The American Physiological Society

Comments on the ILAR update of the *Guide for the Care and Use of Laboratory Animals*

January 28 2009

INTRODUCTION

The American Physiological Society strongly supports the notion that the *Guide for the Care and Use of Laboratory Animals* ought to remain precisely that: a guide. This can be accomplished by making certain that the *Guide* continues to rely upon performance-based standards and the exercise of professional judgment. This approach represents the best way to optimize animal welfare and the successful achievement of the research objective while minimizing non-productive regulatory burden. The *Guide* should facilitate efforts to balance welfare considerations and research objectives. Ultimately there may be nothing more wasteful or inhumane than the needless sacrifice of animal subjects in an activity that has been structured poorly so that it cannot answer the research question.

The APS supports the purpose of the *Guide* as stated in preface to the 1996 edition:

- "to assist investigators in fulfilling their obligation to plan and conduct animal experiments in accord with highest scientific, humane and ethical principles"
- "to assist institutions in caring for and using animals in ways judged to be scientifically, technically and humanely appropriate"[1]

The selection of an animal model is both a scientific and an ethical decision. The APS suggests that the Committee to Update the *Guide* include a preamble with first principles of animal model selection. It should emphasize the need to clarify the scientific question first since that will determine whether an animal model is necessary. If it is determined that the investigation of a particular research question requires an animal model, the rationale for selecting a particular model should be presented to justify why it is the right one to answer the question. Animal models are continually being developed and modified as technical advances and new knowledge is accrued. It would be useful if the *Guide* – perhaps in this preamble – could provide guidance on how to select the right animal model using hypothesis driven criteria including the search for homology and validity.

Animal care and use procedures in scientific research should be established by the IACUC through performance based standards because there is broad agreement that engineering standards cannot anticipate the complex and variable needs of research and animal welfare as science moves forward. Klein and Bayne explain:

> As opposed to more inflexible engineering standards, performance standards are predicated on sound professional judgment, and they facilitate science through an outcome-driven approach, rather than using prescribed processes that are difficult to define or rigid standards that may not fit each institution's circumstances. Performance standards provide criteria for assessing the desired outcome but do not limit the methods by which to achieve that outcome.[2]

Engineering standards can provide a general point of reference, but the use of performance based standards provides flexibility so that personnel can call upon their experience and exercise professional judgment to make adjustments needed to balance research objectives with requirements for humane care and use of animal subjects. For example, while an engineering standard for changing cages might be “1-2 times per week,” the performance standard might be “the frequency and intensity of cleaning and disinfection should depend on what is needed to provide a healthy environment.” This value of this distinction is evident when, for example a rodent model is selected to answer a scientific question regarding diabetes. In that case, changing the cages 1-2 times a week would be inadequate to provide a healthy environment due to increased urine production so the performance-based standard is more appropriate.

Implementing a performance-based approach is essential when research involves procedures that cannot be accommodated using standard animal care practices. When the IACUC has concluded that the research is valid and meritorious but the needs of science and humane care seem to be at odds, performance-based approaches offer the best way to strike reasonable balance between them. The decision-making process to define procedures that can fulfill performance-based standards should engage all relevant parties: Scientists can contribute their expertise concerning research objectives and methodologies, while laboratory animal veterinarians can offer expertise in anesthesia, analgesia and many other aspects of animal care. In some cases, input from specialists outside the institution should be sought to address certain issues. The IACUC should foster a spirit of engagement and cooperation to create an ethos of stewardship that encompasses both scientific and animal welfare imperatives.

Performance-based standards also have the potential to mitigate the growing problem of regulatory burden, which affects many different areas of research. In a 2007 survey conducted by the Federal Demonstration Partnership[3], scientists estimated that 42% of the time they spend on federally funded research was devoted to administrative and regulatory activities. This survey included researchers across all disciplines so it is possible that the burden for life scientists working with animals may be higher. Moreover, as Haywood and Greene have noted, the imposition of additional regulatory requirements is not necessarily beneficial:

> Attempting to minimize the risks of non-compliance through the overzealous application of ‘requirements’ does not necessarily benefit the animals.[4]

Haywood and Greene recommend instead fostering a “culture of compliance based on knowledge of the regulations, dedication to quality animal care, reasoned use of science-based performance standards, and the judicious application of professional judgment.”[4] Doing so provides a “foundation for facilitation of research in the context of animal welfare and regulatory compliance.”[4] This is consistent with a concern expressed by Klein and Bayne:

> The challenge, then, is to have a regulatory environment for animal research that is based on scientific data concerning ways to improve animal welfare, does not burden the cost structure of the institution or waste the public’s money, and provides legitimate benefit to the animals.[5]

The *Guide* should encourage IACUCs to implement performance standards in a manner that minimizes regulatory burden. In addition, APS calls upon the *Guide* Committee to be mindful of the problem of regulatory burden in every aspect of its revision efforts.

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[1] ILAR. 1996. Guide for the Care and Use of Laboratory Animals. Washington: National Academy Press.
[2] Klein, H.J., Bayne, K.A. 2007. Establishing a Culture of Care, Conscience, and Responsibility: Addressing the Improvement of Scientific Discovery and Animal Welfare Through Science-based Performance Standards. ILAR J 48:9.
[3] Decker, RS, Wimsatt L, Trice AG, Konstan JA. 2007 A Profile of Federal-Grant Administrative Burden among Federal Demonstration Partnership Faculty.
[4] Haywood, JR, Greene M. 2008 Avoiding an Overzealous Approach: A Perspective on Regulatory Burden. ILAR J 49:426-434.
[5] Klein, H.J., Bayne, K.A. 2007 48:5.

SUMMARY OF RECOMMENDATIONS

Study Design

The 1996 *Guide* stipulates that an IACUC must evaluate several criteria for every animal study performed. Assessing study design is an important part of the IACUC's review process because these issues are fundamental tenets of the three Rs (Reduction, Refinement, and Replacement). However, it provides relatively little guidance to IACUCs on how to approach such an evaluation.

Recommendations:

The IACUC should consider whether a study is meritorious and ought to be conducted. This must be an informed determination and should include consultations with appropriate experts as needed.

When expert evaluations of the science have already taken place in the grant review process, the IACUC should take this into account when reviewing the protocol. When scientific experts on a peer review panel deem a study to be worthy of sponsored funding, the IACUC ought to have a strong and documented rationale before concluding that such a study should not proceed. Requiring substantive changes to the experimental design at that stage also merits careful consideration.

Repeating studies to validate or build on published literature is an important part of the scientific process. Specific expertise may be needed to determine whether a proposal to repeat a given study is meritorious and necessary or duplicative and unnecessary. The IACUC should consult with appropriate experts if there is concern whether a proposed study should be conducted.

The *Guide* should not mandate the use of drugs made according to specific manufacturing standards or only drugs approved for clinical use. Many considerations apply to drugs used both experimentally and clinically. The use of non-pharmaceutical grade drugs may be necessary when pharmaceutical-grade drugs contain additives and/or preservatives that could influence experimental outcomes.

Minimizing pain and distress

The current *Guide* mandates the proper use of anesthetics and analgesics in research animals as an ethical and scientific imperative and points out that the manifestations of pain or distress are species-specific. Personnel who care for animals or conduct research procedure must be knowledgeable regarding behavioral, physiological, and biochemical indicators of well-being for the species in use.

Recommendations:

The general guidance concerning minimizing pain and distress is adequate but some specific information should be updated. This update of the *Guide* should provide new information about how to minimize pain without diminishing the integrity of the science. It would be helpful to include references to documented efficacious species-specific suggestions for analgesics and anesthetics. This information should be made easily accessible (e.g., on the OLAW web site) so that researchers and IACUC members can refer to it in IACUC protocols.

The *Guide* should not seek to offer uniform or objective measures of "pain." Unnecessary or extended exposure to painful procedures and recovery should be minimized. However, no simple "pain/distress scale" can be applied across all situations and all species. The current practice of assessing animal well-being through daily monitoring of basic behavior, grooming habits and food intake, with longer term monitoring of weight and reproductive performance, offers a reasonable, effective, and objective strategy.

Environmental Enrichment

The 1996 *Guide* states that potential enrichment interventions should be tested to ensure that they improve animal well being, do not inadvertently create an environment that reduces animal well being or endangers animals, and/or do not alter or blurs experimental results. Pre-testing such interventions requires expenditure of resources including animals and is an activity that is generally distinct from the scientific goals of a study. Consequently, the *de novo* development of acceptable enrichment strategies can turn into an "unfunded mandate" when these costs must be absorbed within a fixed budget.

Moreover, environmental enrichment is not necessarily a "win-win" strategy in terms of data quality and animal welfare. Environmental enrichments may have beneficial effects, but numerous peer-reviewed studies have shown that they can also have negative outcomes on research, including increased variability in measured parameters and skewed behavioral and physiological data. Nor do enrichments necessarily represent a net benefit in terms of animal welfare. The 3 Rs, which are widely seen as the primary rubric for implementing alternatives, include reduction, refinement, and replacement. Environmental enrichments may create a serious tension between the objectives of reduction and refinement since at the same time that they may improve conditions for individual animals, they may also increase the number of animals needed to generate valid data.

Some of the evidence offered in support of environmental enrichments is based on the preference of animals for one environmental option versus others. However, preference testing does not provide sufficient evidence to allow a determination of whether environmental enrichment is beneficial or should be a mandatory requirement. Claims for ethical benefits of environmental enrichments must be scrutinized carefully in light of potential negative outcomes, such as the need to increase the number of animals required to obtain valid data.

Recommendations:

The criteria for environmental enrichment in the 1996 *Guide* should be retained. That is, environmental enrichments should improve animal well being without creating an environment that reduces animal well being, endangers animals, and/or alters experimental results.

The *Guide* update should not contain broad mandates for environmental enrichment. Defining what is beneficial and desirable for an animal is not straightforward. Just because a particular environment is not overtly harmful to an animal does not necessarily indicate that it is desirable. An intervention that has a beneficial effect on animals of one species or strain may cause harm in others.

Even the previous experiences of an animal can significantly impact whether the animal responds positively or negatively to an item/situation.

The application of environmental enrichments should be performance-based and subject to professional judgment. Environmental enrichment should be employed only if solid data indicate that the enrichment intervention will enhance animal welfare without jeopardizing experimental design and/or outcomes. IACUCs should also be encouraged to recognize certain aspects of current husbandry practice (cage mates, bedding material, grill-top cages, and even behavioral training) that may in fact provide enrichment.

Animal preferences cannot be viewed as the ideal determinant of what best promotes their well being because animals, like people, may not always make the optimal choices. Some strains of mice will choose to drink an ethanol solution rather than plain water; NHPs and rodents will choose to self-administer psychoactive drugs; and, given a choice between a balanced diet and nutritionally deficient but highly palatable treats, many (if not most) animals will choose the treats

Standard laboratory caging should promote animal well being as reflected by normal growth, development and reproduction with low likelihood of injury, illness, distress, or maladaptive behavior. Animal well being, as defined in this manner, can exist even in housing situations in which an animal cannot perform its entire repertoire of its species-specific behaviors.

Multiple Major Surgeries

The *Guide* provides a broad definition of a major survival surgery: “Major surgery penetrates and exposes a body cavity or produces substantial impairment of physical or physiologic function.” It also lists as examples of major surgical procedures “laparotomy, thoracotomy, craniotomy, joint replacement, and limb amputation.” However, this definition is flawed because opening a body cavity is not necessarily distressful. For example, small craniotomies and “hybrid surgeries” that combine laparoscopy and entry through natural orifices appear to be associated with limited postsurgical pain or distress. In contrast, manipulating subcutaneous tissue –a minor surgery according to the current *Guide*’s definition – can cause significant postsurgical discomfort.

The current definition permits multiple minor procedures that cumulatively could cause considerable distress for an animal, while prohibiting the re-use of animals in studies where the opening of a body cavity may have caused only minimal pain or distress. This flawed approach increases the numbers of animals needed, which clearly contradicts the Three R’s objective of minimizing animal numbers.

Recommendation:

Revise the arbitrary definition of a major surgical procedure currently included in the *Guide.* The definition of major survival surgery should be re-written to focus upon the actual pain or distress of a given procedure. This would allow IACUCs discretion in designating whether a surgery is major or minor.

Housing

Housing densities, temperatures, humidity levels, and environmental enrichment are heavily interrelated. Simultaneous deviations in temperature and humidity pose a much greater challenge to the animal's homeostasis than would a deviation in a single factor. Some of the recommended levels in Chapter 2 of the current *Guide* are vague, and some of the ranges either lack scientific evidence to support them or are at odds with more recent scientific findings.

Recommendations:

Housing densities, temperatures, and humidity levels, and environmental enrichments should be considered as parts of an integrated environment. The *Guide* should utilize performance-based standards for temperature and humidity levels and should provide information to help institutions develop and assess performance-based standards for these factors. Environmental conditions should not be seen as isolated variables and they should not be governed by engineering standards.

The *Guide* should provide information that can help institutions develop and assess performance-based standards for the housing environment. This is a more constructive approach than treating environmental conditions as isolated variables subject to separate ranges, which fails to account for their synergistic impact upon one another.

Guidance concerning housing densities/floor space for rodent species should be revised in light of post-1996 innovations in caging systems and newer publications showing how different housing densities affect rats and mice. This is an important issue because of advances in rodent housing and the fact that these animals play a major role in current research.

The updated *Guide* should provide more flexible guidance on housing temperatures for rodents. The recommended housing temperatures for small rodents in 1996 *Guide* do not necessarily provide an appropriate environment for all species and strains. Providing nesting material is one cost-effective way to blunt the adverse effects of low ambient temperature on rodent homeostasis while also supporting the expression of natural behaviors that may contribute to animal health and well-being.

The updated *Guide* should adopt the FASS guidelines for the care of farm animals including its temperature guidelines. The *Guide for the Care and Use of Agricultural Animals in Agricultural Research and Teaching* of the *Federation of Animal Science Societies* (FASS) is based upon extensive study of the relationship between housing conditions and performance in farm animals. The updated *Guide* should endorse the FASS guide and provide an online link to this document.

The updated *Guide* should facilitate the implementation of performance standards by providing information about health problems that can occur when animals are subject to levels of humidity that are either too high or too low. In contrast to its recommendations for cage density and temperature, there is a great deal of latitude in the 1996 *Guide* concerning humidity, which is allowed to range from 30-70%.

Exercise

In 2006 the American Physiological Society published a *Resource Book for the Design of Animal Exercise Protocols.* This book was developed during series of meetings in 2003 and 2004 involving experts in the fields of exercise physiology and animal research models. It is intended for researchers, Institutional Animal Care and Use Committees (IACUCs), and those involved with research oversight. The authors, who included exercise physiologists and laboratory animal veterinarians, reviewed reference material and drew upon their own experience to compile suggestions about how to design, review, and implement experimental paradigms involving animals and exercise. Their manuscript was then peer reviewed by other exercise physiologists and laboratory animal veterinarians. The APS *Resource Book* is available on the web at http://www.the-aps.org/pa/resources/additional/exercise/book.pdf. The APS urges the committee to consider incorporating this reference into the updated *Guide.*

STUDY DESIGN

The 1996 *Guide* stipulates that an IACUC must evaluate each study in terms of the rationale and purpose of the proposed use of animals, and the justification of the species and number of animals requested. It must also determine whether it would be appropriate to substitute a less-invasive procedure, different species, isolated organ preparation, cell or tissue culture, or computer simulation. In addition, the IACUC must decide whether the proposed study duplicates previous experiments. These are crucial responsibilities. If the IACUC permits a study lacking in scientific merit to proceed, unnecessary animal use may occur. On the other hand, if a committee prohibits a study whose merits were not appreciated, progress may be hindered, possibly preventing or delaying an important scientific discovery. It is therefore essential that committees strike the proper balance between scientific and animal welfare objectives.

Recommendations:

The IACUC should consider whether a study is meritorious and ought to be conducted. It can be difficult for the IACUC to evaluate the merit of a proposal if none of its members have the specific expertise in a given area. This is likely to occur more frequently in the future as the volume of scientific information expands and areas of science become more specialized. IACUCs may find themselves hard pressed to make an informed judgment as to whether a proposal that seems to duplicate previous work ought to go forward. However, repeating experiments can be an important part of the scientific process. Repetition may be needed to confirm a previous finding, particularly in cases where new technological and methodological advances have been made. In such instances, the IACUC needs expert advice to determine whether the research is meritorious or not.

When expert evaluations of the science have already taken place in the grant review process, the IACUC should take this into account when reviewing the protocol. One way IACUCs can gauge whether research is meritorious is to take into account assessments made by funding agency peer reviewers. Most biomedical research is supported by grants, and most funding agencies decide how to allocate their scarce resources on the basis of expert peer review of both the scientific proposal and the experimental design. Government agencies and voluntary health organizations that follow this practice try to recruit leading experts to serve as their reviewers. IACUCs should take advantage of these assessments in evaluating protocols. Bringing in experts outside the committee is another approach IACUCs can take when additional expertise is needed to address questions about the study's scientific merit or its design. The updated *Guide* should recommend that IACUCs take liberal advantage of these two approaches.

When scientific experts on a peer review panel deem a study to be worthy of sponsored funding, the IACUC ought to have a strong and documented rationale before concluding that such a study should not proceed. Requiring substantive changes to the experimental design at that stage also merits careful consideration.

Repeating studies to validate or build on published literature is an important part of the scientific process. Specific expertise may be needed to determine whether a proposal to repeat a given study is meritorious and necessary or duplicative and unnecessary. The IACUC should consult with appropriate experts if there is concern whether a proposed study should be conducted.

Literature searches are not always the most appropriate way to accomplish the "consideration of alternatives" in protocol review. Consideration of alternatives is an important charge to the IACUC in reviewing study design and involves the "3 Rs" of reduction, refinement, and replacement. This is a mandate under the U.S. Government Principles for the Utilization and Care of Vertebrate Animals and has been incorporated into both the Animal Welfare Act and the PHS Policy. Efforts to accomplish reduction, refinement, and replacement are frequently incorporated into the study design by the time the protocol is presented to the IACUC. Nevertheless, because the USDA's AWA regulations emphasize literature searches, some IACUCs believe that they must require database searches for every protocol. However, APHIS Animal Care Policy 12 acknowledges that literature searches are not the only way to identify alternatives. Moreover, required database searches have sometimes turned into a meaningless exercise to fulfill a bureaucratic requirement. The updated *Guide* should encourage IACUCs to accept the most <u>appropriate</u> approach to considering alternatives, whether that is a database search, consultation with a veterinarian, or discussions with other experts. The *Guide* should further clarify that when an alternative has been considered and/or incorporated into the study design, the principal investigators should describe what they have done, but further justification for the selection of the model through a literature search is unnecessary. This will alleviate an unnecessary burden to both investigators and IACUCs.

The *Guide* should not mandate the use of drugs made according to specific manufacturing standards or only drugs approved for clinical use. Many considerations apply to drugs used both experimentally and clinically. The use of non-pharmaceutical grade drugs may be necessary when pharmaceutical-grade drugs contain additives and/or preservatives that could influence experimental outcomes

MINIMIZING PAIN AND DISTRESS

The 1996 *Guide* mandates the proper use of anesthetics and analgesics in research animals as an ethical and scientific imperative. It further notes that since the manifestations of pain or distress are very species-specific, “It is therefore essential that personnel caring for and using animals be very familiar with species specific (and individual) behavioral, physiologic, and biochemical indicators of well-being” (*Guide*, page 64). The *Guide* also incorporates the widely accepted standard, set forth in the U.S. Government Principles for the Care and Use of Animals in Testing, Research, and Training, that unless the contrary is known or established it should be assumed that procedures that cause pain in humans also cause pain in animals.

Recommendations:

The APS believes that the 1996 *Guide* offers an adequate framework for minimizing pain and distress. The *Guide* update should include more recent references to documented, efficacious, species-specific recommendations for analgesics and anesthetics that can be used to minimize pain without diminishing the integrity of the science. It would be particularly helpful if this reference material were made easily accessible (e.g., through the OLAW website) so that researchers and IACUC members had a common point of reference in the formulation and review of research protocols. Several universities have already adopted a strategy making a list of suggested analgesics and anesthetics accessible online so researchers can include in their IACUC protocols. The University of Iowa provides one such resource at http://research.uiowa.edu/animal/?get=aa_regimens. It includes information on pain medications recommended for common research animals such as mice, rats, rabbits, guinea pigs, dogs, cats and macaques.

The *Guide* should not mandate the use of specific drugs or specific manufacturing standards. Many factors must be considered in choosing a pain management/limitation strategy. These include strain or species differences and the potential of certain drugs to affect the dependent variables in a study. Every experiment has its own set of unique issues that must be taken into account in determining whether pain medication is appropriate for a given procedure, and if so, what type. All drugs can have adverse side effects, depending on dose, species and health/treatment condition. Drugs should be selected in consultation with a veterinarian to meet clinical and experimental needs with the dose, frequency, and route of administration designed to provide an appropriate balance between efficacy and risk. For example, it is well known that some non-steroidal anti-inflammatory drugs (NSAID) are harmful to rodents. The NSAID indomethacin produces intestinal inflammation in rodents (6, 7, 9), which might be a counter indication for its use in some cases. For procedures such as subcutaneous pellet implantation or tail vein blood sampling that produce brief and minimal levels of discomfort, the administration of analgesia can be more harmful or distressing to the animal than the procedure itself.

Another issue to be considered is the impact of pain medications on the dependent measures of the study. For example, administration of opiates to alleviate the pain of a procedure could confound the results if measures of pain or immunity are the dependent measures of interest since both are directly impacted by opiates (1, 3, 10, 11, 12). If administration of specific pain medications were mandated, it would create the risk of either confounding any experimental results or requiring significant increases in the numbers of animals. This would create a conflict between the 3 Rs objectives of replacement and refinement since the refinement (through the use of pain relieving drugs) would run counter to reduction (because of the need to increase sample size).

Analgesic choice for transgenic/knockout strains is also a matter of concern. With the dramatic growth in the number of new strains, current analgesic guidelines are rapidly becoming outdated. We are not aware of peer reviewed studies in this area, but it has been observed anecdotally that NSAID effectiveness differs in wild-type vs. cytokine knockout mice.

In addition, a distinction should be made in the manufacturing standards required for drugs used in clinical vs. those used as part of an experimental protocol. Although pharmaceutical-grade compounds should be required in treating clinical problems, there are many instances where it is unnecessary or inappropriate to use clinical-grade compounds, such as in cases where they contain additives or preservatives that would influence experimental outcomes.

The *Guide* should not try to offer a uniform or objective measure of "pain." The 1996 *Guide* requires researchers to design their experiments so as to minimize unnecessary or extended exposure to painful procedures and recovery. However, mandating the use of pain scores or scales would be inappropriate. The study of pain, nociception, and pain measurement is an important, active and complex area of research. The American Pain Society (http://www.ampainsoc.org) currently has 3,200 members who are working to understand these phenomena. The current practice of assessing of animal well-being by the daily monitoring of basic animal behavior, grooming habits and body weight offers a reasonable and objective strategy.

The *Guide* should not mandate housing enhancements such as social groupings or cage features based upon the behavior of animals in non-laboratory settings. Trying to improve animal welfare by specific housing mandates runs the risk of undermining other aspects of animal welfare. This has been noted frequently in situations where social housing causes increased aggression. The vast majority of animals in research are descendants of animals that have lived in laboratory settings for many generations. These animals are far removed from their wild counterparts and have very different needs. This is especially true of transgenic animals. In these cases it does not make sense to base housing requirements on the needs of wild-type animals. Mice are known to display very different levels of aggression across strains. Some mice are better served by being housed in isolation, while others are better served in group housing environments (8). Activation of the stress response is a powerful force that impacts nearly all aspects of physiology (2, 4, 5). Scientists always seek to control the research environment so as to avoid the unintentional activation of the stress response. One important aspect of this is to ensure that housing environments do not perturb basal physiological responses. Therefore, the goal as stated in the 1996 *Guide* remains valid: to provide housing that is adequate and humane. The APS urges this committee not to mandate specific housing requirements that go beyond this performance-based goal.

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ENVIRONMENTAL ENRICHMENT

Refinement of the care and use of animals in research has been ongoing for many years. This has led to numerous informal best practices and relatively high but general standardization, particularly with regard to animal housing, genetics and health status. This trend has likely contributed significantly to a substantial decrease in the number of animals needed for research and testing.[17 18] Recent interest in the implementation of environmental enrichment (EE) for research animals as a form of refinement has generated many studies seeking to determine how various environmental perturbations intended to promote well being in laboratory animals affect behavioral and physiological parameters that are commonly measured. The findings and conclusions of these studies are mixed, particularly with regard to the application of EE interventions across species, strains, genders, and ages; whether or how they impact the animals and the science; and, in some cases, whether the effects are positive, negative, or neutral in terms of animal well being.

The 1996 version of the *Guide for the Care and Use of Laboratory Animals* contains only a limited discussion of EE, noting the need to consider factors such as the availability and suitability of enrichments in "planning for adequate and appropriate physical and social environment, housing, space and management." It also set forth the performance goal that "animals should be housed with a goal of maximizing species-specific behaviors and minimizing stress-induced behaviors. This is a good approach in light of the conundrums and lack of consensus evident in the research on EE.

Standardization of EE programs would be problematic at present because no consensus currently exists concerning the definition of enrichment and how to determine its efficacy.[7] In general, EE for laboratory animals refers to the use of housing conditions that offer enhanced sensory, motor, and cognitive stimulation of brain neuronal systems in comparison with standardized, relatively impoverished, housing conditions.[15] EE paradigms typically incorporate features such as social housing, larger enclosures, the presence of objects that may be changed periodically to provide novelty, three dimensional structures for climbing or exercise, foraging opportunities, and hiding or nesting areas.[9 15] For example, solid-bottom bedded cages, which are a standard method of rodent housing, may be viewed as providing enrichment, as compared with wire bottom cages, because bedding can be used for the species-specific behaviors of burrowing and foraging, and they may also reduce aggression.[3 7] However, the interpretational conundrums, varying conclusions, and lack of consensus in the literature preclude a rational basis for mandating the use of EE in research settings. Rather, the approach described in the 1996 *Guide* should prevail, namely, the exercise of professional judgment in how to meet performance standards combined with flexibility in terms of when and how EE should be applied.

Achieving optimal housing conditions for animals is a laudable goal, whether applied to research, agricultural production, zoos, or even pets. As such, the consideration of interventions to improve animal well being is warranted under many if not most circumstances. However, the complexities and uncertainties that permeate the application of changes in housing in the research environment warrant careful consideration of their benefits and costs of such interventions and suggest that a continued reliance upon professional judgment and performance standards rather than rigid requirements.

Literature review

A brief overview of some of the studies that have evaluated EE from various perspectives illustrates the complexity and inconsistency that pervade its use. The most basic approach is to evaluate the impact of given forms of EE on measured variables in normal animals. Several such studies report, for example, that EE either changes the mean values of body weights, organ weights, or hematological parameters, or does not influence mean values but increases variation in mean values of some measured variables. [16 20 41 53 54] In another study, housing males and female mice of four inbred strains in the same cage type led to the conclusion that EE can affect experimental results; it does not necessarily improve well-being; and it may create conflicts between achieving "refinement" versus "reduction." [55] The latter comment arises because if EE increases the variability of measured parameters, it would potentially require an increase in the number of animals needed to achieve reasonable statistical power and perhaps even change the experimental results. [53] In another example, a comparison of C57Bl/6 and 129S6/SvEv mice found that housing in an enriched versus a standard environment increased exploratory activity in the plus-maze and reduced habituation in the locomotor activity test in C57Bl/6 mice, whereas 129S6/SvEv mice showed increased hot plate latencies and reduced aggression.[1] EE accentuated strain differences in the plus-maze, locomotor activity, hot plate and forced swim tests, whereas strain differences in the plus-maze and resident-intruder tests were not conserved across environments.[1] Similarly, in rats, the impact of enrichment on the response to various procedures depends on both the rat strain and the form of enrichment used.[30] Finally, of potential concern as an issue of animal well-being, some studies report that EE is associated with increased aggression. [22 23] Such potential adverse outcomes and demonstrated complexity could directly jeopardize animal well being and have the further adverse effects of increasing the number of animals needed and/or altering experimental results, which also has the potential to influence research findings, particularly if different strains of a species are being compared. Because the same enrichment design can have positive, negative, different, or no effect, based upon the strains used and the variables studied, considerable forethought is necessary before the introduction of enrichment designs into experimental plans.[53 55]

Another experimental approach to the assessment of EE evaluates the impact of cage size and housing density on normal animals with and without additional enrichment. Such studies find complex interactions between housing density and EE, with additional influences including gender, cage size, and the specific measure of behavior used. For example, male pups born in enriched cages showed more anxiety-like behavior and less exploration than did males born in non-enriched cages. [64] However, EE improved reproductive performance regardless of cage size; pups from non-enriched cages weighed less than pups from enriched cages, and fewer survived to weaning age. [64] In another example, mice housed under various densities were all provided with basic enrichment of paper bedding and a plastic house. Singly housed mice developed lower fecal corticosterone levels over time, reaching a minimum from 14 days onwards.[27] In group housed mice, housing density did not affect fecal corticosterone, aggression, or stereotypy. [27] Providing additional enrichment reduced fecal corticosterone in group housed but not singly housed mice. [27] Taken together, these representative studies illustrate that inbred strains of mice vary in their responses to EE and support the conclusion that the scientific determination of an optimal cage density standard or EE strategy that can be uniformly applied across all mouse strains will be a difficult task. [34]

A final type of study evaluates the impact of EE on animal models of disease. Considerable evidence indicates that EE can modulate brain development and promote recovery from brain damage; however, consensus has not been reached concerning which aspects of enrichment are either crucial or optimal with regard to causing those effects. [9 16 45] Changes induced in the brain by exposure to EE include modifications in structure and neurogenesis, changes in cognitive function, and alterations in brain chemistry; benefits have been reported for the treatment of depression and mental retardation, cognitive dysfunction in Alzheimer's and aging models, functional deficits in neurodegenerative disease, and epilepsy. [15 38 45] Furthermore, as in studies of normal animals, findings from these studies are also complex. For example, in one study, hamsters were housed individually in cages of four different sizes with or without enrichment for 14 weeks. [33] Mean basal rectal temperatures were significantly higher in hamsters maintained in small cages, but the injection of lipopolysaccharide (LPS) induced a greater febrile response in animals housed in large cages, with no effect on variability.[33] Therefore, in this study, cage size appears to influence thermoregulatory homeostasis differently under basal conditions and in response to experimental manipulations. [33] As a final example of this tremendous complexity, cholecystokinin-2-receptor-deficient (CCK2R-KO) and control mice housed under EE or standard housing showed significant genotype x environment interactions in plus-maze, hot plate, restraint-induced analgesia, and water maze tests. [2] As compared with corresponding wild-type littermates, CCK2R-KO mice housed in standard conditions had higher measures of anxiety and restraint-induced analgesia and worse performance in the water maze when housed under standard but not enriched conditions. [2] In contrast, a genotype-dependent phenotype was detected in the hot plate, rotarod and locomotor activity tests among mice housed in enriched, but not in standard conditions. [2] Finally, the phenotype of CCK2R-KO mice in the hot-plate and rotarod tests was gender-dependent. [2]

Many studies find no effect of EE on facets of physiology and behavior or measured experimental endpoints. For example, one study evaluated the impact of nesting material on mice and detected no major differences in behavioral and physiological parameters under different housing conditions, concluding that supplying nesting material will not jeopardize the outcome of experiments. [58] Another reported that EE does not increase individual variability in behavioral tests or the likelihood of obtaining conflicting data in replicate studies, concluding that housing conditions of laboratory mice can be enriched ("improved") without affecting the standardization of results. [66] In a transgenic mouse model for Alzheimer's disease, EE had comparable effects in wild type and transgenic mice, with both strains showing higher levels of exploration and locomotor behavior with EE; learning and memory measures were not affected by EE in either strain.[21] As a final example, housing in standard or enriched cages did not affect either mean values or variation of behavioral parameters recorded in a behavioral test involving treatment with the anxiolytic drug diazepam.[4] However, since these examples, like all other studies of this type, evaluate only a limited number of conditions, parameters, and strains, broad conclusions seem unwarranted in light of these clear limitations in scope. The thoroughness of such studies and the associated scope of their conclusions require careful scrutiny before EE suggestions are implemented. For example, one group that had previously validated a series of neurologic and behavioral screening batteries for several commonly used inbred strains across laboratories investigated whether subtle changes in cage environment could affect outcomes in behavioral tests. [56] The study identified several significant and distinct genotype-environment-test interactions; for example, strain phenotype distribution patterns for open-field center entries and total distance traveled can be reversed depending on the form of enrichment used, while prepulse inhibition of the acoustic startle response is not affected by the enrichment condition. [56] These findings indicate that subtle variations in cage environment may confound the interpretation of data collected in behavioral studies, and that the impact may vary depending on the specific behavior studied. [56]

Because of these considerations, the purported benefits of EE in laboratory settings must be carefully scrutinized before the interventions are imposed. Otherwise, changes in responses, skewing of data, inconsistencies between different forms of EE, and increased variability may confound data or increase the number of animals needed to generate valid data. As stated by others, "the provision of enrichment should be evaluated in the context of the health of the animal and research goals on a case-by-case basis," "ultimately, the decision to include a particular type of enrichment should be based on a consideration of the safety of the animal and the staff, whether the enrichment has a demonstrable beneficial effect on the animal, and whether the potential effects of the enrichment are experimentally relevant." [7] Furthermore, "minor cage supplementation intended for improvement of animal well-being may alter important aspects of an animal's physiology and development in a manner not easily predicted from available research," and "we do not understand the mechanisms by which rodents respond physically to environmental changes sufficiently to implement them to a knowledgeable manner." [9]

Table 1: Questions and issues relevant to regulatory requirements and performance-based programs for environmental enrichment

	Question	Issues
1.	What is enrichment?	Variation across species and strains in terms of environmental needs complicates the development of highly inclusive, prescriptive, or preemptive statements about EE. Consensus on such a definition will be difficult to achieve, but is necessary before abandoning local performance-based regulation and reliance on professional judgment in favor of regulatory mandates. In addition, enriched housing must be differentiated from adequate housing, with an associated determination of whether EE is essential or adequate housing is acceptable.
2	What is enrichment intended to accomplish?	The goals of EE should be carefully defined, with measurable outcomes that are known to benefit the animal in a significant way. These goals should be defined in terms of both the stimulation of positive species-typical behaviors and/or the prevention of abnormal or undesirable behaviors.
3	How is enrichment related to normal standardized housing and a natural habitat?	Some items of the animal environment might be viewed as EE if these were not already standard or experimental components of normal animal care. Examples include some features of "standard" caging (e.g., bedding, grilled cage tops) and behavioral training of non-human primates. Mandated use of EE would require that its form be defined and somewhat standardized for each species across labs to facilitate consistency and replication across labs.
4	Who should determine whether a given enrichment intervention benefits and/or does not harm the animal or the science?	At present, an IACUC or regulatory agency may demand implementation of enrichment. However, since a number of experimental situations could be negatively affected by EE, any requirement for enrichment should only be implemented after consultation with investigators. Moreover, an investigator should never be forced to employ EE unless there is solid evidence that the manipulation would not interfere with experimental outcomes.

5	What is “enough” enrichment, and how can that be measured?	Common indices of animal well being are variables such as food intake, growth, and appropriate breeding performance. Standard laboratory housing normally meets these performance goals. Even though promoting “psychological well being” is mandated by law for non-human primates, defining and measuring this outcome is itself a complex task.
6	What are the potential negative consequences of EE?	Variation in the caging environment between research groups will contribute to greater variability in measured outcomes and may therefore reduce statistical power and require using more animals in the experimental design. Also, despite good intentions, some EE interventions may overtly harm the animals or confound interpretation of the data.
7	What are potential negative consequences of social housing?	Categorizing rodents, NHP, and other animals as “social” and housing them accordingly overlooks many potential adverse consequences of their social natures and social housing. Social animals can be highly territorial and/or develop hierarchical relationships that may adversely affect both high- and low-ranking animals (e.g., prevention of access to food, need for constant vigilance to retain a dominant status). Furthermore, the presence of both dominant and submissive individuals among caged groups causes variability among animals in the group in terms of features like hormonal profiles and neurogenesis.

Recommendations:

EE should be based on solid data indicating that the enrichment intervention will enhance animal well being without jeopardizing experimental design or outcomes. The need for and implementation of EE is subject to many considerations and should be implemented based on the combined judgment of all professionals involved and assessed based on performance standards. IACUCs and research staff should work together to provide an adequate environment that meets animal needs yet is cost effective and standardized. Together, these groups should be able to determine which enrichment implements could/should be used in conjunction with standard animal housing, and the decision and selection should be based on scientific data. The issues of housing and enrichment are intertwined. The Animal Welfare Act already mandates EE for nonhuman primates. In addition, the literature suggests that IACUCs and/or animal resource facilities are implementing housing modifications that they believe will enrich an animal's environment, but not necessarily with any scientific evidence to support its benefit.

The *Guide* update should not contain broad mandates for environmental enrichment. Defining what is beneficial and desirable for an animal is not straightforward. Just because a particular environment is not overtly harmful to an animal does not necessarily indicate that it is desirable. An intervention that has a beneficial effect on animals of one species or strain may cause harm in others. Even the previous experiences of an animal can significantly impact whether the animal responds positively or negatively to an item/situation. In addition, whether targeted EE and behavioral management programs produce better research models from a behavioral standpoint needs further exploration so that data, rather than anecdotes, can be evaluated for merit.

Animal preferences cannot be viewed as the ideal determinant of what best promotes their well being because animals, like people, may not always make the optimal choices. [9] [14] [19] For example, some strains of mice will choose to drink an ethanol solution rather than plain water, NHPs and rodents will choose to self-administer psychoactive drugs, and given a choice between a balanced diet and nutritionally deficient but highly palatable treats, many (most?) animals will choose the treats. A review that assessed 40 studies published between 1987 and 2000 concluded that mice ‘prefer’ a more complex cage compared to a standard cage because they will work for access to cages with shelter and raised platforms. [48] However, a key issue is not whether EE is preferred by animals, but whether it should be mandatory, assuming housing is adequate to promote animal health.

Standard laboratory caging should promote animal well being as reflected by normal growth, development and reproduction with low likelihood of injury, illness, distress, or maladaptive behavior. However, animal well being, as defined in this manner, can exist even in housing situations in which an animal cannot perform its entire repertoire of its species-specific behaviors. [42] Furthermore, assessment of even these seemingly routine benchmarks is complex. For example, one group reports that EE was associated with fewer mouse pups born, fewer litters per dam, and an earlier age-related decline in production in breeding females, but had no significant effect on breeding index (young weaned/female/week), with a complicating interaction with type of caging system used. [52] [54] In contrast, another group reports that EE improved reproductive performance, pups from non-enriched cages weighed less than pups from enriched cages, and fewer survived to weaning age. [64] [65] A related perspective that may merit consideration is that the behavioral needs of animals that have been bred for generations under laboratory conditions may differ substantially from those of similar wild or ancestral species, and the behavioral needs of laboratory species, as with other domestic species, have likely adapted well to the confined and controlled conditions in which they live. [43]

A commonly used form of EE is the provision of a nest box or nesting material. However, the use of even such seemingly benign interventions can create variable results in terms of benefit to the animal. For example, one study reported that even the location of a plastic nest box within the cage influenced whether mice used or avoided it. [32] The complex dome-shaped, multilayered nests that are constructed by wild mice are not commonly seen in the laboratory setting, perhaps because of inappropriate nesting material rather than poor nest-building ability of research strains. [25] Providing naturalistic nesting materials can improve nest quality, as assessed using a 'naturalistic nest score' system, and these naturalistic materials can be sterilized for use in common housing systems. [25] Although provision of nesting material may reduce aggressive behavior in some strains of mice, [59] [60, 61] providing a shelter can increase aggression and physiologic indices of stress in other strains. [22] [40] [59]

Other issues and considerations

Positive contact with humans is another common EE recommendation. Animals of many species (e.g., dogs, cats, ferrets, rabbits) that become socialized to humans at a young age are easier to handle while on study. [13] Exposure of many animal species to humans, unique situations, and objects early in life can also reduce the negative consequences of novel situations and objects that they may experience later in life in a research environment. [31] For example, an environment that is enriched with toys and food treats may make rabbits more sociable towards their human caretakers, with no adverse effects on reproduction, although effects on research have not been evaluated. [28 29 49] However, although these approaches may be feasible in some situations, their application to large numbers of animals (particularly rodents) can be problematic from the perspective of disease risk and personnel time.

The response of animals to their environment is influenced by genotype, gender, and age, among other factors. [6 43, 48 57] Studies of the impact of the environment on animals are always limited by design, leaving open the question of whether other crucial but unmeasured parameters are (or are not) changed or made more variable. These limitations mandate that enrichment interventions should be carefully evaluated prior to implementation in a research setting to determine that the actually improve animal well being, do not create an environment that reduces animal well being or endangers animals, and/or do not alter experimental results.[7] Such considerations support basing the need for and implementation of EE on the combined judgment of all professionals involved. IACUCs and research staff should work together to provide an adequate environment that meets animal and research needs yet is cost effective and well defined and controlled. Together, these groups should be able to determine which enrichment could and should be used in conjunction with standard animal housing based on scientific data and experimental goals.

A concern that is sometimes raised regarding standardized (non-enriched) housing is that like standardized genetics, it may generate results that are specific to the specific experimental constraints. [67] Although this is likely the case in at least some circumstances, precisely controlled experimental conditions are nonetheless essential to the ability to measure, quantify or even detect the subtle and complex biological signals that are fundamental to advancement in modern biomedical knowledge. This process of standardization in animal housing, genetics and health status has likely contributed significantly to a substantial decrease in the number of animals needed for research and testing. [17 18]

Although standardization of environments reduces variation across groups, experiments and laboratories, [46 64] controlling inter-laboratory variation can be difficult even when such standardization is part of a study by design. [12 61] The use of different types of EE can increase variability within and between labs. [52] To minimize data variability, housing density should be strictly controlled. [33] Concerns regarding enrichment are its undefined impact on the animals, increased variability in the data, and poor definition across labs and in publications (contributing to discrepant results across labs), potential for animal harm, and where to draw the line with regard to adequate housing versus optimal housing versus preferred or even beneficial housing.

Table 2: Key concepts relevant to changing housing conditions for research animals [9]	
1	Animal welfare and EE comprise different, not synonymous, dimensions of animal housing, and EE does not guarantee improved animal welfare.
2	Animals' environmental preferences are not a guideline to their well-being and can be physically detrimental.
3	The term 'housing supplementation" may better describe cage additions beyond feed, water, and bedding. This term establishes no expectations with regard to the effects on the animals, and thus can be used to describe an experimental condition when the effects on animal well-being

	are not yet known or are not the subject of a primary question.
4	In many cases, neither laboratory animal science experts nor researchers can be certain whether supplementing a standard rodent cage compromises animal well-being or research results. When either outcome is in question, EE should not be mandated by the institution or oversight agencies.
5	Alterations in housing that clearly promote better health, reproduction, and fitness are beneficial to both the animals and those who use and care for them. However, attempting to improve emotional states that cannot yet be reliably identified or measured may not help either the animals or the research as intended.
6	Variability can be difficult to control both within and between laboratories. For this reason, the potential for small environmental differences to significantly affect research results should not be underestimated.
Adapted from [9] and reprinted with permission.	

Non-human primates

EE issues relevant to NHP require special consideration since the Animal Welfare Act specifies that these animals must be housed in a manner that promotes their psychological well being. Considerable evidence indicates that NHPs housed under impoverished conditions develop behavioral abnormalities, including, in the most extreme example, self-injurious activity. [26] In part because of these problems and the AWA legislation, hundreds of peer-reviewed publications have evaluated the effects of EE or behavioral management procedures in NHP. This body of literature now provides a scientific foundation for the care and management of captive NHP and is available to guide regulations and policies related to EE for NHP. Behavioral management programs in which NHP are taught to cooperate in the conduct of research, veterinary care, and animal management procedures are becoming an increasingly important feature of NHP care and use programs. [5, 36 37 51] Such behavioral training can increase species-typical behavior, improve social interactions, reduce physiological changes associated with research, veterinary and animal management procedures, and diminish abnormal behaviors (e.g., stereotypy). [10 11 35 50] Avoiding or minimizing the development of abnormal behavior not only improves the well being of these animals but also enhances their value for education and research. [26]

Socialization is the central foundation for promoting and maintaining the psychological well-being of NHP. Social housing is now viewed as the “default” system, with a well justified reason required for maintaining animals in individual housing. Such reasons could include health problems, severe social incompatibility, and scientific research that cannot be accomplished with socially housed subjects. Facilities housing NHP must have the caging, equipment and staff expertise appropriate for forming and maintaining the animals in social groups. Social housing is particularly important for young NHP because of the long-term negative consequences of early social deprivation (e.g., deficits in breeding and parenting, expression of stereotypic and self-injurious behavior). [8 39 46] Research protocols that require individual housing or social isolation should use adult rather than young animals unless young primates are essential for the work.

Although standardization of EE for NHP across facilities might be desirable to reduce possible confounding factors in research, it will be difficult to achieve. Many aspects of animal husbandry vary across facilities, including caging, feeding routines, management procedures, interactions between animals and staff, and the specific studies being conducted in the animal population. All of these considerations will influence the feasibility, selection and implementation of EE procedures. In addition, recalibration of critical dependent measures may be necessary as EE programs develop. Furthermore, EE procedures will continue to change (improve) over time. Such variation may require transitional assessment to delineate effects, perhaps recalibrate critical dependent measures, and allow the animals to adjust prior to their use for data collection. For example, an intervention or dependent measure that has been used for individually-housed NHP may require different implementation or generate different outcomes when used among socially-housed animals. Such variation may require transitional assessment to delineate differences and allow appropriate adjustments prior to the use of these animals for data collection. Including information on EE procedures in publications is crucial to facilitating the confirmation and extension of published results.

EE must be managed carefully to minimize the likelihood of introducing health problems. If EE devices, interventions, and opportunities are not well managed, potential problems that may arise include ingesting materials that are toxic or may cause intestinal blockages, becoming entangled in or strangled by enrichment devices, transmission of disease, and aggressive competition, wounds or stress related to living with conspecifics. However, none of these problems are unique to enrichment (for example, disease can be transmitted by an improperly sanitized cage), and most of these potential problems can be avoided. EE should be offered in ways that facilitate safety. For example, established cleaning procedures should be routinely used for EE devices, and the devices should be tested to verify that the sanitation is effective. Strangulation hazards can be minimized by using care in the attachment and presentation of EE devices in the cage. Risks associated with social housing can be moderated by taking precautions when introducing NHP to one another, careful monitoring of socially housed NHP, and applying behavioral management strategies to address severe aggression. EE that is appropriate to the species, sex, age and background of the animals can reduce aggression, decrease or eliminate abnormal behaviors, and substantially improve the general well being of captive NHP. [27] The tremendous benefits to NHP well-being should be weighed against the potential health risks, which are relatively small.

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MULTIPLE MAJOR SURVIVAL SURGERIES

Recommendation:

Revise the arbitrary definition of a major surgical procedure currently included in the *Guide.* The definition of major survival surgery should be re-written to focus upon the actual pain or distress of a given procedure. This would allow IACUCs discretion in designating whether a surgery is major or minor.

The tenet that it is undesirable to subject an animal to multiple major survival surgeries was established in the Animal Welfare Act and Animal Welfare Regulations. The following is stipulated in 9 CFR, Part 2, Section 2.31 (d)(1)(x) of the Animal Welfare Regulations:

> *No animal can be used in more than one major operative procedure from which it is allowed to recover unless justified, such as when required by or related to other surgical procedures, in writing, by the principal investigator; required as a routine veterinary procedure or to protect the health or well-being of the animal as determined by the attending veterinarian; or in other special circumstances as determined by the Administrator upon request. This requirement and the exceptions to it were contained in revised proposal Secs. 2.30(f)(1) and 2.35(b)(3)(ii)(D), except that reference to endangered species or marine mammals and the provision that cost savings is not adequate justification for multiple use of animals are removed. Such concerns may be addressed to the Administrator as an element of the special circumstances which might justify multiple major operative procedures.*

This regulation acknowledges that there may be legitimate reasons to conduct multiple major survival procedures in the same animal, including for scientific necessity and routine veterinary care. Moreover, the Animal Welfare Regulations indicated that under special circumstances the same animal may be utilized in multiple surgical studies because it provides a mechanism to request approval to do so. USDA's APHIS Animal Care Policy 14 supplies further guidance on how to request permission to employ animals in multiple surgical studies.

Interestingly, the Animal Welfare Act and Regulations do not stipulate which surgical procedures constitute a major procedure. Under the AWA, this determination is left to the discretion of the IACUC. By contrast, the 1996 edition of *Guide* offers specific guidelines including this definition of a major survival surgery: "Major surgery penetrates and exposes a body cavity or produces substantial impairment of physical or physiologic function." The 1996 *Guide* also provides examples of a major surgical procedure, including "laparotomy, thoracotomy, craniotomy, joint replacement, and limb amputation." No references are provided to justify this definition, nor the validity of the notion that opening a body cavity is necessarily painful or distressful for an animal. To the contrary, there is evidence from human surgical studies that a craniotomy is often associated with minimal levels of postsurgical pain (Atkinson et al., 1993; Conway, 1984; Dunbar et al., 1999; Bonica, 1990). New "hybrid surgeries" are also emerging that combine laparoscopy and natural orifice surgery techniques (Pearl, 2008). Such methods result in very limited postsurgical pain or distress, although a body cavity is penetrated. In contrast, manipulation of subcutaneous tissue, which is considered a minor surgery according to the definition in the current *Guide*, can result in significant postsurgical discomfort. As such, the arbitrary definition of a major surgical procedure provided in the *Guide* could permit the performance of multiple minor procedures that cumulatively cause considerable distress for an animal, while prohibiting the reuse in multiple surgical studies of animals that have experienced an opening of a body cavity that resulted in minimal pain or distress. The latter could be a violation of a major tenet of animal subject research: that the number of animals employed and euthanized during experimental studies should be minimized.

The *Guide* does mention one "special circumstance" that justifies utilizing an animal in more than one study involving major surgery: to conserve scarce animal resources. A 1990 National Research Council report ("Important Laboratory Animal Resources: Selection Criteria and Funding Mechanisms for their Preservation") is cited as guidance to evaluate whether an animal falls into this category. This report deals mainly with preservation of mutant and transgenic lines, although some of the principles stipulated within it are more broadly applicable: importance, validity, replaceability, versatility, and use of the model. Under some situations, a common species that has recovered from a major survival surgery could seemingly fall into the category of such a scarce resource. For example, after multi-year recovery from an experimental transplant surgery, an animal could be viewed as a valuable resource and as such should be eligible for another study employing major surgery in a case where the experiment requires the use of this particular unique model and the additional surgery would not result in undue pain or distress for the animal. Such a circumstance could be viewed as permissible by the Animal Welfare Act, if properly evaluated by an IACUC and approved by the USDA Administrator. However, it is unclear whether the standards promulgated in the *Guide* offer this flexibility.

The *Guide's* specious definition of a major survival surgery, as well as its failure to properly allow an evaluation of special circumstances for using an animal in more than one study involving major surgery, should be re-evaluated. The well-being of individual animals and minimizing the pain and distress they experience should be paramount in the consideration of using them in multiple surgical studies. However, such determinations should be left to the judgment of an IACUC as well as to the USDA when an Animal Welfare Act-covered species is involved.

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HOUSING, ENVIRONMENT, AND MANAGEMENT

Chapter 2 of the 1996 *Guide* includes specific ranges as guidelines for housing densities, temperatures, and humidity levels. While such engineering standards can sometimes provide helpful benchmarks, the APS strongly recommends the use of performance standards for animal housing. This is particularly important because some of the ranges recommended in the 1996 edition of the *Guide* are unclear or lack supporting evidence, and others are at odds with more recent research findings. Therefore the housing, environment, and management chapter of the *Guide* should be updated to include more current research where available and to address gaps in supporting documentation.

Recommendations:

Housing densities, temperatures, and humidity levels, and environmental enrichments should be considered part of an integrated environment because these factors are heavily interrelated. The tolerance of individual animals to variation in the environment is also influenced by body size, strain, age, gender, breeding status, and health issues. The combined effect of temperature and humidity can increase the homeostatic challenge, while other factors such as the presence of nesting material can allow some species to adapt to suboptimal conditions. Lighting, cage and rack systems, and ventilation of primary and secondary enclosures may also cause the environment to be more or less suitable to an individual animal's needs.

The *Guide* should provide information that can help institutions develop and assess performance-based standards for the housing environment. This approach is more constructive than is treating environmental conditions as isolated variables subject to separate ranges, which fails to account for their interactions with one another.

The updated *Guide* should adopt the FASS guidelines and provide an online link directing readers to this document. The *Guide for the Care and Use of Agricultural Animals in Agricultural Research and Teaching* of the *Federation of Animal Science Societies* (FASS) is based on extensive study of the relationship between housing conditions and performance in farm animals. The updated *Guide* should endorse the FASS guidelines rather than duplicate or contradict this effort.

Housing density

Recommendation:

Guidance concerning housing densities/floor space for rodent species should be revised in light of post-1996 innovations in caging systems and newer publications showing how different housing densities affect rats and mice. Rodents play an increasingly important role in current biomedical research. Moreover, since the 1996 edition of the *Guide*, a number of studies have been published investigating the influence of different housing densities for rats and mice. During this period there have also been significant advances in rodent housing systems. Consequently, housing density guidelines for these species should be reassessed in light of new housing options and findings concerning floor space requirements.

Several studies investigating the impact of housing mice at various densities indicate that some strains can be housed at about twice the density (i.e., half the floor space) currently recommended in the *Guide* without any significant impact on stress hormones, behavior, or immunologic function (Fullwood 1998, McGlone 2001, Smith 2004, Smith 2005). Although the temperature and levels of CO_2, and NH_3 in the cages increased along with the number of animals per cage, all remained within tolerable limits for animals housed in individually ventilated cages (Smith 2004, Smith 2005). Similarly, an earlier, pre-1996 study found that housing mice at these increased densities had no effect on body or adrenal weights (Peters 1990). Other studies found that certain aspects of immune function and mortality improved in mice maintained at a higher housing density (Fullwood 1998, McGlone 2001). Although one recent study found minor changes in plasma corticosterone levels and T-helper lymphocytes in female BalbC mice housed at higher density, the changes were small and did not occur in C57Bl6 mice (Laber 2008). This and other studies indicate that the appropriate housing densities depend on the physical environment and the strain being housed.

Under some conditions, housing rodents at lower densities than those recommended in the 1996 *Guide*, including single housing, may be advisable or necessary. For example, single housing may be preferable if aggressive male mice would otherwise injure cage mates. In addition, for certain scientific objectives, group housing can introduce additional biological variability, particularly in behavioral and endocrinologic studies. If rodents are group housed for such studies, a larger number of subjects may be needed to detect small but physiologically important experimental effects and to achieve statistical significance.

Several studies demonstrate that the responses to differing housing densities are strain dependent (Smith 2005, Peters 1990, Laber 2008). Although mice of the FVB/NJ strain display increased aggression at higher housing density, C57BL/6, BALB/c and NOD/LtJ mice do not (Smith 2004, Smith 2005). Corticosterone levels and lymphocyte function were affected in female BALB/c mice housed at higher densities, but not in C57BL/6 mice (Laber 2008). Environmental enrichment also has an influence (Hunt 2006), as do age, gender, genetic modifications and the type of cage and cage-rack. The number and complexity of these variables provide a strong rationale for avoiding required compliance with engineering standards for mouse housing densities.

Some have suggested that housing density guidelines that are lower than those recommended in the 1996 *Guide* would reduce variation between studies from different laboratories (Laber 2008). Certainly it would be inappropriate to change housing conditions in an ongoing study. However, the APS opposes rigidly normalizing housing densities for the sake of inter-study comparability. Housing densities should be determined for specific studies based upon factors such as experimental design, the goals of the research, cage and racking system, and providing appropriate care for the animals based on strain and other factors previously discussed. The updated *Guide* should provide information resources yet assign local IACUCs the responsibility of determining appropriate rodent housing densities using performance-based criteria.

One important aspect of housing that was not addressed in the 1996 *Guide* is space requirements for dams with litters. Among mice, particularly in trio-breeding settings, densities may easily exceed the weight-based recommendations for floor space per animal given in Table 2.1 of the 1996 *Guide*. Three recent studies that investigated how space affected mouse litters (Whitaker 2007, Davidson

2007, O'Malley 2008) consistently found no significant impact on weight gain or corticosterone levels in newborns raised at different densities, including some that far exceeded those currently recommended. These studies further found no significant abnormalities in behavioral tests or reproductive performance in the adult mice as a result of the differing housing densities. The APS suggests that in the updated *Guide,* dams and their litters be considered as a single biological unit prior to weaning, with appropriate housing densities for dams and litters of various strains determined by the local IACUC.

In summary, most mice seem to thrive in higher housing densities than are recommended in the 1996 *Guide* without significant physiological compromise, and the same seems to hold for rats (Sharp 2003, Bean 2008). However, the fact that so many factors contribute to the determination of what is appropriate underscores the need for a performance-based approach rather than a single set of floor space requirements.

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Housing temperatures for rodents

Recommendation:

The updated *Guide* should provide more flexible guidance on housing temperatures for rodents. The recommended housing temperatures for small rodents in 1996 *Guide* do not necessarily provide an appropriate environment for all species and strains. The ambient temperature (Ta) at which small rodents are housed can have a large impact on energy expenditure and core temperature (Tc) homeostasis. The 1996 *Guide* recommends that small rodents be housed at Ta ranging from 18-26°C, regardless of differences in body size (Table 2.4). Because small rodents are largely dependent on metabolic regulation for thermal homeostasis (Phillips 1995), prolonged stimulation of metabolic rate during housing under cool ambient conditions can alter normal food and water consumption, reproductive fecundity, and general activity profiles.

Small animals typically live in burrows where they huddle in small groups to conserve heat and energy; however, these behavioral mechanisms of heat conservation are not typically available in laboratory animal facilities. Because the threshold Ta for the stimulation of a shivering response is ~20°C in mice (Oufara 1987) and ~15-18°C in rats (Stoner 1971), some laboratory rodents housed under the current range of Ta recommended by The *Guide* may be residing under cool ambient conditions that chronically stimulate increases in metabolism. This has the potential to stimulate thermoregulatory adaptations that are unaccounted for in experimental studies.

Nonconformity in experimental designs between studies has resulted in large variability of reported thermoneutral (TNZ) values for small rodents. For example, the TNZ of rats has been reported to span a 12°C range (22-34°C; Clarkson 1972; Gordon 1987; Gwosdow 1985; Herrington 1940; Pace 1983; Poole 1976; Swift 1939). Strain differences within a species are known to exist – e.g., hairless mice are more sensitive to low Ta, with a TNZ that is ~2-4°C higher than that of albino mice (Mount, L.E., 1971). Strain differences in TNZ values have also been reported for the rat (Gordon, C.J., 1987).

Data in mice and rats indicate a 24-hour profile of preferred Ta (behavioral preference in a thermal gradient) with relatively warm temperatures (~33°C in mice; 28°C in rats) selected during the lights-on (sleeping) period and ~2-4°C lower temperatures selected during the lights-off (active) period (Gordon, C.J., 1987; Gordon, C.J., Becker, P., and Ali, J.S., 1998; Leon, L.R., 2005). The selection of cooler Ta during the lights-off period is a behavioral response to increases in metabolic heat production during the nocturnal period when increases in food consumption and activity profiles are typically observed. However, the 1996 *Guide* does not consider circadian variability in thermal requirements of rodents.

Overall, the data indicate that (1) the preferred Ta of mice and rats is higher than those currently recommended by The *Guide*, (2) strain differences are apparent in mice and rats (Gordon, C.J., 1987; Leon, L.R., 2005; Mount, L.E., 1971), and (3) circadian variation in preferred Ta is evident. The updated *Guide* should provide flexible guidance and suggest simple strategies that facilities can use to meet the wide-ranging needs of various species and strains of rodents. For example, providing nest-building materials may minimize the effects of low ambient temperatures. Nesting is a behavior that is naturally expressed in both wild and laboratory rodents under the proper environmental conditions (Hess et al., 2008). As shown by Gordon (2004), nest building is sufficient to blunt the effects of low ambient temperatures on metabolism and circadian core temperature fluctuations in mice. Providing nesting material represents a cost-effective method to blunt the adverse effects of low ambient temperature on rodent homeostasis while also supporting the expression of natural behaviors that may contribute to animal health and well-being.

Housing temperatures for farm animals

Recommendation:

The updated *Guide* should incorporate housing temperature guidelines for farm animals in the FASS *Guide for the Care and Use of Agricultural Animals in Agricultural Research and Teaching*. The 1996 *Guide* recommendations are particularly problematic for newborn animals and could be improved greatly by referencing animal care guidelines developed by other professional societies, including those formulated for farm animals and non-mammalian species.

The 1996 *Guide* recommends a room temperature of 16-27°C (61-81°F) for "farm animals and poultry" (page 32, table 3.2). Some temperatures within this range are clearly inadequate for some species and can cause severe distress. For example, a room set at the maximal allowed temperature of 81°F would cause severe distress and a high-rate of mortality due to cold exposure for day-old chickens and newborn pigs. Given the breath of species included under this definition and the detailed guidelines available for these animals (see FASS reference), reference to farm animal guidelines would be preferable to providing ranges.

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Humidity

Recommendation:

The updated *Guide* should facilitate the implementation of performance standards by providing information about health problems that can occur when animals are subject to levels of humidity that are either too high or too low. In contrast to its recommendations for cage density and temperature, the 1996 *Guide* allows a great deal of latitude concerning humidity, which can range from 30-70%. However, high humidity can strongly influence animal well-being by challenging water and temperature homeostasis. This may affect scientific results, particularly in studies involving salt and volume homeostasis. On the other end of the spectrum, low humidity is thought to cause skin lesions and necrosis of the tail in rats ("Ringtail," Njaa 1957, Totton 1958, Taylor 2006).

Humidity should be evaluated in conjunction with temperature and other factors (housing density, cage ventilation, and rapid air changes versus static caging). This assessment can best be accomplished through a performance-based approach overseen by the local IACUC rather than by imposing an engineering standard. The updated *Guide* could facilitate the implementation of performance standards by directing IACUCs to sources of information about health problems that may occur in various species due to high or low humidity. In addition, the updated *Guide* should emphasize an integrated view of all housing conditions.

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Additional References

We recommend referring readers to additional resources on animal care guidelines such as the ones below.

By Species

Farm Animals

Federation of Animal Science Societies. 1999. *Guide for the Care and Use of Agricultural Animals in Agricultural Research and Teaching.* Savoy, IL

Banner Committee (1995). *The Report of the Committee to Consider the Ethical Implications of Emerging Technologies in the Breeding of Farm Animals*. HMSO, ISBN: 112429653

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Reptiles and amphibians

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Fish

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Invertebrates

National Research Council (1981). *Laboratory Animal Management: Marine Invertebrates.* National Academy Press: Washington, DC, 382p. NAL call number: SF407.M37M37

Animal Care Guidelines and Resources

Canadian Council on Animal Care

Guide to the Care and Use of Experimental Animals, vols. 1, Second ed. 1993 (120 pp.) and 2 1984 (208 pp.). Canadian Council on Animal Care, 1105-151 Slater, Ottawa, Ontario KIP 5H3, Canada.

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Special topics

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