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With What Should We Replace Nonhuman Animals in Biomedical Research Protocols?

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Abstract

Historically, most discussions about nonhuman animal experimentation consider what has become known as the 3 *R*'s: refinement, reduction, and replacement. Refinement and reduction receive the most attention, but recent modeling advances suggest that suitable replacement of nonhuman animal testing would bolster human research and increase translatability to human health outcomes. This article discusses these modeling advances and advocates their use, especially as replacements to nonpredictive nonhuman animal protocols, and discusses growing momentum in biomedical research communities and federal agencies that favors replacement of animal testing.

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Three *R*'s

The principles known as the 3 *R*'s (*replacement*, or substituting animals for insentient, nonanimal models; *reduction*, or reducing the number of animals used to gain information; and *refinement*, decreasing the severity of pain-inducing procedures used on animals) have been a staple framework guiding the use of animals in biomedical research for more than 50 years.[1,2] Historically, however, emphasis has been placed on refinement and reduction, with less effort devoted to replacement due to the former being seen as more achievable than the latter.[3] Today, there is a worldwide call by scientists and animal advocates to shift to a 1-*R* principle: replacement.[4] This call stems not only from the increased global concern for nonhuman animal suffering, but also from the growing recognition of the lack of translatability of nonhuman animal research and recent technological advancements in human disease and biological modeling.

While the principles of reduction and refinement arguably lead to some reduction in harm to animals and improvements in medical research, they nevertheless remain rooted in a paradigm that perpetuates the use of nonhuman animals for biomedical research and drug development. Moreover, it is now well known that the lack of translatability of nonhuman animal research testing to human outcomes is causing failures in therapeutic development at an alarming rate. More than 80% of all drugs and

vaccines found safe and effective in preclinical trials, including those based on animal testing, fail during human clinical trials.[5] Much of this failure rate can be attributed to the physiological and pathological differences between humans and nonhuman animals.[5] Additionally, even with attempts to reduce the failure rate by changing nonhuman animal study design protocols, which can be costly and time-consuming, animal research has yet to translate reliably to humans.[6] This article discusses human-relevant models that can replace animal testing, the ethical questions spurring the growing momentum in biomedical research away from animal testing, and the federal actions that support this shift.

Human-Relevant Models

Research methods that more faithfully reproduce human biology and physiology than nonhuman animal models offer a path toward a new paradigm for biomedical research that is fundamentally more accurate, predictive, efficient, and effective.[4] Recent scientific developments have led to multiple "human-relevant" research models—those based on human biology—that have the potential to lead to improved understanding of human biology, disease pathophysiology, and therapeutic development.[7] Unlike traditional in vitro systems, these newer in vitro models utilize 3-dimensional architecture of human tissues and organs.[8]

Some of these in vitro models are referred to as microphysiological systems (MPS) or, more commonly, as organ-on-a-chip or organ chips. By combining human cell sourcing, organ-specific microenvironments, and tissue-relevant forces, MPS more closely emulate human biology than traditional 2-dimensional in vitro models. MPS can also be integrated into multi-organ and potentially full human-body systems.[8] Another alternative to traditional in vitro models is organoids, stem cell-derived, 3-dimensional culture systems or "mini organs" that are proving useful for closely examining organ-specific functions and diseases. Both MPS and organoids can be considered human-relevant models in that they are based on and represent human physiology as opposed to animal physiology.[9]

These methods more faithfully recapitulate human physiology than nonhuman animal tests and have the potential to predict human drug safety and effectiveness more accurately. Over the past few decades, improvements in these models have led to brain organoid screening systems that can help identify gene mutations, vulnerable cell types, and gene regulatory networks underlying autism spectrum disorders[10]; kidney-on-a-chip models that can be used to predict kidney-related toxicity of cancer drugs[11]; and lung tissue models than can revolutionize infection research.[12] Researchers in this space are working on organoid and organ chip models of nearly all major organs in the human system,[8] with certain specialists aiming to build full human-on-a-chip models.[13,14]

Additionally, the biological differences between humans and other animals, combined with the lack of diversity in nonhuman animals bred for research, lead to results that do not account for the genetic and ethnic diversity of humans.[9] Human-relevant models not only represent human physiology better than animal tests, but also can represent the physiology of specific populations, ethnicities, or individuals.[13,15,16,17,18,19] Models that better represent the diversity of human populations can lead to better predictions of clinical trial outcomes.[20] Thus, the future applications of human-relevant methods far exceed those of animal testing.

Critics of human-relevant models note that these models are not yet fully proven and are not at a point at which they can fully replace all animal testing.[21,22,23,24] This criticism, while fair, serves as an impediment to rather than a facilitator of the improvement of human-relevant methods. It reinforces the status quo in governmental funding instead of boosting government funding for improved human-relevant methods. Very little funding goes toward human-relevant methods currently,[25] but with government funding and support to validate their reliability and improve their complexity, they could flourish, as did the Human Genome Project.[26]

Is It Ethical to Keep Using Animals for Biomedical Research and Drug Development?

Despite rats, dogs, cats, and humans sharing many biological features, subtle differences in physiology, biochemistry, and genetic expression between humans and other animals can significantly mislead research and therapeutic development. Species differences result in drugs and vaccines that seem promising in animal tests failing when tried in humans. For example, thousands of drugs that worked in animal tests for stroke, HIV, immune system disorders, and other diseases failed when tried in humans.[6,27,28,29,30] These failures are primarily due to toxicities not predicted by animal tests or to a lack of efficacy.[6,27,28,29,30]

One of the main safety problems caused by drugs is liver toxicity.[31] A groundbreaking study found that, in a set of 27 drugs, human liver chip methods identified nearly 7 of every 8 drugs proven to be hepatoxic during clinical use after they were deemed safe by animal tests.[32] Twenty-two of these drugs were determined to be safe by animal tests but later caused the death of 208 patients and required liver transplants in 10 others.[33] The drugs were subsequently pulled from the market or given black box labeling.[33]

There is also strong reason to believe that many drugs that may be effective and safe in humans are prematurely delayed or discarded due to misleading results in animal tests.[6] Certain drugs, such as cyclosporine, are widely and successfully used but were initially delayed because of animal test results that did not apply to humans.[6]

Both the abandonment of potentially useful treatments and the numerous unsafe treatments that proceed to clinical trials after nonhuman animal testing call into question the opportunity costs of the current research paradigm. The continued costly focus on animal testing impedes the development of better, more accurate organ-on-chip and other human-relevant research methods. The lack of translation of nonhuman animal tests to humans is especially alarming, as there are no approved treatments for many diseases, including sepsis.[27] Additionally, the poor translatability from animals to humans leads to suffering in nonhuman animals that is disproportionate to any perceived knowledge gained. Therefore, a shift in focus toward the development and use of human-relevant research methods should be at the forefront of industry, academia, and governmental funding and priorities.

Human-Relevant Methods

In 2013, Francis Collins, then director of the National Institutes of Health (NIH), published his thoughts on the failures of animal testing and a need to move toward human-relevant methods.[34] Since that time, the US government has demonstrated interest in the pursuit of human-relevant testing methods. This interest comes in the form of agency actions and legislative initiatives.

Federal agency actions. While important steps have been made, agency actions have thus far been small in impact and have fallen short in essential ways. A more robust shift away from animal testing has yet to happen or to be determined as a goal for the future.[5] In the 2012 International Animal Research Regulations report, the National Research Council stated that almost half of NIH funding goes to testing that involves animal use.[35] Much of this funding is in the form of grants. Between FY 2008 and FY 2015, more than 70% of projects awarded NIH grants used mouse models.[25,36] However, the US government has a few initiatives that have the potential to support human-relevant research. Under the umbrella of the NIH, the Advanced Research Projects Agency for Health (ARPA-H) was founded on the idea that increasing direct federal funding of transformative and innovative research will drive—as well as quicken the application and implementation of—scientific breakthroughs to improve health. ARPA-H has shown an interest in more accurate human-relevant models by requesting research proposals using human cells to 3D-print organs.[37] Another center within the NIH, the National Center for Advancing Translational Science, is home to many programs focused on advancing human-relevant research methods. These programs include the 3-D Tissue Bioprinting Program and the Tissue Chip for Drug Screening Program.[38] Although these federal agency programs constitute a good start in funding research methods that are more accurate for human biology than animal testing, human-relevant testing methods are still not a top priotity.[25]

Legislative initiatives. In December 2022, the US president signed into law the FDA Modernization Act 2.0. The FDA Modernization Act 2.0 is a bipartisan-supported act that updated the Federal Food, Drug, and Cosmetic Act by lifting the legal requirement for animal testing and allowing drug applicants to use non-animal methods, such as organ chips, for therapeutic safety and efficacy testing.[39] While this new law does not end the use of animals in drug testing, it does establish that human-relevant methods, including organoids and organ chips, can be used to determine a drug's safety and efficacy for the purpose of gaining approval from the US Food and Drug Administration (FDA). In theory, this act opens the door for drug manufacturers to embrace human-relevant test models. However, by not mandating the use of more accurate human-relevant methods in place of animal testing, and by not making the replacement of animal testing a programmatic priority within the FDA, this new law may not lead to any practical changes within the drug development field in the near future.

While the FDA Modernization Act is the most significant bill supporting human-relevant research methods, other bills have been introduced in recent years, including the Humane Research and Testing Act of 2021 (HRTA) and the Humane and Existing Alternatives in Research and Testing Sciences Act of 2022 (HEARTS Act).[40,41] The HRTA would have established a center dedicated to human-relevant research methods within the NIH. This center would fund and incentivize scientists to develop novel, more effective methods to replace nonhuman animal testing.[40] While the HRTA has not been reintroduced in the 118th congressional session, its concept of a center was integrated into the HEARTS Act, which will require NIH to provide incentives for the use of nonanimal research methods.[41] Both the HRTA and the latest version of the HEARTS Act actively incentivize a shift away from animal testing in favor of non-animal, human-relevant methods.

Conclusion

The US federal government has recognized the importance of human-relevant research methods as ethical alternatives to the use of animals and as beneficial for medical

advancement. The poor translatability of nonhuman animal research and the high failure rate of drugs in development reflect the immense limitations of animal testing. However, the United States has yet to fully prioritize a shift away from animal testing, as reflected by its funding programs. To better support the discovery, development, and use of innovative human-relevant models, researchers, physician groups, and patient advocacy groups should demand that comprehensive governmental funding of such research be a priority. Not only is such a funding priority more humane for nonhuman animals, but it is also a necessity for human health.

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