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We are writing on behalf of People for the Ethical Treatment of Animals—PETA entities have more than 9 million members and supporters globally—to provide recommendations on current work to update the guidelines that will result in the 9th edition of the *Guide for the Care and Use of Laboratory Animals*.

General recommendations

1. To effectively update the 8th edition of The Guide, NASEM's Standing Committee for the Care and Use of Animals in Research must consider our evolving understanding of animals' biological, psychological, and social characteristics. Additionally, they should incorporate advancements in human-relevant, non-animal research methods that can serve as alternatives to animal use. The 8th edition of The Guide was published in 2011. Over the past 13 years, we have gained significant insights into the ethological needs of animals, numerous factors that confound data obtained from animals in laboratory settings, and the challenges with reproducibility and translation of research findings.

2. According to the 8th edition of The Guide, this document exists to "assist institutions in caring for and using animals in ways judged to be scientifically, technically, and humanely appropriate." Also: "Recommendations in the Guide are based on published data, scientific principles, expert opinion, and experience with methods and practices that have proved to be consistent with both high-quality research and humane animal care and use." As such, in situations for which the implementation of human-relevant, animal-free methods would be scientifically appropriate, the Guide should recommend these methods be adopted in place of using animals. Indeed, The Guide has the opportunity to position itself in the leading role in the transition to animal-free innovation and openness. To achieve this, The Guide should include the following:
   - Recommendation that personnel—including scientists, regulators, and grant reviewers—must receive training and education on the intrinsic and extrinsic factors that limit reliable translation of data from animal models to the clinical realm as well as the capabilities and functionalities of non-animal methods (also called new approach methodologies or NAMs) and their benefits.
   - Recommendation that personnel—including scientists, research personnel, regulators, and grant reviewers—must receive training on animal methods bias (resources available at www.animalmethodsbias.org) as well as steps that can be taken to ensure that this bias does not encourage the approval of protocols that use animals.

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- Advocating for a cost-benefit analysis of employing non-animal methods versus experiments on animals.
- Ensuring that information on procurement, training, funding, and use of non-animal methods is transparent and widely available.

3. Project evaluation should consist of the following:
   - A systematic review of relevant literature on similar animal models and research questions before an animal experiment is approved.[1] Systematic reviews have been shown to improve study design and reporting, facilitate data exchange, reduce repetition, promote collaboration, and help researchers acquire new skills and insights.[2]
   - A thorough harm-benefit analysis carried out prior to the use of animals in research and mandated as part of the approval process. Harms to animals must include not only procedural harms that would occur within proposed experiments, but also the lifelong harms associated with life in a laboratory and, in the case of some species, re-use in multiple projects. Expected benefits to humans, animals, or the environment must be assessed in an evidence-based context that includes previous failures (such as the failure of similar experiments to provide meaningful benefits, [3] which could be assessed by the systematic reviews suggested in the previous bullet point) and take into account confounding variables inherent to laboratories, such as stress inflicted on animals.
   - The assessment of the compliance of the project with the 3Rs, with an increased focus on replacement.[4]
   - A more stringent and transparent assessment and assignment of the classification of the severity of procedures.
   - The searches for both alternatives to the use of animals and alternatives to proposed painful procedures should be conducted in appropriate databases using a variety of effective keywords and appropriate terminology, preferably under the consultation of a research librarian.

4. Researchers on PHS-supported studies should be required to comply with the ARRIVE guidelines when publishing their work. The ARRIVE guidelines improve reporting so that studies are described in enough detail that they can be replicated and have a greater chance of contributing to science. PHS-supported researchers should also be required to publish all data, even if it is negative.[5]

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[3] Animal Procedures Committee. Review of the assessment of cumulative severity and lifetime experience in non-human primates used in neuroscience research. 2013 Accessed July 2024 https://assets.publishing.service.gov.uk/government/uploads/system/uploads/attachment_data/file/261687/cs_nhp_review_FINAL_2013_corrected.pdf

[4] Rawle F. The Role of Review and Regulatory Approvals Processes for Animal Research in Supporting Implementation of the 3Rs. NC3Rs; 2023.

[5] du Sert NP, Hurst V, Ahluwalia A, Alam S, Avey MT, Baker M, et al. The ARRIVE guidelines 2.0: updated guidelines for reporting animal research. PLOS Biol, 2020;18(7):e3000410. https://doi.org/10.1371/journal.pbio.3000410

5. The number of animals used by each institution—including species not covered by the Animal Welfare Act (AWA)—should be reported and published on the institution's website.
6. IACUC composition: Committee members filling the non-scientist and unaffiliated roles should be individuals who represent the interests of the local community, as Congress intended. These individuals should serve a maximum term of five years to maintain the effectiveness and objectivity of the position. Individuals who use animals in research should make up no more than 1/3 of committee membership. Imbalanced IACUC composition favoring researchers and institutional interests can lead to groupthink, polarization of opinions and the suppression of dissent. Nonaffiliated IACUC members have reported feeling intimidated and report that their input is often dismissed or diluted because they are outnumbered.[6] It has been reported that IACUC members believe that membership comprised of more nonscientists and community members would improve the committee's functioning.[7]
7. Facilities should be required to create more naturalistic living environments to reduce the chronic stress endured in animals in laboratories and lessen the experimental confounds caused by this stress. The committee should not wait until every last study is conducted to determine whether adding more space or cage complexity will reduce these factors, but instead use common sense to appreciate that animals engaged in stereotypic behaviors or self-mutilation will not produce good science.

Recommendations for Chapter 3: Terrestrial Housing

1. Laboratory environments inherently cause stress to animals,[8] with environmental factors within laboratories accounting for as much as 42% of data variability.[9] To reduce stress and data variability, animals should be provided with sufficient complexity within their enclosures to allow expression of a wide range of normal behaviors, like running, walking, climbing and jumping.
2. The institution's Animal Care and Use Program should include clear, species-specific protocols for assessing the success of social housing, determining the compatibility of conspecifics, and for introducing unfamiliar conspecifics.
3. Despite the robust language in the 8th edition of the Guide, recommending social housing for social species, animals in laboratories are still often housed alone. Single housing, a psychosocial stressor, and nursery rearing of all animals, especially nonhuman primates, should be prohibited as they cause major behavioral problems and reduce welfare.[10,11] It is well established that nonsocial housing alters animals' behavior and physiology. Singly

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[6] Schuppli CA, Fraser D. Factors influencing the effectiveness of research ethics committees. J Med Ethics. 2007;33(5):294-301. doi:10.1136/jme.2005.015057

[7] Graham K. A study of three IACUCs and their views of scientific merit and alternatives. J Appl Anim Welf Sci. 2002;5(1):75-81. doi:10.1207/S15327604JAWS0501_7

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housed primates exhibit stereotypic and depression-like behavior.[12] Additionally, animals housed in isolation have poor welfare outcomes.[13,14,15] Single housing of animals used in experiments is also associated with critical physiological and behavioral changes. Animals housed alone exhibit higher blood pressure, higher fecal cortisol and heart rate, altered neuroendocrine functions,[16] and abnormal immunological functioning[17,18] compared to socially housed animals. A recent meta-analysis found that housing conditions can increase the morbidity of induced stress-sensitive conditions, such as cancer, strokes, anxiety, cardiovascular disease, and depression in rodents. Single housing impairs associative learning and memory, frequency of food retrieval, and affective reactivity to complex objects[19] of animals used in experiments. There is considerable evidence that the change introduced by housing conditions affects outcomes across *all species and biomedical research fields*.[20,21] There is a clear need for stronger language in *The Guide* on social housing which recognizes single housing as a significant experimental confound and pushes laboratories do to better.

4. According to the American Society of Primatologists, "single housing should not be assumed in research involving the application of experimental appliances (e.g., telemetry implants, head caps, eye coils, tethers), the administration of substances exerting psychosocial effects, or use of restricted/controlled diets. Such research has been successfully performed on socially housed subjects." The group added: "Anticipated financial constraints or lack of resources such as appropriate caging are not a sufficient reason to fail to provide social housing."[22]
5. Animals should be socially housed in stable groups of compatible individuals, with additional outdoor access. Social living improves the replicability, reproducibility, and external validity of experiments. For nonhuman primates, socialization is the best form of enrichment, and animals value social housing over other forms of enrichment, though those must also be provided. Conflict and aggression between conspecifics are natural and do not

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[13] Baker KC, Bloomsmith MA, Oettinger B, et al. Benefits of pair housing are consistent across a diverse population of rhesus macaques. Appl Anim Behav Sci. 2012;137(3-4):148-156.

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mean animals need to be separated or isolated. According to a 2022 review,[23] enrichment such as nesting material or other items that can be manipulated and in which animals can hide are promising tools to minimize aggression among group-housed mice. The review recommended using less aggressive strains of mice whenever possible. For primates, "behavioral managers should regularly monitor the subordination signaling network (i.e., the power structure), sex ratio, and conflict policing behavior present in their social groups to determine whether each group has the appropriate group composition to promote their own natural conflict management mechanisms."[24] "Enrichment that is species, sex, age and background appropriate can dramatically reduce aggression,"[25] whereas "human activity increases aggression."[26] Another way to reduce this issues with aggression is to conduct experiments in naturalistic settings, such as forgoing laboratory-based experiments for field studies.[27]

6. Animals' basic needs should not be optional and relegated to the category of 'enrichment.' Many recent studies demonstrate the positive effects of more complex and naturalistic environments on abnormal behavior,[28] aging, immunity, and cancer in rodents[29] and reduced trauma in nonhuman primates.[30] Laboratories should be mandated to explore housing and husbandry frameworks developed by organizations outside of the laboratory space and engage in consultation with animal welfare and protection organizations.
7. Standard cages for mice[31] and rats[32] interfere with important natural behaviors and increase morbidity and mortality in rodents used in experiments. This contributes to the failure of animal research to translate into clinical trials and lowers its reproducibility.[33] Home cages should be enlarged and enrichment, such as running wheels, igloos, tube mazes, wood logs, and other naturalistic objects should be mandatory.[34] Wire mesh or wire grid floors should be prohibited in rodent cages and only solid floors should be allowed. Cages should have a

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height of at least 12 cm for mice, 18 cm for hamsters, and 26 cm for rats to allow for normal postural stretching. The height of cages should allow rodents to stand on their hind legs, stretch up fully, and climb the bars of the cage lid.

8. Enclosures for nonhuman primates must be significantly increased in size[35] and should include double vertical space (more space reduces stereotypical behaviors).[36] Nonhuman primates must be given the ability to climb, swing, jump, and retreat vertically.
9. Cold stress affects animals' health and well-being[37] and this has been shown to be especially true for mice.[38] Typical laboratory temperatures of around 20°C can lead to a 50-60% increase in metabolic rates above basal levels. Laboratory temperatures should be kept closer to 26°C and nesting material should be provided for animals to help regulate their temperature. Only eight to 10 grams of nesting material is needed to eliminate thermal stress for mice.[39]
10. Young nonhuman primates should not be separated from their mothers until they are behaviorally independent. The committee should instruct facilities to follow the guidelines of the International Primatological Society, which recommends that nonhuman primates remain in contact with their mothers for 12-18 months. The group adds: "There is unlikely to be any greater productivity through early weaning, in seasonally breeding species, such as rhesus monkeys. Even in non-seasonal breeders [such as long-tailed macaques], any slight increase in productivity must be offset against the resulting behavioural abnormalities of the offspring"[40]
11. Only positive reinforcement training, such as those using treats, praise, or other rewards to encourage desired behaviors, such as that employed by clicker training, should be used as these methods improve welfare, reduce abnormal behavior and fear,[41] and have the potential to improve data variability.
12. The chasing of rodents around their cage and the handling of rodents by the tail should be prohibited, as tail handling has been associated with reduced welfare. Mice and rats can be directed into a plastic or cardboard tunnel (cores of toilet paper rolls can be repurposed for this task) or similar structure and lifted from the enclosure or can be directed forward into the cupped hands of the researcher or staff.

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13. Light affects animals' circadian biology which alters their physiology, morphology, and behavior.[42,43] Other animals' perception of light is different than humans. For example, rodents see ultraviolet light. The light used in animals' housing environment must be determined based on species-specific needs. For rodents, this might be violet pumped LED light that provides UV cone stimulation without negative UV exposure. Complete darkness is not recommended, but animals should have a space where they can hide from light.[44]

Recommendation to Appendices: Species-Specific References—Environment, Housing, Management, and Veterinary Care

1. As *The Health Research Extension Act of 1985* was intended to provide statutory authority for protections for all laboratory animals—not just vertebrate animals,[45] invertebrates (including, at minimum, cephalopod mollusks, decapod crustaceans, and insects) should be included in the next update to *The Guide*. For this change to happen, the definition of the "animal" must be updated and species-specific standards for husbandry and housing of invertebrates should be added.

We would like to thank the Board on Animal Health Sciences, Conservation, and Research (BAHSCR) and the Standing Committee for the Care and Use of Animals in Research for its work in updating *The Guide* and for considering our recommendations, which we would be happy to meet to discuss these at their convenience.

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