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How Should the 3 *R*'s Be Revised and Why?

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Abstract

The Principles of Humane Experimental Technique established what many know today as the "3 *R*'s"—refinement, reduction, and replacement—when it was published in 1959. Since their formulation, these principles have guided decision-making for many about nonhuman animal subjects' uses in laboratory-based research. Discussion about how to amend or replace the 3 *R*'s is ongoing, driven mainly by philosophical ethics approaches to nonhuman animal rights and by scientific advancement. This article explores merits and drawbacks of possible updates to and interpretations of the 3 *R*'s.

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The 3 *R*'s

Russell and Burch published *The Principles of Humane Experimental Technique* in 1959, which established the "3 *R*'s" as key principles that govern use of nonhuman animals in a laboratory setting.[1] Today, the 3 *R*'s is the most well-known ethical framework for conducting scientific research using nonhuman animals. The 3 *R*'s—refinement, reduction, and replacement—are almost universally accepted by responsible scientists throughout the world and form the basis of many legal and regulatory systems that govern laboratory nonhuman animal use.[2,3,4,5,6,7]

However, it is no longer obvious that the 3 *R*'s as originally conceived represent a sufficient framework for the use of animals in research. Since the initial formulation of the 3 *R*'s, there has been considerable discussion about how to amend or replace them, driven in part by the writings of philosophers such as Peter Singer[8] and Tom Regan,[9] as well as by organizations that advocate for nonhuman animal rights. Furthermore, science and scientific methods have advanced in the past 6 decades, and the need for, and use of, nonhuman animals has changed. In addition, the translatability of animal models to human conditions has been called into question.[10] These factors raise the question about how to revise the 3 *R*'s for modern-day science.

Key Lessons

Several lessons have been learned since the implementation of the 3 *R*'s ethical framework in the mid-20th century. First, the integration of ethical principles into practice requires substantial time. The 3 *R*'s took over 30 years to take hold within the scientific community.[4] Therefore, it is unsurprising that the 3 *R*'s would warrant revision, given their long history. And yet there are many aspects of the 3 *R*'s that have endured; these aspects are a testament to the original wisdom and utility of the framework and help to account for its worldwide adoption.

A crucial pillar of the 3 *R*'s is the notion that humane science is necessary for both scientific and ethical reasons. Scientific results obtained from animals deprived of necessities or animals experiencing unmitigated pain, stress, or distress have little-to-no scientific merit.[11] Scientists must care about animal welfare so that their research can yield meaningful results. Fortunately, most scientists understand that the strength of scientific results is not independent of animal welfare and that good science comes from well-cared-for nonhuman animals.[11,12]

Yet, what is meant by humane treatment or well-being of nonhuman animals is less clear. The 3 *R*'s are premised on a pain-and-stress avoidance model that seeks first to avoid pain and stress by replacing animal models with nonanimal alternatives (which evolved from the original intent of replacing "higher-order" nonhuman animals with "lower-order" nonhuman animals), then to minimize total pain by reducing the number of animals, and finally to minimize individual pain by refining the pain-inducing procedure. The problem with this utilitarian model is that the consequence of the action cannot be known until the action is taken. Although an experiment may yield a very strong positive outcome that could warrant an animal's subjection to some pain, that outcome cannot be known before the experiment is conducted. Additionally, when applied, the principles can conflict with one another, giving rise to the need to better define each of the 3 *R* concepts.

Although the use of animal models under a 3 *R* ethical framework has yielded substantial scientific progress, there are instances in which animal models have not accurately predicted human responses. Some of these failures indicate that the animal model information cannot be relied upon when assessing toxicity of potential drugs in humans.[13] Furthermore, drugs for some disease types, such as Alzheimer's, have repeatedly seen successes in animal models and yet failed in human clinical trials.[14] Finally, although the extent of the problem is unknown, there are instances in which a drug could be fatal to nonhuman animals but a major success in humans (eg, aspirin).[13] A retrospective evaluation of the 3 *R*'s framework suggests that it is insufficient and that a different or modified ethical framework is needed.

Importantly, the particular ethical framework adopted by scientists is only one of the factors that influences scientists in their choice of methods. The 3 *R*'s may predispose a researcher to use a scientifically sound animal alternative, but that choice may be impeded by lack of regulatory approval. The regulatory state is an intermediary between what the scientific literature and ethical analysis support, on the one hand, and what is legally permissible, on the other. Vanda Pharmaceuticals tried to use the former to challenge the latter in filing suit against the US Food and Drug Administration in 2019.[15] This suit was unsuccessful but demonstrates a clear instance in which a drug product could not be brought to market without data from nonhuman animal subjects despite the scientific experts determining that animal models were unnecessary.

Finally, although the 3 *R*'s are codified in the laws of other countries, in the United States they are not explicitly required by law but only incorporated into guidelines by reference, such as through the National Research Council's *Guide for the Care and Use of Laboratory Animals*.[16] Some call for the codification of the 3 *R*'s, while others treat the *Guide* as synonymous with the law.[17,18] Regardless, it is clear that the 3 *R*'s are widely used by US-based researchers. Therefore, we can conclude that ethical mandates need not necessarily be enshrined in statutes or regulations but may be captured at a lower level, such as in guidance documents.

Systemic Changes to Modern Clinical Science

Changes are needed not only to the ethical framework but also to the system within which the ethical framework functions. Training is one of the most important gaps to address. The Animal Welfare Act (AWA) of 1966 requires animal care personnel to be trained in welfare practices.[19] However, Russell and Burch envisioned a far more expansive form of training, which remains an ideal.[1] A 2014 study found that 58% of scientists who had signed up to take a laboratory animal sciences course were not aware of the 3 *R*'s prior to the course.[20] This survey included career scientists who had been conducting animal research for years. Ethics training for scientists and everyone working with animals in research must occur regularly. Ethics training for personnel working with animals in the lab should include a survey of normative ethical theories (eg, utilitarianism, deontology, virtue ethics) and cover both how the laws and regulations have incorporated some of these ethical approaches, as well as ethical gaps in the legal framework like the failure to require facilities to report *all* nonhuman animal use numbers so that reduction can be assessed on a larger scale. Most importantly, the training must not be superficial but instead be substantive, employing appropriate pedagogical methods to ensure staff's engagement in the course, retention of the content, and application of the content in laboratory settings.

But training should not be limited to the 3 *R*'s directly. Rather, in order for the ideas of the 3 *R*'s to be fully accessible, it is necessary to address the knowledge gap between those actively conducting research and those developing innovative technologies. Currently, in the event that a painful procedure will be used, there is a requirement for researchers to search for an alternative and address why an alternative to nonhuman animal models cannot be used.[21] Yet, in practice, this requirement is met primarily pro forma.[22,23,24,25] This requirement can be satisfied by merely checking a box and writing a simple sentence on a form submitted to a local Institutional Animal Care and Use Committee (IACUC), but doing so often does not reflect a concerted effort to identify plausible alternatives. Part of the problem is that the dissemination of information concerning animal alternatives is lacking. The number of alternatives is exploding, but there is no clear pathway for regulatory acceptance of new methods in the United States. This is a major limitation for the adoption of alternatives. New methods will not be widely accepted in science and research without a clear regulatory pathway.

New Ethical Framework

Several ethical frameworks have been proposed to succeed the 3 *R*'s framework. Some propose a justice-based model that aims to end nonhuman animal testing completely.[26,27] Some proponents of this model omit an acknowledgement that this transition could not occur overnight or fail to provide a proposed plan for such a transition.[28] The absence of these 2 features is a major limitation of these approaches, particularly within the context of a discourse focused on practical application. For the

purpose of remaining grounded in the practical, the ethical framework described below focuses on filling a key gap in the 3 *R*'s model.

Experimental strength has been identified as a key missing component of the 3 *R*'s model.[10] This criticism is to some extent due to the shortcomings of the 3 *R*'s' utilitarian foundations. Interestingly, the IACUC—the body responsible for reviewing nonhuman animal research protocols under the AWA and the Public Health Services Policy—is implicitly instructed to refrain from this type of review.[29] Nevertheless, many may find it difficult in practice to avoid identifying this omission as a weakness of the system.

One proposal to incorporate experimental strength extends the 3 *R*'s to what has been coined the 3 *V*'s.[30,31] These additional elements comprise (a) construct validity, (b) internal validity, and (c) external validity. Each of these elements represents a unique aspect that, when taken together, provides a better assessment of overall experimental strength. *Construct validity* refers to the model's capacity to speak to the scientific objective of interest. *Internal validity* refers to design rigor (eg, sample size, statistical model, use of control groups). *External validity* refers to the extent to which the results are widely generalizable or only narrowly applicable. Taken together, the 3 *V*'s aim to reduce the occurrence of animal research that provides little-to-no meaningful information. The 3 *V* approach is also consistent with the evolution of science after the 3 *R*'s were first conceived by addressing the most pressing problems that confront animal models today.

Next Steps

Science advances when it respects and incorporates ethical principles. The introduction of the 3 *R*'s marked a fundamental shift in uses of nonhuman animal subjects.[32] However, reviews of the 3 *R*'s framework over the past several decades indicate room for improvement. For science to truly operate ethically, everyone involved must be taught—and express in their actions—the principles. Furthermore, the principles must be regularly reinforced. Knowing the 3 *R*'s is only one step, and that alone is insufficient. The 3 *R*'s can and should catch up with the scientific advances of the past few decades and seek to address some of the framework's limitations that have been uncovered. Efforts made to develop new replacement models must be given their full chance of success by identifying a clear regulatory approval pathway, and there must be systematic ways to disseminate information about newly available alternatives.

Finally, even with all this in mind, a revision of the 3 *R*'s is warranted. Russell and Burch provided a cornerstone of animal research practice. Yet the best models are refined over time as use and experience reveal gaps. The addition of the 3 *V*'s, which serves to address experimental validity, is one possible revision. The moment is ripe to implement changes and strengthen the 3 *R*'s so that they can continue to be a useful tool for 21st-century science.

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