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Confronting the bias towards animal experimentation (animal methods bias)

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Laws and policies are in place around the world to promote the replacement and reduction of nonhuman animals in science. These principles are rooted not just in ethical considerations for animals, but also in scientific considerations regarding the limitations of using nonhuman animals to model human biology, health, and disease. New nonanimal research approaches that use human biology, cells, and data to mimic complex human physiological states and therapeutic responses have become increasingly effective and accessible, replacing the use of animals in several applications, and becoming a crucial tool for biomedical research and drug development. Despite many advantages, acceptance of these new nonanimal methods has been slow, and barriers to their broader uptake remain. One such barrier is animal methods bias, the preference for animal-based methods where they are not necessary or where animal-free methods are suitable. This bias can impact research assessments and can discourage researchers from using novel nonanimal approaches. This article provides an introductory overview of animal methods bias for the general public, reviewing evidence, exploring consequences, and discussing ongoing mitigation efforts aimed at reducing barriers in the shift away from animal use in biomedical research and testing.

KEYWORDS

animal methods bias, peer review, alternatives to animal experiments, scientific publishing, biomedical research, drug development

Introduction: animal and human-based preclinical research methods

Animal experiments are frequently performed for basic research (with the aim to gain knowledge without specific applications) and for applied research (applying knowledge, for example, to try to find new drugs for humans and to test for their toxicity or safety). According to the directive on the use of animals in science in the European Union, animal experiments must be replaced whenever possible, and EU Member States should make a substantial effort to reduce and replace animal use in science ([European Parliament, 2010](#)). A similar principle is applied in other regions, including the United States: the 3Rs principle to replace, reduce, and refine animal use in science ([Russell and Burch, 1959](#); [Office of Laboratory Animal Welfare, 2015](#)). These principles are rooted not just in ethical considerations for animals, but also in scientific considerations regarding the limitations of using nonhuman animals to model human biology, health, and disease.

Animal tests are often expensive, take a long time to conduct, and can give misleading results ([Meigs et al., 2018](#)). Approximately 92% of drugs in

development fail to pass human clinical trials—mostly due to failures during safety and efficacy testing—despite safe and effective findings demonstrated in preclinical tests (Thomas et al., 2021). A recent economic analysis estimated that the use of more predictive preclinical nonanimal technologies instead of animal tests could save over \$24 billion (Ewart et al., 2022). There is increasing recognition among government, academic, and industry scientists that nonanimal research methods have the potential to overcome some of the scientific limitations of animal-based methods (Baran et al., 2022; Gribaldo and Dura, 2022; Ingber, 2022; Advisory Committee to the Director Working Group on Catalyzing the Development and Use of Novel Alternative Methods to Advance Biomedical Research, 2023).

In recent years, *in vitro* (in a dish) and *in silico* (computational) research models have become increasingly effective and accessible, replacing the use of animals in several applications, and becoming a crucial tool for preclinical research. These promising new models use human biology, cells, and data to mimic complex human physiological states and therapeutic responses (Shaker et al., 2021; Loewa et al., 2023). Examples of innovative, human-biology based *in vitro* models are organoids, organs-on-a-chip (also called organ chips and tissue chips), and induced pluripotent stem cells. Organoids are three-dimensional cell aggregates (also called spheroids) consisting of multiple cell types and designed to imitate physiological processes. Human organ-on-a-chip systems are microdevices composed of three-dimensional cells and fluids to simulate physiological processes in human organs. Induced pluripotent stem cells are adult human-derived cells that have been genetically reprogrammed to a stem cell-like state and then further engineered to one of a variety of cell types that can be found throughout the body.

Animals have been used in research for so long that the scientific community has been slow to accept novel nonanimal methods. Some of these new methods have high entry costs and can be difficult for researchers to operationalize in their laboratories, highlighting the need for more funding and expanded infrastructure (Busek et al., 2022). Acceptance of new nonanimal methods can also be improved with good laboratory practices to ensure that high-quality experiments are performed and that findings can be reproduced by other researchers, and with thorough model evaluation to confirm that experiments are suitable for their intended use (Pamies et al., 2022; van der Zalm et al., 2022).

Other barriers to the broader use of nonanimal methods are more psychological, though, such as a bias or preference for animal-based methods. This *animal methods bias* may be especially prevalent when research that uses nonanimal methods is being assessed during subjective evaluations of studies for publication or proposals for grant funding. By impacting publications and funding awards, animal methods bias can be a barrier to the sharing and uptake of novel nonanimal approaches, standing in the way of improved preclinical predictiveness and further complicating drug development. The concept of *animal methods bias* is further expanded on below, including an overview of current evidence, how it impacts research assessments, and ongoing efforts to mitigate its harmful effects on human health research.

Animal methods bias: the bias toward animal experimentation in research and publishing

Publishing plays a crucial role in the advancement of science, helping to translate research findings into medical interventions. It also impacts researchers' careers, playing a role in hiring decisions and other evaluations. The publishing process is not without biases, though. According to the Catalogue of Bias, a database of psychological, methodological, and reporting biases created by The Center for Evidence-Based Medicine at Oxford University, publication bias is defined as "when the likelihood of a study being published is affected by the findings of the study" (DeVito and Goldacre, 2019). But what if the likelihood of a study being published is affected by the *methods* of the study, namely, animal or animal-free methods?

In his article, *Is it Time for Reviewer three to Request Human Organ Chip Experiments Instead of Animal Validation Studies?*, Dr. Donald Ingber questioned why animal data is still considered the gold standard in human health research, while presenting evidence that organ chips may better suit this purpose (Ingber, 2020). He framed this issue as a problem with peer review, describing an increasingly common anecdote about reviewer requests for animal experiments even though the author(s) explained why they did not use animals for their experiments.

Animal methods bias in publishing is thus a newly defined type of publishing bias, describing a preference for animal-based methods where they are not necessary or where nonanimal methods are suitable, which affects the likelihood of a manuscript being accepted for publication or introduces a significant delay to manuscript acceptance. Animal methods bias affects other aspects of research too, including the review of grant applications, when researchers apply for funds to enable their animal-free projects but are held back by biased assessments of their proposals. It can be likened to another kind of bias called *scholarly bias*, the favoring of perspectives, theories, or methods that align with one's own (Langfeldt et al., 2023).

To further understand when and why this occurs, which in turn informs solutions, the first author of this article and colleagues conducted a small survey to assess the experiences and perceptions of authors and reviewers related to animal- and human biology-based experiments during peer review (Krebs et al., 2023b). Respondents represented a broad range of biomedical research and related fields, primarily worked in academic (74%) and industry (10%) sectors, and in the United States (32%).

Twenty-one of the 68 total respondents indicated that they have performed animal-based experiments for the sole purpose of anticipating reviewer requests for them. In other words, they did not think the experiments were necessary outside the context of review. Thirty-one of the 68 total respondents indicated that they have been asked by reviewers to add animal experiments to a study that otherwise has no animal-based experiments. Among those 31 respondents, just three indicated that they felt the request was justified, while 14 respondents felt that it was sometimes justified, and 11 did not think the request was justified (three respondents did not provide an answer to this question).

When asked to elaborate on their perceptions of these requests, respondents expressed that reviewers ask for animal

experiments out of habit, not because it is necessary or relevant. Some respondents also indicated that more prominent journals are more likely to request or expect animal experiments, which acts as an incentive for conducting animal experiments or as a punishment for researchers who use animal-free, human biology-based methods. Overall, the survey identified the following consequences of animal methods bias during manuscript peer review: the conduct of animal experiments which would have otherwise not been performed, as well as negative career repercussions, including delays in publication, rejection, or withdrawal of papers, and being forced to publish in less-prominent journals.

The survey also asked questions about respondents' experiences as reviewers, and specifically regarding reasons for making requests for additional animal experiments. Respondents indicated that their preference for animal methods or their lack of awareness of appropriate animal-free methods were reasons for making requests for additional animal experiments.

Because of the pressures to publish, researchers may feel compelled to comply with reviewer requests for animal experiments even when they disagree with their necessity. Alternatively, failing to comply with such requests may result in negative career consequences. Altogether, animal methods bias affects how nonanimal research is published and may even discourage researchers from using these methods. In other words, it is a barrier to the uptake and dissemination of nonanimal research—important research that holds promise for improving preclinical predictiveness and rates of translation from drug discovery to clinical trial approvals.

How to mitigate animal methods bias

An April 2022 workshop to address animal methods bias in scientific publishing was convened among academic and industry researchers, journal editors, government representatives, and advocates in order to: (1) explore a range of stakeholder perspectives, (2) describe the current state of animal- and nonanimal-based experimental systems, (3) describe animal methods bias in publishing and related biases in publishing and peer review, and (4) identify potential causes, consequences, and potential mitigation strategies for animal methods bias in publishing (Krebs et al., 2022).

Barriers to addressing animal methods bias were identified, including:

- The high-pressure nature of the research environment,
- Impact factor, an index measuring the impact of scholarly literature that represents the annual average number of citations to articles published in each journal over the past 2 years,
- Financial stakes,
- Animals as the “gold standard,” seen as the default method by the research community,
- Institutional inertia and psychological lock-in (see Gluck, 2019), and
- Lack of knowledge or desire to learn about animal-free methods.

Workshop attendees also identified recommendations for addressing animal methods bias geared toward the scientific community, journals and publishers, and funders, governments, and policymakers. Recommendations included the following:

- Build awareness about animal methods bias among editors, peer reviewers, and the scientific community more broadly, especially early-career researchers;
- Increase authors' confidence in their ability to challenge reviewers' requests for animal experiments, such as through the Author Guide for Addressing Animal Methods Bias (Krebs et al., 2023a);
- Provide educational materials for reviewers, as recently acknowledged by the US National Institutes of Health to ensure the better evaluation of nonanimal research (Advisory Committee to the Director Working Group on Catalyzing the Development and Use of Novel Alternative Methods to Advance Biomedical Research, 2023);
- Mandate that requests for addition of animal methods be scrutinized by other reviewers; and
- Prioritize funding for animal-free, human biology-based methods, including to improve accessibility and training for researchers.

After the workshop, attendees formed the Coalition to Illuminate and Address Animal Methods Bias (COLAAB) to continue to explore and address this issue.¹ The COLAAB is currently gathering additional evidence of animal methods bias and its consequences and developing and implementing tools for overcoming it.

Conclusion

New nonanimal methods hold great promise for advancing biomedical research and drug development. Although a lot of work remains to improve the acceptance of nonanimal methods within the scientific community, researchers are increasingly turning to them to answer their research questions. Researchers should be able to do so without unfair requests or expectations for animal experiments from reviewers who prefer their own methodologies or are ill-equipped to evaluate novel ones.

Animal methods bias is a serious issue that adds additional and unnecessary work for researchers who use animal-free approaches, and it perpetuates the idea that animal-free approaches are not sufficiently valuable on their own. Animal methods bias is a symptom of a research ecosystem that rewards animal use and disincentivizes a shift toward potentially more reliable human-biology based research methods, and is therefore a barrier to changing the *status quo* from its reliance on animals.

To advance biomedical research and get safer and better drugs to more patients, researchers, drug developers, and funding agencies must address animal methods bias. Measures that empower researchers to confront unfair requests for animal experiments, prevent reviewers from making such requests, and advance the standardization, evaluation, and infrastructure for nonanimal research approaches will all be important. The public can play a

¹ www.animalmethodsbias.org

role too. Consumers and taxpayers have power in the market and with publicly funded research, and they are already helping to turn the tide by demanding cruelty-free cosmetics and supporting lawmakers' shifts toward animal-free research and testing approaches.

Author contributions

CK: Conceptualization, Project administration, Writing—original draft, Writing—review and editing. KH: Conceptualization, Writing—original draft, Writing—review and editing.

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Conflict of interest

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