**June 2012**

**Animal Care & Use Matters**

NUS is AAALAC Accredited

Closing of CM pharmacy in MD 4 and CeLS Vivarium.

 Standard Operating Procedure (SOP) of Comparative Medicine(CM) on pathogen screening for biological materials.

More IACUC Post-Approval Monitoring (PAM)

Emergency contact number for CM/OSHE/IACUC

**IACUC/ Protocol Matters**

 Mouse Cage Density and Trio Breeding

 Guidelines on PI and Contact Person

 All IACUC protocols will be approved for a maximum duration of 4 years with no further extensions

Launch of Genetically Modified Mice database

Importance of submission of Annual Protocol Review (APR) and Annual Breeding Protocol Review (ABPR)

**Animal Care and Use Matters**

**NUS is AAALAC Accredited**

AAALAC International is a private, non-profit organization that promotes the humane treatment of animals in research, teaching and testing through voluntary accreditation and assessment programs. AAALAC stands for the "Association for Assessment and Accreditation of Laboratory Animal Care” and is widely regarded as the “gold standard” to achieve in the animal care and use program. We are therefore very proud that NUS is officially listed as an AAALAC accredited institution on 22 Nov 2010. We would like to urge all researchers to continue to uphold the good animal care and use practice that was promised to AAALAC. AAALAC will be having another site visit to NUS sometime in 2013.

**Closing of CM Pharmacy in MD 4 and CeLS Vivarium**

Comparative Medicine (CM) has decided to have only one (1) pharmacy/consumable store at**MD2**.  As such, stores located in MD4 and CeLS vivarium have ceased operations since Monday, April 2nd 2012.

 The reasons for closing two of CM’s pharmacies are as follows:

1. Regulatory recommendations on reevaluating our current procedures/processes.
2. Staffing, consumables being underutilized.
3. MD4-Closing for renovation.

Researchers may contact CM Operation Director Mr Peter Cullen should they have any further queries/enquiries.

**Standard Operating Procedure (SOP) of Comparative Medicine (CM) on Pathogen Screening for Biological Materials**

CM has recently come up with a SOP supported by IACUC for the testing of murine pathogens in biological materials.

All murine derived and non-murine(including human) derived cell lines that have been passaged through rodents or have been exposed to rodents outside CM animal facilities will **need to be submitted** to CM for pathogen testing prior to being used in animals in NUS.

On the other hand, murine cells derived from donor animals located in the same facility and room as the recipients as well as non-murine derived biologicals that have NOT been passaged through rodents or exposed to murine products and commercially derived biologicals for which the vendor can provide negative pathogen screening results, need not be submitted to CM for testing. A copy of the SOP is available on IACUC’s website. Researchers can also contact CM at 65166410 or [lacacca@nus.edu.sg](mailto:lacacca@nus.edu.sg) for enquiries or to arrange for the testing of their biological materials.

**More IACUC Post-Approval Monitoring (PAM)**

In an effort to foster better communication between IACUC and the research community as well as to collect evidence of good performance, IACUC office would be conducting more routine Post-Approval Monitoring (PAM) sessions with PI and researchers. IACUC fully understands that the nature of science is an on-going process and as a result, PIs may have made some minor adjustments to their experimental setup. Therefore, PI are encouraged to treat PAM sessions as a valuable opportunity for them to check and clarify with IACUC on whether any small changes that they have made has reached a point where the protocol will need to be amended. This means that PAM will provide researchers with a chance to correct any deviations from their approved protocol if necessary.

IACUC will be conducting post-Approval Monitoring (PAM) on a more frequent basis. It is hoped that PIs and researchers would be able to provide IACUC office staffs with their full cooperation and support and to regard PAM as an effective bridge for closing the gap between regulation and practical science ( J.G.Collins.2008. Post approval Monitoring and the Institutional Animal Care and Use Committee(IACUC). ILAR J 39: 388-392)

**Emergency contact numbers for CM/OSHE/IACUC**

OSHE/ Campus Security           **6874 1616**

IACUC                                           **6516 2644**

**Contact list for ANIMAL Emergencies**

Please call the Emergency Veterinarian Number **90013073**. If unanswered, please leave your contact number. If one does not hear back within 10-15 min, please call the private mobile number of the vet on call. If there is still no response, please call any of the other vets.

Vet on Call

|  |  |  |
| --- | --- | --- |
|  |  |  |
| **Jan 2012** | **Feb 2012** | **Mar 2012** |
| Dr Jonnathon- 9747 0951 (HP) | Dr Shannon- 9780 6052 (HP) | Dr Enoka- 9080 3872 (HP) |
| **Apr 2012** | **May 2012** | **June 2012** |
| Dr Anna- 9227 7016 (HP) | Dr Jonnathon- 9747 0951 (HP) | Dr Shannon- 9780 6052 (HP) |
| **Jul 2012** | **Aug 2012** | **Sep 2012** |
| Dr Enoka- 9080 3872 (HP) | Dr Anna- 9227 7016 (HP) | Dr Jonnathon- 9747 0951 (HP) |
| **Oct 2012** | **Nov 2012** | **Dec 2012** |
| Dr Shannon- 9780 6052 (HP) | Dr Enoka- 9080 3872 (HP) | Dr Anna-9227 7016 (HP) |

**IACUC/Protocol matters**

**Mouse Cage Density and Trio Breeding**

IACUC currently allows 2 types of breeding. The first is “paired breeding” which consists of one male and female mouse housed in the same cage with the male removed prior to the birth of the first litter to avoid post-partum breeding OR the first litter weaned promptly at 21 days of age.

The second type of breeding is “trio breeding” which consists of 2 female mice and 1 male mouse being housed in the same cage with the pregnant female removed once it is obviously pregnant or when the plug is identified, and a new female may then be introduced to the cage once the pregnant female is removed.

Some revisions were made recently to the previous IACUC policy on “weaning and extended weaning”. In particular, this revision now includes and describes an alternative method on trio-breeding in addition to the approved trio breeding method described above. For this alternative method, **upon providing justifications and obtaining approval** from IACUC, PIs may be allowed to keep 2 females mice with their litters together with the male mouse in the same cage provided the pups are weaned promptly at day 21. The policy has now been renamed “Mouse cage density and trio breeding” and a copy of this policy can be obtained from IACUC website <http://www.nus.edu.sg/iacuc/policies.html>

**Guidelines on PI and Contact Person**

IACUC had at its 93th meeting, approved guidelines for the requirement of Principle Investigator-ship (PI) of an IACUC protocol. Besides full time members of the faculty, IACUC will include the following 3 criteria when accessing the suitability of an applicant to be the PI of an IACUC protocol.

1. Holder of an appropriate research grant or funds to carry out the intended animal work
2. Holder of a primary appointment and is salaried by NUS
3. Have a laboratory or research program to carry out research in NUS

In addition, at the 94th IACUC meeting, the guidelines for the contact person in the PI’s absence were approved. The contact person should be a primary appointment holder who is salaried by NUS, and who has appropriate animal / OSHE training & OH clearance. He/she should be given the authority in the absence of PI to make urgent decisions on animal welfare issues. . Both PI and the contact person must sign to officialise the agreement.

**All IACUC protocols will be approved for a maximum duration of 4 years with no further extensions**

At the 95th IACUC meeting, a proposal to revise the current maximum approval period from 3 years to 4 years was approved in view of the fact that actual animal work is often carried out months after IACUC approval due to administrative and logistical issues and difficulties such as staff recruitment. This policy will take immediate effect for all new IACUC protocol applications. PIs must however take note that there **will be no extension of the protocol beyond 4 years.** Work beyond 4 years will need a fresh protocol application to IACUC.

**Launch of Genetically Modified Mice Database**

NUS IACUC understands that sharing of valuable scientific resources such as transgenic mice can make for a more effective use of resources (Ref: NIH Statement on Sharing and Distributing Mouse Resources).

NUS IACUC office has therefore set up a database for genetically modified mice strains that researchers in NUS are breeding. This database has since been uploaded onto NUS IACUC’s website and made available to all researchers in NUS. Researchers who wish to use any genetically modified mice strain(s) in the database are **advised to read the disclaimer carefully** before contacting the relevant PI.

The IACUC office will seek PI’s permission to include his/her genetically modified mice into the database whenever it comes across a new breeding protocol or amendment involving genetically modified mice. PIs will have the final decision as to whether they would be able to share their strain(s) of genetically modified mice with other researchers. The database will be updated periodically.

**Importance of Submission of Annual Protocol Review (APR) and Annual Breeding Protocol Review (ABPR)**

Mandated by the local regulatory authority “The Agri-Food and Veterinary Authority (AVA)” as well as the “Association for Assessment and Accreditation of Laboratory Animal Care International (AAALAC)”, The submission of the APR and ABPR form serves as an important platform for IACUC to review the status of an approved protocol and the problem(s) encountered thereof in the past year. PIs are welcomed to provide IACUC with any feedback. Please note that the annual renewal of protocol is not automatic.

**Special Article**

**Post-approval Monitoring and the Institutional Animal Care and Use Committee (IACUC)**

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**Abstract**

Monitoring of the use of live vertebrate animals in research, teaching, and testing after approval of their use by an institutional animal care and use committee (IACUC) are receiving increased attention in the laboratory animal community. In this article the author provides his opinions on the value of postapproval monitoring (PAM) to the overall self-regulation that is the responsibility of an IACUC. PAM must never supersede or replace federally mandated IACUC responsibilities, but an efficient PAM process can provide significant additional information that enables an institution to be confident that it is meeting both the letter and the spirit of the federal regulations developed to ensure humane animal care. PAM personnel should be excellent communicators and able to maintain a professional demeanor in challenging circumstances. Their knowledge of laboratory animal care, invasive procedures, and regulations will enable them to align the pursuit of scientific research with adherence to these regulations. An effective PAM program involves knowledgeable individuals who can, on behalf of the IACUC, monitor new procedures and personnel and provide IACUC-mandated training or retraining.

**Key Words:** institutional animal care and use committee; postapproval monitoring

**Introduction**

Most of the material in this article is a matter of personal opinion so it seems prudent to share with readers some of the background that has helped to form those opinions. Before becoming chair of an institutional animal care and use committee (IACUC1) I was involved in efforts to educate the public about the use of live vertebrate animals in research, teaching, and testing. Those activities included frequent interactions with people who did not have a good understanding of the process of science and the role of animals in that process. Those interactions helped me realize that (1) the image of the "mad" or "crazy" scientist makes it easy for members of the public to believe that awful things happen behind closed laboratory doors; (2) the general public is unaware of the level of regulatory oversight that governs the use of live vertebrate animals in research, teaching, and testing; and (3) members of the public who support the use of live vertebrate animals in research, teaching, or testing want to know that there are programs in place to ensure that those animals receive humane care.

Further informing my opinions is my experience of more than 10 years as chair of an IACUC at a major research university, a term that began at a time when the institution's IACUC processes were in need of improvement. One of the very early tools that contributed to an improved IACUC was a nascent postapproval monitoring (PAM1) system. That, and the good fortune to hire an outstanding professional as director of the IACUC office, laid the groundwork for a much improved IACUC process. Throughout my tenure the individuals involved in postapproval monitoring, acting on behalf of the IACUC, have become an essential component of that improved process.

More recently, I have been privileged to serve as a core faculty member in the national IACUC 101 Program and have also participated in many AAALAC (Association for the Assessment and Accreditation of Laboratory Animal Care International) site visits. Those experiences have revealed opportunities for IACUCs to be better informed about the day-to-day operations of their animal programs.

My experiences have led me to conclude that a PAM process can be a valuable adjunct to the mandated self-regulation that is the responsibility of an IACUC. It is my hope that this article will help the reader appreciate the benefits of postapproval monitoring of IACUC-approved uses of live vertebrate animals in research, teaching, and testing.

**What Is the IACUC Process Really All About?**

The overarching theme of the articles in this issue of the *ILAR Journal* is animal use oversight. For readers not fully acquainted with the IACUC process, following is a brief overview of IACUC responsibilities and functions.

The use of live vertebrate animals in research, teaching, and testing is a privilege, not a right. Associated with that privilege are a number of responsibilities. In the United States oversight for the responsible use of animals depends on self-regulation at the local institutional level. The institutional animal care and use committee is the local body charged by the federal government to ensure self-regulation. As stated in the National Research Council's *Guide for the Care and Use of Laboratory Animals* (the *Guide;* NRC 1996, 9), each institution "must appoint an IACUC . . . to oversee and evaluate the institution's animal program, procedures, and facilities to ensure that they are consistent with the recommendations in this *Guide,* the AWRs [Animal Welfare Regulations], and the PHS [Public Health Service] Policy."

The charge to an IACUC is broad and far reaching. In brief, the committee is responsible for oversight of all live vertebrate animals, all processes that could affect the animals, and processes that could affect humans who work with the animals. The US Department of Agriculture's Animal Welfare Act and Regulations (AWAR 1989) and the Public Health Service Policy (NIH 2002) have listed the following functions that an IACUC must perform as it acts on behalf of the institution to oversee the use of animals:

1. At least once every 6 months, review the research facility's program for the humane care and use of animals.
2. At least once every 6 months, inspect all of the institution's animal facilities.
3. Prepare reports of IACUC evaluations based on facility and program reviews and submit those reports to the relevant institutional official (IO).
4. Review and, if warranted, investigate concerns involving the care and use of animals at the research facility.
5. make recommendations to the io about any aspect of the research facility's animal program, facilities, or personnel training.
6. Review and approve, require modifications to, or withhold approval of the components of proposed activities related to the care and use of animals.
7. Review and approve, require modifications to, or withhold approval of proposed significant changes in the care and use of animals in ongoing activities.
8. Exercise as necessary the authority to suspend an activity involving animals.

Depending on the species used and the source of funding, most IACUC members are familiar with the Animal Welfare Act (9 CFR) and the PHS Policy on Humane Care and Use of Laboratory Animals (PHS Policy). Many of us are also acquainted with AAALAC International's accrediting process, which relies on the *Guide.* Each of those documents outlines sometimes complex do's and don’ts. The intent of the documents is stated more simply in the US Government Principles for the Utilization and Care of Vertebrate Animals Used in Testing, Research and Training (NIH 2002, 4-5) and in the NASA Principles for the Ethical Care and Use of Animals (NASA 1998). In essence the IACUC is responsible for ensuring that research animals at the committee's institution are used in a humane way in procedures that are likely to improve animal or human health, will advance knowledge, and are for the good of society.

I believe that a well-functioning IACUC also provides the public with two very important assurances. The first relates to the desire of the public to be assured that "mad" scientists are not doing evil things behind closed doors. An institution should be able to point with pride to the self-regulatory oversight provided by its IACUC. Ongoing inspections and evaluations as well as the willingness to investigate concerns can do much to assure members of the public that humane animal care is viewed as an essential component of successful research, teaching, or testing. Going beyond the minimum standards of the regulations strengthens the argument that humane care is important to the institution.

The second assurance relates to public concern about the expenditure of public funds. Society demonstrates its support of the use of animals in biomedical research through the allocation of public funds to that research. The presence of a well-functioning IACUC is one example of the complex oversight process designed to ensure the appropriate expenditure of those funds.

It is clear to anyone familiar with the responsibilities of an IACUC that valid, up-to-date information about an animal program is essential if the committee is to meet its responsibilities. PAM can be a very valuable tool in obtaining that information.

**What is PAM?**

Other articles in this issue address, in detail, various ways to implement PAM (Banks and Norton 2008; Plante and James 2008); in this article I offer a basic definition and then provide a skeletal outline of the PAM process in use at my institution (and thus the one with which I am most familiar).

PAM can be briefly defined as any effort focused on determining what happens to animals after IACUC approval has been granted for their use in research, teaching, or testing. It can even be as simple as having husbandry staff monitor the condition of animals after surgical procedures.

At my institution 3.5 FTEs are devoted to PAM and they fulfill three major roles on behalf of the IACUC. The first is to conduct comparative reviews, which involve the review of approved protocols followed by a scheduled meeting with lab staff to discuss current activities in the laboratory (these lab meetings are independent of the semiannual facility inspections). The second role of these individuals is to oversee new procedures, new investigators, or procedures/investigators about which the IACUC has a concern. Their third role is to provide training and/or retraining as the need arises. There is a more complete description of these tasks later in this article.

**Why Would There Be a Change in an Approved Animal Use Activity?**

According to the definition of scientific method in Wikipedia (<http://wikipedia.org/>), scientific researchers propose hypotheses as explanations of phenomena and design experimental studies to test these hypotheses; the experimental steps must be repeatable in order to predict dependably any future results. The Oxford English Dictionary (<http://www.oed.com/>) defines scientific method as systematic observation, measurement, and experiment, and the formulation, testing, and modification of hypotheses. Note that in both definitions the formulation and testing of hypotheses is central. Although the popular press would have us believe that every new scientific finding is an absolute, in fact there is no such thing as a scientific truth. The best that scientists can do is to state that current hypotheses have not yet been proven invalid. Scientists are trained to continually test hypotheses and to do that requires that they change experimental plans as they gain new knowledge. The very nature of the scientific method requires change, which explains the need for ongoing modification to already approved animal use, and the approval process for modifications needs to be as rigorous as the initial one. PAM can help investigators determine when a series of small changes has reached the point that a protocol needs to be modified.

**Why Bother with PAM?**

IACUC members are charged with ensuring that the animals entrusted to the institution's care are treated humanely. It is not enough to approve a well-written and -justified protocol; the IACUC must also ensure that the natural process of change that is science has not resulted in significant modifications to what was initially approved. Without knowing what is really being done to the animals the committee is at a great disadvantage and lack of follow-up after protocol approval can lead to difficulties for both investigators and institutions. So institutions should "bother" with PAM in order to ensure that all laboratory personnel adhere to the approved procedures of each protocol and that essential personnel (IACUC members, the attending veterinarian, the IO) are as knowledgeable as possible about the animal program.

Because PAM also provides extra opportunities for communication with the individuals who work directly with the animals, it can reveal the need for training or retraining. Often the author of a protocol does not have day-to-day responsibility for animal use; and although principal investigators are expected to ensure that all their laboratory staff are fully trained, that can be a challenge. As an additional training resource, PAM personnel can be especially helpful at institutions where many individuals did not learn English as a first language.2 Differences in language and cultural understanding of the role of animals in society can create challenges in educating employees. It is not uncommon, for example, to find that respect for a lab chief makes it difficult for a student or technician to admit that they do not understand the specifics of animal handling or care. The presence of a neutral third party with the requisite skills to provide additional training can often ensure that regulations are understood and followed.

In these ways PAM can be valuable to an institution's efforts to meet regulatory responsibilities while supporting the advance of scientific knowledge.

**How Does PAM Differ from IACUC Responsibilities?**

As stated earlier, an IACUC has a clear charge to perform specific functions. PAM must not supersede or replace the IACUC in meeting those institutional responsibilities. Instead, PAM can be integrated into IACUC processes; for example, the IACUC may choose to use PAM as an additional source of information about laboratory conditions. If an IACUC decides to use PAM to evaluate an institution's programs or facilities the committee must maintain responsibility for evaluating and disseminating the resulting report.

Although it seems apparent that information obtained by PAM would be of value to an IACUC there is no need for direct interaction, so PAM may also function completely independently of the IACUC. For example, an attending veterinarian may decide that s/he would like to use PAM to more closely monitor animal health at a facility.

Whether or not PAM is associated with an IACUC, it is essential to establish clear lines of authority and reporting. There is an inborn conflict in any PAM process: on the one hand PAM personnel should be viewed as a resource by investigators, but PAM staff also need to be able to identify and deal with problems. What happens if they uncover a significant deviation from an approved protocol? What happens if PAM reveals a surgical procedure conducted by an inadequately trained individual? What happens if PAM personnel encounter unapproved housing of experimental animals? The reader can doubtless add other scenarios to this list.

Knowing about a problem and not dealing with it may be worse than not knowing at all. If an institution decides to implement PAM it must establish clear lines of authority and responsibility to address issues such as the following:

* Under whose authority do the PAM staff conduct inspections?
* What standards are they using for their inspections?
* Who determines whether something is a problem?
* If the PAM staff encounter unapproved research or a significant deviation from a protocol, what is done with that information?
* If they encounter unapproved research or a significant deviation from a protocol, are they authorized to inform lab members that the work may not continue?
* If they encounter problems that will require retraining, who will oversee and document the training?
* If the principal investigator or laboratory staff disagree with PAM findings what recourse is available?

These questions touch on some of the administrative issues that an institution must address if a formal PAM process is to be implemented. Although, as stated above, there is no requirement that PAM be associated with an IACUC, it is easier to address such questions if the PAM process occurs on behalf of the IACUC. An additional advantage of such an arrangement is that it enables researchers to better understand the process and reduces confusion among lab staff by presenting a unified presence and consistent communication about regulations, responsibility, standards, and training.

**What Skills Should PAM Personnel Have?**

The first and probably most important skill for personnel involved in PAM is the ability to listen and communicate well, especially in potentially adversarial situations. PAM personnel must be confident in their knowledge base and in their ability to maintain professional composure in challenging situations. In an ideal world interactions would always be collegial, but when they are not someone needs to keep a level head. Good listening skills are especially important when interacting with colleagues for whom English is not the primary language. In addition, broad experience with laboratory animal care and, if possible, invasive procedures typically used at the institution are also of great value. Needless to say, a thorough and clear understanding of regulations is necessary. With these skills PAM personnel can effectively bridge the gap between regulation and practical science.

**How Can an IACUC Use PAM to Its Advantage?**

**Provide firsthand observations.** For the IACUC to provide adequate oversight it must have ways to gather information about animals, what is being done to them, and the conditions in which they are maintained. Because PAM personnel constantly interact with laboratory staff, they become quite knowledgeable about the animal program. At our monthly IACUC meetings, for example, we frequently call on PAM staff to clarify issues, something that they are able to do because of their ongoing, direct contact with the individuals who work with the animals. As the PAM staff conduct comparative reviews on behalf of the IACUC, they observe firsthand the reality of the conditions in a laboratory and the level of expertise of the individuals who work with the animals. They thus provide the IACUC with a presence throughout the research facility.

**Monitor procedures and personnel.** With more than 450 principal investigators and more than 600 active IACUC protocols, our IACUC frequently encounters procedures with which members are not familiar, procedures that have not been previously published, and new laboratory staff who will be performing invasive procedures or whose expertise may be in question. In those situations PAM personnel provide an invaluable service to the IACUC by, for example, observing a new procedure or a person with questionable expertise. PAM personnel make it possible for the IACUC (1) to approve the start of a project knowing that there will be immediate feedback if any unexpected problems arise that must be addressed before the project may continue and (2) to support the process of science with confidence that the animals receive humane care. Essential aspects of this monitoring are both the close coordination between PAM and veterinary personnel and the PAM personnel's authority, on behalf of the IACUC, to inform an investigator that unapproved work may not continue and will be reported to the IACUC.

**Perform IACUC-mandated training or retraining.** When problems arise as a result of a laboratory staff member's lack of training or understanding of accepted procedures, mandatory training (or retraining) is an essential step toward allowing that person to work again with animals. PAM personnel, on behalf of the IACUC, are uniquely positioned to provide or oversee that training. Frequently they have already interacted with members of the laboratory and so are seen as a resource. Because they are working on behalf of the IACUC, their records provide documentation of completion of the desired training and they can return to the laboratory at any time to evaluate the success of the training.

There are many other ways in which PAM can support an IACUC but the above three have been of particular value to the IACUC at my institution.

**How Do You Know If Your Program Needs PAM?**

If your veterinary and husbandry personnel know the lab staff, the protocols, and the animals, you probably have PAM. Many programs have excellent PAM procedures already in place; and in fact most programs with which I am familiar have at least some components of a PAM process. If your institution is confident that the inevitable errors and accidents associated with an animal program are appropriately communicated to your attending veterinarian and IACUC then your postapproval monitoring is excellent.

If, on the other hand, your IACUC never receives a report about an error or accident associated with your animal program, your institution needs PAM. In spite of the best efforts of individuals dedicated to the humane care of laboratory animals, human error, equipment malfunctions, and accidents will occur. If an IACUC is not made aware of such events it cannot determine whether an incident was isolated or is an indication of a broader problem that must be addressed. Unfortunately, some IACUCs have not yet fully grasped the importance and complexity of program review and oversight—approving a well-written protocol is just the beginning of the process.

**Summary**

Remarkable advances in the life sciences are announced daily. Although the popular press and many scientists get caught up in the sensationalism of such announcements, many of which are premature, at the heart of the process is an increased knowledge base that continues to improve the quality of life for both human and nonhuman animals. Nonhuman animals are essential surrogates for humans in many of the experiments that lead to those advances. As a community charged with providing those animals with humane care we must use all of the tools at our disposal to meet that obligation. In addition to carefully reviewing proposed animal uses, the IACUC must ensure that the approved use adheres to the standards agreed to at the time of approval.

Postapproval monitoring is essential for institutions and IACUCs to meet their obligation to provide self-regulation of the use of live vertebrate animals in research, teaching, or testing. Such animal use is a privilege, not a right. We must demonstrate to society that we are worthy of that privilege.

1Abbreviations used in this article: IACUC, institutional animal care and use committee; PAM, postapproval monitoring

2The Council of Graduate Schools reported that from 1996 through 2006 the overall enrollment of international students in graduate programs increased by 4% per year, and by 5% per year in the health sciences between 2000 and 2006 (Redd 2007). A few minutes spent listening to the remarkable number of languages spoken during lunch in a cafeteria at most major research institutions is a reminder that US research is increasingly dependent on individuals from many backgrounds and cultures.

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