criteria were based on these factors, and scored at each maintenance interval. Brevital patency checks were conducted, as necessary, during self-administration testing at the completion of testing, and/or prior to euthanasia. Animals with patent catheters displayed a total loss of muscle control within 20 s of administration and regained muscle control within 30 min. Scoring of the femoral vein catheter indicated that approximately 80% of the catheters were patent for at least 1 mo, with approximately 50% at 3 mo, and approximately 6% at 10 mo. At the time of necropsy, no study animals were noted with signs of infection. If adequate care and proper aseptic techniques are used when preparing and maintaining indwelling catheters on the technically demanding SA studies, animal use can be maximized while potentially providing more robust and scientifically valid data.

P297 Interobserver Reliability Assessment of the Functional Observational Battery in the Beagle Dog and Nonhuman Primate

TS Fenton*, EA Lambert, EL Lashley

WIL Research Laboratories, Ashland, OH

The functional observational batteries (FOBs) of the beagle dog and nonhuman primate are a series of evaluations for detecting physiologic and behavioral deficits. They consist of a neurologic evaluation and an assessment of physiologic functions, such as body temperature, blood pressure, respiration rate, and heart rate. To avoid variability, the FOB should be performed by the same individual throughout the course of a study, but due to limitations this is not always possible. In order to ensure there is little to no variability between observers conducting the FOBs, an inhouse interobserver reliability assessment study is performed every 2 y, in which the observations of all trained personnel are compared individually to those of a designated trainer who has previously shown proficiency and consistency in performing the FOB. The study is conducted in 3 phases and includes the administration of 2 positive control articles known to elicit neurologic and physiologic responses. Phase I is designed to ensure consistent and accurate use of all equipment and to gain a strong knowledge of the behavior of normal, untreated animals of the species being evaluated. This also serves as a training period for newly trained personnel. Phase II is conducted in a group setting consisting of experienced personnel and trainees simultaneously observing positive control article-treated animals, allowing for open discussion and interpretation of the findings. Phase III is also conducted in a group setting, such that personnel simultaneously observe both positive control article-treated and vehicle-treated animals but with no open discussion of the findings. All personnel are blinded to the treatment of each animal during this phase. At the completion of phase III, all data are evaluated for interobserver reliability. All instances of failing to meet the predetermined criteria are discussed by the study director and study assessors who make the final decision of whether interobserver reliability is achieved. All personnel that demonstrate a sufficient level of interobserver reliability are considered approved to perform FOBs on study.

P298 Aqueous Stability of Ivermectin Drench Reconstituted in Mice Polycarbonate Water Bottles

VH Monterroso*[1], G Cherala[2,3], B DuBois[2,3], N Koewler[1]

[1]Comparative Medicine, [2]College of Pharmacy, Oregon Health and Science University, Portland, OR; [3]College of Pharmacy, Oregon State University, Portland, OR

Fur mites in mice housed in modern research facilities are a persistent problem, and it has been reported that fur mites exist in 30% to 50% of the research facilities in the US. In recent years, incidence and prevalence of fur mite infections have increased, and different modalities of treatment have been described. Ivermectin is effective in the treatment of fur mites during adult and larval stages. However, delivery of the drug has been a challenge in large populations of rodents. Ivermectin has been delivered in various forms including food, topical solutions, drinking water, and others. However, the aqueous stability of ivermectin is not known under the vivarium environmental conditions. The objective of this work was to determine the week-long stability of the ivermectin aqueous solution. Ivermectin drench solution (0.08%) was diluted in reverse osmosis (RO) water (autoclaved), for a final concentration of 0.008 mg/mL. Final solution was maintained in polycarbonate water bottles at 4 °C, room temperature, or 37 °C for up to 7 d. Aliquots were collected at 0900 on day 0, 1, 2, 3, 4, 5, 6, and 7. Aliquots were stored at −80 °C until batch analysis using HPLC. Results showed that ivermectin concentration did not alter samples maintained at all 3 temperatures over a 7-d period. In conclusion, our data suggests that ivermectin (drench) reconstituted (0.008 mg/mL) in RO drinking water is stable for at least 7 d at room temperature, providing an adequate ivermectin treatment (for example, fur mites) of targeted mice populations.

P299 Progressive Body Weight Trends Predict Development of Bone and Gastrointestinal Syndrome in the Common Marmoset (*Callithrix jacchus*)

VK Baxter*[1], NP Sotuyo[2], GC Shaw[1], EK Hutchinson[1], KA Metcalf Pate[1]

[1]Molecular and Comparative Pathobiology, [2]Department of Biomedical Engineering, Johns Hopkins School of Medicine, Baltimore, MD

Gastrointestinal (GI) disease, such as marmoset wasting syndrome, and bone disease are 2 widespread causes of morbidity and mortality in captive common marmoset (*Callithrix jacchus*) colonies. Unfortunately these conditions are usually diagnosed at a point beyond which therapeutic intervention can curb the progression of disease and when long-term research investments in the animals have already been made. Previous

work by our group has shown that bone disease and GI disease are associated in marmosets (referred to here as bone and GI syndrome, or BGS), and animals with BGS possess lower terminal body weights compared with unaffected controls. We hypothesize that progressive body weight trends can predict which animals will develop BGS. To investigate this, we collected progressive weight data from 5 animals that died of natural causes and were diagnosed with GI ± bone disease at necropsy and from 5 age- and sex-matched unaffected marmosets for comparison. Peak body weight, time of peak weight, and percent weight loss at death were determined for each animal, and percent peak body weight was graphed against time prior to death. Affected marmosets tended to have lower peak body weights than unaffected marmosets (median 386 g compared with 456 g, Mann–Whitney [MW] $P = 0.095$) and reached their peak body weight at a significantly earlier time before death (median 264 d compared with 3 d premortem, MW $P = 0.016$). Marmosets with GI ± bone disease died at 62% of their peak body weight on average compared with 98% for unaffected marmosets (MW $P = 0.0079$). Affected animals lost an average of 0.14% of their body weight per day, while unaffected animals alternatively maintained their weight with an average daily gain of 0.04% body weight (MW $P = 0.0079$). Weight loss of greater than 0.05% body weight per day or greater than 18% of peak body weight total could significantly distinguish between animals that would be diagnosed with BGS at necropsy and unaffected animals (Fisher exact $P = 0.018$) at 8 to 9 mo or 2 to 3 mo prior to death, respectively. Therefore, progressive weight loss can indeed predict development of BGS in marmosets before they reach the terminal stage of disease, providing a window of opportunity for possible therapeutic intervention.

P300 Early Detection of Clinical Disease in Guinea Pigs Experimentally Infected with *Mycobacterium tuberculosis*

W Tuttle*

MIP, Colorado State University, Fort Collins, CO

Guinea pigs are a common model used for vaccine research against *Mycobacterium tuberculosis* due to their similarities in immune response to humans. Unfortunately, guinea pigs are a very stoic species and do not show clinical signs of illness until their health status is significantly compromised. Common criteria for euthanasia include pallor or cyanosis of the ears, nose, and feet, as well as difficulty breathing. On necropsy, these animals tend to have disseminated tuberculosis infection, which is evident by visible granulomas on the lungs, liver, and spleen. In order to find earlier endpoints for guinea pigs experimentally infected with *Mycobacterium tuberculosis*, we monitored indicators of illness over the course of disease. Twenty female Ducan Hartley guinea pigs of the same age were obtained. Each week they were weighed and given a physical exam. Prior to inoculation blood and urine were collected for CBC, chemistry, blood gas, urinalysis, and urine culture for baseline data. Ten animals were then vaccinated with BCG, and all animals were incoluated with an aerosolized form of *Mycobacterium tuberculosis*. Every 30 d postinoculation the same samples were obtained for diagnostics. Starting at 90 d postinoculation, there were decreased neutrophils despite a normal white blood cell count. Additionally, as early as 30 d postinoculation the hematocrit is elevated and the p02 is decreased. Once the guinea pigs reached visible signs of clinical disease they were euthanized. At necropsy we collected blood and urine for the parameters already listed, as well as blood culture. All major organs were taken for culture and histology. Grossly, the lungs, liver, and spleen have disseminated tuberculoid lesions, regardless of the time of death postinoculation. Our study shows that guinea pigs infected with tuberculosis show few signs of clinical disease aside from blood gas abnormalities. Because so little blood is necessary for this test, blood gasses may be used as a clinical indicator of progression of disease in guinea pigs experimentally infected with *Mycobacterium tuberculosis*.

P301 Research Animal Coordinator Certification: A New Tool for Fostering Compliant Behavior in Animal Care and Use

WL Wade*

Office of Animal Welfare Assurance, Duke University, Durham, NC

Our institution's Animal Care and Use Program offers a training and certification program for individuals wishing to serve as their laboratory go-to person. Referred to as the Research Animal Coordinator Certification (RACC) program, individuals who participate in this program receive specific and detailed training concerning animal care and use regulations, requirements, and policies at our institution—the goal of which is to facilitate research, enhance understanding, and minimize noncompliance. Those who achieve certification may be designated by their principal investigator (PI) as the laboratory coordinator for all animal activities and may provide in-lab guidance regarding animal care and use (serve as an extension of the IACUC and veterinary staff). The RACC program benefits the PI and research laboratory (smoother research protocol application, more efficient review/approval, and decreased risk of noncompliance issues) while also benefiting the RACC candidate (enhancing their value to the research team, improving personal research skills, enhancing their knowledge of how to get things done). The RACC program is voluntary, and offered at no cost. Managed by the Office of Animal Welfare Assurance (OAWA) with significant contributions from the Division of Laboratory Animal Resources (DLAR) and the Institutional Animal Care and Use Committee (IACUC), the RACC program uses a multimodal educational approach (for example, lectures, web-modules, meetings, one-on-one discussion, and hands-on learning). The Research Animal Coordinator Certification program uses a "small class" approach, generally having 6 to 8 RAC candidates in a class (which facilitates discussion during the face-to-face discussions). There are 2 phases to the RAC training program, and the successful candidate completes both phases over a period of 6 to 7 mo.

P302 Cell Tracking in Nonhuman Primates Using Magnetic Resonance Imaging

Y Ito*[1,2], H Koie[2], H Shibata[1], S Okabayashi[3], Y Katakai[3], C Ohno[3], K Kanayama[2], Y Yasutomi[1], N Ageyama[1]

[1]Tsukuba Primate Reserch Center, Tsukuba, Ibaraki, Japan; [2]College of Bioresource Science, Nihon University, Fujisawa, Kanagawa, Japan; [3]The Corporation for Production and Research of Laboratory Primates, Tsukuba, Ibaraki, Japan

Many types of regenerative medical techniques, including stem cell transplantation, have been investigated with a view to clinically treating conditions such as coronary syndrome and Buerger disease. Transplanted cells can improve blood flow at infarcted regions in small laboratory animals. Magnetic resonance imaging (MRI) is a noninvasive method that can identify and trace many types of transplanted cells labeled with superparamagnetic iron oxide (SPIO) and/or fluorescent iron particles (FIP), thereby determining regenerative effects at local sites as well as the fate of such cells. Therefore, we investigated the regenerative effects of transplanted cells in a nonhuman primate model. The first cynomolgus monkey (*Macaca fascicularis*) was intramuscularly transplanted on triceps surae muscle, and the second monkey was intravenously transplanted with peripheral blood mononuclear cells colabeled with ferucarbotran and ferumoxide. We also cultured mesenchymal stem cells (MSCs) aspirated from ilium and/or tuber ischia of monkeys and analyzed them by FACS. Then we also implanted FIP-labeled mesenchymal stem cells derived from bone-marrow directly to 5 hearts of monkeys. They were assessed by acquiring T1- and T2-weighted MRI sequences using a 3T MR scanner on days 0 and 7. Labeled cells were detected at the triceps surae muscle in the first monkey and detected at the liver in the second monkey. Specimens of triceps surae muscle were also stained with Berlin blue to confirm the remaining labeled cells. No clinical manifestations were evident in the monkeys throughout these procedures. Clear MRI images of the monkey heart after direct implantation with FIP-labeled MSCs were also acquired. In summary, tracking transplanted cells labeled with SPIO and FIP in nonhuman primate using MRI is a safe and efficient system that might help to reveal the mechanism of symptomatic improvements in human regenerative medicine.

P303 Antinociceptive Effects of Sustained-Release Buprenorphine in Postlaporatomy Mice (*Mus musculus*)

H Chum*[1], A Kerk[2], K Jampachaisri[3], M Hsieh[2], C Pacharinsak[1]

Vol 52, No 5
Journal of the American Association for Laboratory Animal Science
September 2013

[1]Department of Comparative Medicine, [2]Department of Urology, Stanford University, Stanford, CA; [3]Naresuan University, Phitsanulok, Thailand

Effective postoperative pain management is essential for the care and welfare of laboratory animals. A sustained release formulation of buprenorphine (Bup-SR) has been recently introduced to veterinary medicine, and its use is becoming more widespread due to its long duration of action. Here we evaluated the effects of Bup-SR in postlaporatomy mice compared with Bup-HCL, an established standard of care. Postoperative antinociception was assessed by 2 blinded evaluators on day 0 and days 1 to 3 postoperatively through the use of behavioral scoring and the modified mouse grimace score (mMGS). Mice were assigned to 1 of 3 experimental groups: Bup-HCl (0.1 mg/kg SC, BID × 3 d; $n = 5$); Bup-SR/0.6 (0.6 mg/kg SC, once; $n = 6$); Bup-SR/6 (6 mg/kg SC, once; $n = 5$). Statistical analyses yielded no significant difference for both behavioral assessment and mMGS between Bup-HCl and Bup-SR/0.6 on days 0, 1, 2, and 3. However, animals in the Bup-SR/6 group showed signs of severe lethargy leading to death or euthanasia. Our findings indicate that: 1) Bup-SR 0.6 mg/kg provides antinociception in postlaporatomy mice; 2) high doses of Bup-SR have negative effects and should be used with caution.