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WEILL CORNELL MEDICAL COLLEGE EDITION

In Focus

Center of Comparative Medicine & Pathology
Research Animal Resource Center
Laboratory of Comparative Pathology

Tattoo systems to ink toes, foot pads and/or tails are available for identification of neonate and adult rodents.

Image credit:
http://www.animalid.com/Lab_Animal_Identification_Systems/NEO9_Neonate_Rodent_Tattooing_System.php?vClip=CD-Neonate-Rat.wmv#clipHolder

Mouse Identification Methods: Time Tested & New

Appropriate recordkeeping is a crucial part of conducting biomedical research. This often requires the need to identify and track individual animals. For large animals, tattoos are the most common method used. For rodents, such as mice, ear tags or punches are widely used. There are, however, various other options available that should be considered when determining what method best suits your needs. The various methods available for identifying mice will be reviewed.

When a temporary method is sufficient, non-toxic permanent markers may be used to mark the tails or fur for up to 3 or 4 days[1]. When treatments are administered, this method can be used to identify which animals have been treated as it can differentiate animals for short durations.

For studies requiring long-term identification, more permanent methods such as tattooing or ear punches/notching are recommended.

The use of implantable microchips and ear tags should also be considered. Toe

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Sustained Release Formulation of Buprenorphine Developed

Buprenorphine (proprietary name: Buprenex), a partial μ-opioid receptor agonist, is the most commonly used post-operative analgesic in laboratory animals. Its principal drawback is the need for repetitive and frequent dosing (every 4-6 hrs in rodents, 6-8 hours in dogs and cats) to provide sustained post-operative analgesia. ZooPharm Inc. has developed a liquid matrix designed to slowly release buprenorphine and maintain therapeutic levels for up to 72 hours. The drug named Buprenorphine SR™, was developed and intended for clinical use in dogs and cats.

This sustained release (SR) formulation is administered subcutaneously and has been evaluated clinically in dogs and cats and in both an orthopedic surgical and a thermal nociception model in Sprague-Dawley rats. When evaluated for feline post-ovariohysterectomy (spay) pain control, Buprenorphine SR™ was equally effective as the traditional formulation with a comparable side effect profile[2]. In the rat models, the analgesia provided by Buprenorphine SR™ was comparable to the traditional formulation, except that the

Cont. on pg. 5

SR
VETERINARY TECHNOLOGIES

Buprenorphine SR™, sustained release, developed and intended for clinical use in dogs and cats, may have murine applications.

Image credit:
http://www.wildpharm.com/documents/Buprenorphine info sheet.pdf

Inside: Mouse Facial Expressions & Pain

Ivermectin Anthelminthic Shown to Have Previously Unrecognized Biologic Effects on Cre Recombinase

Ivermectin, the antiparasitic agent used by RARC to treat murine fur mite infestations was recently reported by researchers at the University of Pennsylvania to induce unexpected changes in tamoxifen inducible Cre systems.

The authors noted activation of a tamoxifen-regulated Cre recombinase fusion protein in the absence of tamoxifen treatment in the progeny of mice treated with ivermectin-containing feed. Even after cessation of ivermectin treatment, the offspring of treated dams continued to show deletion of the loxP-flanked 'stop' cassette for expression of YFP for up to 15 weeks.

This change, however, was not observed in the breeders or any other adult mice weaned prior to the onset of treatment that were provided ivermectin-containing feed. The authors propose that ivermectin metabolites transmitted transplacentally and/or via lactation were responsible for the tamoxifen-independent changes, which may explain the difference observed between the two genotypically identical groups.

Ivermectin-induced CreT2-mediated deletion did not occur in all tissues as is seen with tamoxifen treatment. Ivermectin only initiated deletion of the loxP-flanked 'stop' cassette in the spleen and thymus. Further examination of the hematopoietic cells showed a bias for deletion in T cells.

All mouse strains/stocks imported into WCMC/MSKCC from atypical vendors, e.g., other academic mouse colonies, are treated with ivermectin-containing feed in quarantine to eradicate undetected fur mite infestations as part of RARC's Biosecurity Program. As the risk of

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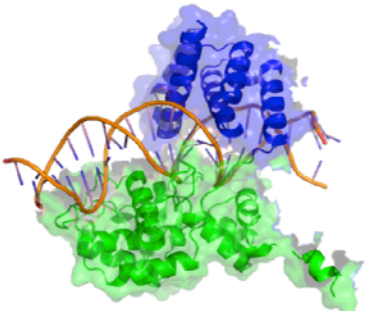
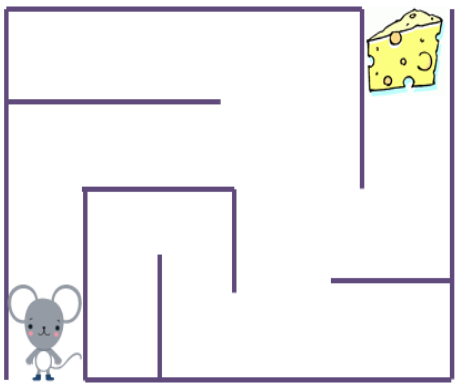


Pymol generated image of Cre recombinase bound to DNA substrate (side view). (Williamog1990, Pymol)

Image credit:
http://en.wikipedia.org/wiki/File:CreRecom%2BDNA%28sideview%29.png

John Sibley
Manager of the CCMP's Laboratory of Comparative Pathology

Selamectin, familiar to pet owners as one of the widely used topical, systemically acting, broad-spectrum parasiticides, can be used alternatively to ivermectin.
Image credit:
http://www.revolutionpet.com/revolutionpet.aspx



EnCCoMPass – We are doing our best to simplify the process:
Easier navigation within the system
Repeat data entries a thing of the past
Pre-Approved Techniques provided to optimize approval
Reviewer comments within the document

John Sibley joins the Laboratory of Comparative Pathology

We are pleased to announce that John Sibley, BS, Medical Technology, MT(ASCP), has recently joined the CCMP as Manager of the Laboratory Comparative Pathology (LCP). John graduated from Plattsburgh State University with a BA in Biology in 1975 and from Upstate Medical University in 1976 with a BS in Medical Technology. He worked at Ayerst Laboratories, which later became Wyeth Pharmaceuticals, for over 35 years where he oversaw the clinical pathology laboratory for all of Wyeth's preclinical drug studies prior to joining the LCP. Throughout his career, John has been developing technologies for small volume clinical pathology testing. This technology is highly desirable when evaluating hematology and clinical chemistries in rodents when euthanasia is not a viable or desirable option for sample collection. This expertise is highly desirable as many investigators and RARC staff require clinical pathology data from our mouse models and patients. John also has considerable experience in anatomic pathology having managed this resource throughout his career. John can be reached at (646)-888-2421 or sibleyj1@mskcc.org

Ivermectin Effects on Cre Recombinase, Cont. from pg. 1

inadvertent fur mite introduction remains high and ivermectin is the most effective acaricide currently available, it will continue to be utilized. Users of tamoxifen inducible Cre systems should request that their animals be treated with an alternative antiparasitic (topical Selamectin) if they are concerned about the effects of Cre recombinase. Although selamectin is related chemically to ivermectin, it has not yet been reported to have this similar effect.

For more detailed information see:

1.Corbo-Rodgers E. et al. Correspondence: Oral ivermectin as an unexpected initiator of CreT2-mediated deletion in T cells. Nature Immunology 13:3, 197-198 (2012)
2. Ricart Arbona R.J., Lipman N.S., Riedel E.R., Wolf F.R. Murine Fur Mite Treatment and Eradication I: Toxicological Evaluation of Ivermectin-Compounded Feed. JAALAS. 49:564-70 (2010)
3. Ricart Arbona R.J., Lipman N.S., Wolf F.R. Murine Fur Mite Treatment and Eradication II: Diagnostic Considerations. JAALAS. 49:583-587. (2010)
4. Ricart Arbona R.J., Lipman N.S., Wolf F.R. Murine Fur Mite Treatment and Eradication III: Treatment of a Large Mouse Colony with Ivermectin-Compounded Feed. JAALAS. 49:633-637. (2010)

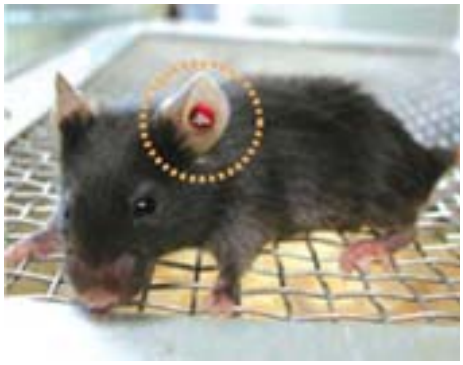
First EnCCoMPass Module to be Released

WCMC's and MSKCC's IACUCs are pleased to announce a new web-based protocol submission and review system – *EnCCoMPass Protocol*. This new system provides functionality that will make the protocol development, submission and review process easier and quicker. *EnCCoMPass Protocol* features include a navigation pane which provides easy navigation between sections of the protocol form and flexibility to move between sections of the protocol during the protocol development process. Unlike eSirius, *EnCCoMPass Protocol's* navigation pane flags incomplete sections, allowing submitters to revisit and complete them at any time.

EnCCoMPass Protocol has been built to reduce the need for repeat data entry when the same information is required in multiple question sets. All previously entered information will automatically migrate to the relevant sections as necessary. *EnCCoMPass Protocol* also provides instructions and examples as additional guidance to aid the author during protocol development. Links to key RARC and IACUC guidelines and policies are available throughout the system as well as links to informative websites and common search engines used to complete the search for alternatives section of the protocol form. In addition, *EnCCoMPass Protocol* contains many Pre-Approved Techniques (PATs). PATs are pre-written descriptions of commonly used *in vivo* techniques such as device implantation, blood withdrawal, imaging and dosing techniques. PATs are a great tool for optimizing submission, review and approval of protocols. Finally, *EnCCoMPass Protocol* offers significant enhancements which simplify the reviewer-submitter communication process. The reviewer's comments will now appear within the protocol system allowing the PI to communicate with the reviewer and IACUC directly within the system. All current protocol data which exists in eSirius will be migrated to the *EnCCoMPass Protocol* system to ensure as seamless a transition as possible.

WCMC's IACUC looks forward to releasing this new system and receiving your feedback. Watch for broadcast notifications from the IACUC as the release date approaches.

Mouse Identification Methods, cont. from pg. 1



Several new ear tag options are now offered such as the small, circular one seen above that provides numbering from 1 to 999 and is available in five colors.
Image credit:
https://www.stoeltingco.com/stoelting/1465/Physio

clipping is another technique but its use is limited to pre-weanling animals.

Tattoos can be applied to neonates (on the toe or foot pad) as well as on adults (on the tail, the toe, or foot pad). Tattooing is useful for identifying a large number of animals with a unique ID. However, there is the risk of cross contamination between animals if the equipment is not sanitized properly. There are two commonly used tattooing systems available: the AIMS™ and the Aramis Micro Tattoo Systems. For best results, special training is available from the systems' manufacturers. In a trial conducted by Abbott Laboratories, the Aramis Micro Tattoo system was used successfully to tattoo dots in set patterns on the ears of adult mice as an alternative to ear notching/punches[2]. A relatively new system, the Labstamp system from SOMARK[3], is an automated system for tail tattooing.

Ear punching/notching has the advantage of simultaneously providing tissue that can be utilized for genotyping. The punch or notch must be placed properly so that it is not misread if placed at the edge of the pinnae (i.e., making a punch look like a notch), or worse, causing harm to the animal by placing it too close to the base of the ear. As with tattooing, there is also the risk of cross contamination if the equipment is not properly sanitized. Also, the size of the mouse's ear limits the maximum number that can be generated in contrast to tattooing. It is also important to check the identification of the animals regularly, as it is not uncommon for the notches/punches to close over time.

Microchips are available in sizes small enough to implant into adult mice. Along with the transponder and applicator, a reader is needed. Importantly, there have been reports of tumors, mainly sarcomas, associated with the transponders[4,5] making them undesirable in carcinogenicity studies.

There are several new ear tag options in addition to the commonly used metal ear tags. Although ear tags are generally easy to apply, proper tag placement and orientation is critical. Improper placement can result in injury to the animal and increase the risk of the tag becoming caught and potentially torn out. A small, circular ear tag (about 3mm for mice) that does not extend beyond the ear is available from Stoelting. They are numbered up to 999 and are available in five colors. Much like the traditional ear tags, these ear tags require an applicator. Aside from its small size allowing it to fit within the boundaries of the pinnae, another advantage is that fewer mistakes are possible when placing. There is even an ear tag that contains a 3-D barcode (see example in the box to the left). This MinID tag system from ZonotID requires a scanner to read the barcode, which then can be downloaded to a computer as the animal is identified.

In cases where neonatal mice must be identified and no other method is suitable, toe clipping may be justifiable. Utmost care must be taken when conducting this procedure. WCMC's IACUC policy requires the technique to be performed by a trained individual and is limited to pups less than 21 days of age. Genotyping should be performed using the tissue acquired from the amputation, if conducted. For full details, the toe clipping policy is available on the IACUC's website.

When determining which identification method is best suited to your study, it is important to weigh the benefits and disadvantages of each. It is also important to consider the age of the mice, how many animals will need to be identified, and the duration of the study, as well as any special training needed. The method selected should meet the needs of the study and minimize animal discomfort.

Jax Mice Now Available with Microchip Ear Tags

Jackson Laboratories (JAX) has recently announced a new system of ID tags that will be affixed to certain mouse strains beginning in May 2012[6]. The select strains that will arrive pre-tagged are as follows:

- **B6.V-Lepob/J (heterozygotes only) – stock # 000632**
- **B6.BKS(D)-Leprdb/J (heterozygotes only) – stock # 000697**
- **B6.Cg-Foxn1nu/J (heterozygotes only) – stock # 000819**
- **C57BL/6J-ApcMin/J – stock # 002020**
- **B6SJL-Tg(SOD1*G93A)1Gur/J – stock # 002726**
- **B6CBA-Tg(HDexon1)62Gpb/1J – stock # 002810**
- **C57BL/6-Tg(TcraTcrb)1100Mjb/J – stock # 003831**
- **B6CBA-Tg(HDexon1)62Gpb/3J – stock # 006494**

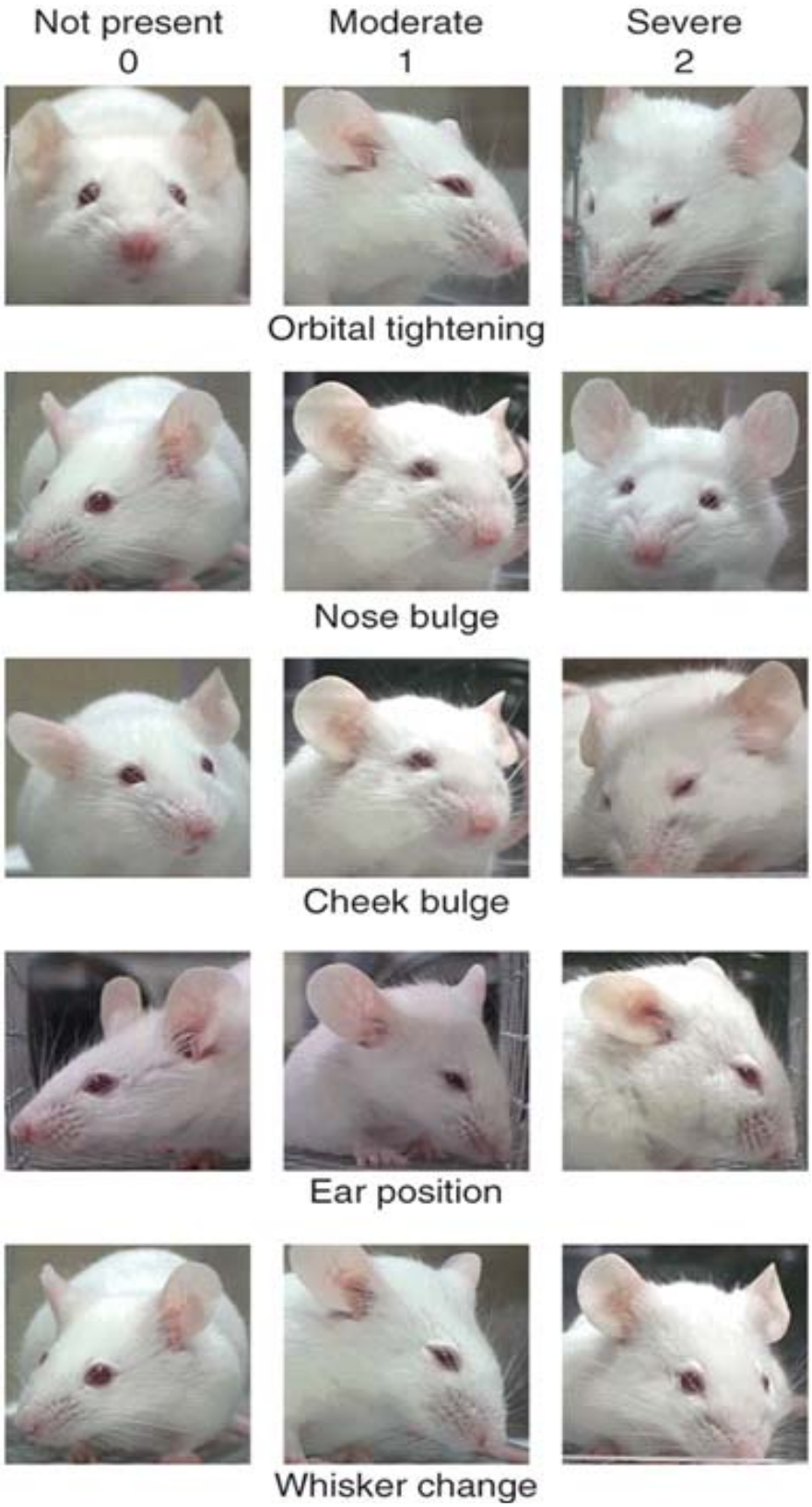
These tags are designed to be read by commercially available scanners, and can be removed using a skin staple remover. These tags, however, were not designed to be MRI compatible. According to JAX, these tags will be made available for other mouse strains beginning June 1st, upon request. Currently, this ID system is not available for purchase. See the JAXTag website for more information.

http://www.jax.org/jaxtag

References:

1. BASIC BIOMETHODOLOGY FOR LABORATORY MICE http://www.theodora.com/rodent_laboratory/identification.html
2. Mouse Ear Tattoo: A Quick and Easy Alternative to Ear Punches and Tags http://www.ketchum.ca/lab/mouse-ear-tattoo.html
3. http://www.somarkinnovations.com/about-labstamp
4. Blanchard KT, Barthel C, French JE, Holden HE, Moretz R, Pack, FD, Tennant RW, Stoll RE. Transponder-Induced Sarcoma in the Heterozygous p53+/- Mouse. 1999. Tox Path vol 27 (5): 519-527
5. Le Calvez S, Perron-Lepage MF, Burnett R. Subcutaneous Microchip-Associated Tumors in B6C3F1 Mice: A Retrospective Study to Attempt to Determine Their Histogenesis. 2006. Exp and Tox Path vol 57 (4): 255-265
6. http://www.jax.org/jaxtag

Facial Expressions Useful to Evaluate Pain in Mice



Images of mice exhibiting behaviors associated with pain shown for each of the five facial expressions in the Mouse Grimace Scale.

Image credit: *http://www.nature.com/nmeth/journal/v7/n6/full/nmeth.1455.html*

Whereas facial expressions can signify pain in humans, there is a paucity of published information on facial expression and pain in veterinary medicine. Langford et al., created and validated a murine grimace scale (MGS) to help characterize signs of pain in mice[1]. The scale is based on five facial expressions that mice demonstrated before and after being subjected to a standard nociceptive assay. Interestingly, three of the five facial expressions observed in mice are also putative pain expressions in humans. These were orbital tightening as well as nose and cheek bulge. Ear position and whisker change or whisker movement were also incorporated into the mouse grimace scale. Digital photos of the mice were accurately scored 70-80% of the time, however when a video camera was utilized the scoring accuracy increased to 97%. Limitations were seen in cases of neuropathic pain which is chronic pain due to nerve injury or damage.

Matsumiya et.al., recently reported on the use of the MGS to assess post-operative pain management after a laparotomy[2]. They demonstrated that the MGS was a dependable tool to assess spontaneous pain which had moderate duration of action. The analgesic buprenorphine was shown to be very effective as confirmed using the MGS.

Therefore, MGS is an effective tool which can be employed and should be considered for mice in Category D undergoing potentially painful procedures such as a laparotomy.

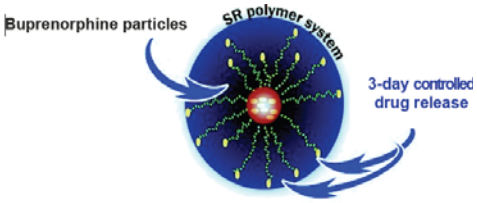
Consult RARC's Education & Quality Assurance section for training on using the MGS.

References:

1. Langford DJ, Bailey AL, Chanda ML, Clarke SE, Drummond TE, Echols S, Glick S, Ingrao J, Klassen-Ross T, Lacroix-Fralish ML, Matsumiya L, Sorge RE, Sotocinal SG, Tabaka JM, Wong D, van den Maagdenberg AM, Ferrari MD, Craig KD, Mogil JS. Coding of facial expressions of pain in the laboratory mouse. Nat Methods. 2010 Jun;7(6):447-9. Epub 2010 May 9.

2. Matsumiya LC, Sorge RE, Sotocinal SG, Tabaka JM, Wieskopf JS, Zaloum A, King OD, Mogil JS. Using the mouse grimace scale to reevaluate the efficacy of postoperative analgesics in laboratory mice. J Am Assoc Lab Anim Sci. 2012;51(1):42-9.

Sustained Release Buprenorphine, cont. from pg. 1



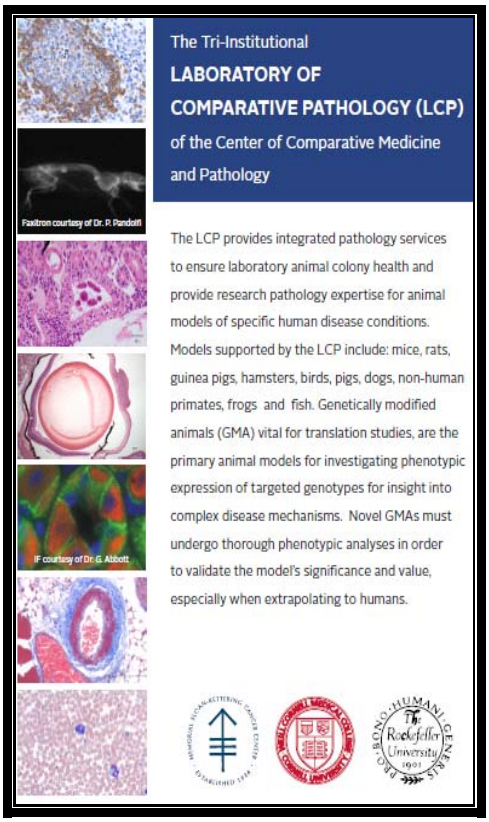
SR[tm] Provides a consistent 72-hour release profile with consistent drug absorption
Image credit:
http://www.wildpharm.com/documents/Buprenorphine_info_sheet.pdf

analgesic effect lasted as long as 72 hours. Systemic side effects, usually observed with multiple dose administration (e.g., weight loss) were not observed in rats; however, skin irritation/ulceration[1] developed at the injection site.

The ulcerative crusting lesions appear within the first 48 hours after injection at and around the injection site Altering technique by slowly withdrawing the needle, pinching off of the injection site to prevent any of the drug from seeping out onto the skin, and ensuring the injection was administered subcutaneously and not intradermally decreased the likelihood of lesion development. The lesions were more common and severe in Lewis rats. The potential for lesion development precludes us from recommending the current formulation for use in rodents. As the manufacturer suspects the solvent used in the formulation, NMO, to

Cont. on pg. 6

Register Your Mice in the GEM Database



The CCMP, in conjunction with the Mouse Genetics Core, maintains a database of genetically engineered mouse (GEM) lines and stocks maintained at both WCMC and MSKCC. The database can be accessed from either of RARC's web sites. A listing of the strains and stocks currently available as well as an online form to submit database entries can be found on the sites or directly at http://intranet.med.cornell.edu/research/rarc/gem_db.html.
Participation is voluntary.

In addition to assisting other laboratories in achieving their research aims, registration is another way in which investigators whose research is federally funded can meet federally-mandated sharing requirements. Importantly, sharing of lines/stocks reduces the need to import them from other institutions or vendors reducing costs; the time to gain access to the animals, as quarantine is typically avoided; the costs associated with importation including per diem and testing in quarantine; the likelihood of colony contamination in that every atypical vendor import poses a risk to our colonies; and, it may also help in establishing intra- or inter-institutional collaboration.

Office of Laboratory Animal Welfare
OLAW
http://grants.nih.gov/grants/olaw/olaw.htm

NIH's OLAW Provides Assistance to Investigators Preparing the Vertebrate Animal Section for NIH Grant Submissions

Every NIH grant application involving the use of vertebrate animals must include the "Vertebrate Animal Section" (VAS).

The Office for Laboratory Animal Welfare (OLAW) has recently published a concise factsheet with detailed instructions on how to properly complete the VAS. See the OLAW factsheet at:
http://grants.nih.gov/grants/olaw/VASfactsheet_v12.pdf

Soon a link to the OLAW fact sheet along with other useful information will be added to our website
http://weill.cornell.edu/research/research_support/comp_med/

We hope these two resources will make your application process easier. RARC staff are also available to assist.

All newly purchased bottles display a large white arrow at the bottom indicating proper bottle orientation as demonstrated in the photo below.

!Flooded Cage Update!

RARC staff have been working diligently to identify, prevent, and/or eliminate the cause(s) of flooded mouse cages which has increased in incidence over the last 4 months. Several factors have been identified including the use of yellow tape, whose adhesive contains plasticizers which weaken the themoplastic, to assist staff in bottle positioning as well as the bottle's age. Beginning in March and continuing through the summer, bottles manufactured before 2007 will be replaced. RARC will no longer identify the drill hole or bottom of the bottle with yellow tape. All newly purchased bottles will display a large white arrow at the bottom indicating proper bottle orientation for animal water access. Additionally, RARC's cage wash staff have been testing bottles for leaking by placing them top down overnight; bottles that leaked are removed and evaluated. This process has decreased the number of flooded cages by over 80%.

Please remember that investigative staff should leave any flooded cages they find intact after relocating the cage occupants to a clean and dry cage, and notify RARC management so that the cause can be investigated. Questions or concerns should be directed to RARC's Husbandry and Operations management at rarcho@med.cornell.edu.

Sustained Release Buprenorphine, cont. from pg. 5

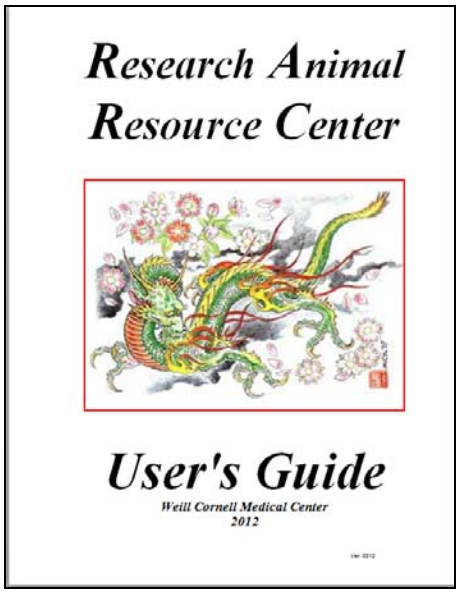
be the source of the problem they have replaced it with triacetin for use in rodents.

In conjunction with Zoopharm we are currently evaluating this formulation in mice. The concentration (0.5 mg/ml) is also more appropriate for use in rodents. Early results are promising. If the results demonstrate the drug to be both safe and effective, it will be made available for use at WCMC.

References:

1. Foley PL, Liang H, Crichlow AR. Evaluation of a sustained-release formulation of buprenorphine for analgesia in rats. J Am Assoc Lab Anim Sci. 2011; 50(2):198-204.

2. Catbagan DL, Quimby JM, Mama KR, Rychel JK, Mich *PM*. Comparison of the efficacy and adverse effects of sustained-release buprenorphine hydrochloride following subcutaneous administration and buprenorphine hydrochloride following oral transmucosal administration in cats undergoing ovariohysterectomy. Am J Vet Res. 2011; 72:461–466



Did you know that the 2012 RARC User's Guide is available via the WCMC intranet? Please visit:
http://intranet.med.cornell.edu/research/rarc/edu_tra/WCMC_User_Guide/index.html

Have suggestions for topics in the next InFocus? Please contact EQA with ideas and comments on the content of the newsletter. Your feedback is important to us!