Request for Information (RFI): Catalyzing the Development and Use of Novel Alternative Methods to Advance Biomedical Research

I am responding to this RFI:	on-behalf-of-an-organization
Name	Emily R. Trunnell, Ph.D.
Name of Organization	People for the Ethical Treatment of Animals
Type of Organization	nonprofit-research-organization
Type of Organization - Other	
Role	scientific-researcher
1. Please provide feedback on the use of novel alternative methods to study human biology, circuits, systems, and disease states, including how novel alternatives:	Translating basic science and pre-clinical research into meaningful, affordable outcomes for patients is a critical challenge in biomedical research. Despite decades of research and billions of dollars invested in animal-based models of human biology, circuits, systems, and disease states, effective treatments for many debilitating and deadly human diseases remain elusive. The “translation gap” between data emerging from biomedical research and understanding/treating human health is due, in part, to the limitations of animal models. Species differences in anatomy, physiology, and gene expression—affecting developmental trajectories, metabolism, immune responses, disease susceptibility, and more—make translating data from an animal experiment into a human-relevant preventative measure, treatment, or cure extremely difficult. Animal models are often oversimplified and

1. Please provide feedback on the use of novel alternative methods to study human biology, circuits, systems, and disease states, including how novel alternatives:	pathology, with targets that may be meaningful in an animal laboratory but are ultimately inadequate for humans. Poor study design combined with the confinement and unnatural conditions of laboratory life further undermine the internal validity of animal research. Depending on the disease area of interest, novel drugs for humans fail in clinical trials between 90 and 100% of the time. The vast majority (90%) of “highly promising” basic science discoveries (most of them from experiments on animals) make no difference at all for human patients (Contopoulos-Ioannidis 2003). The failure of animal-based research models and assays is contributing to the increased costs of drug development and the public’s declining trust in science. If our finite public funds are to be used responsibly, they must fund reliable research and test methods that lead to effective treatment of diseases and protection of human health. Motivated by both the ethical concerns surrounding animal-based experimentation and testing as well as the limited translatability of animal-based data, advances in novel, non-animal methods (a.k.a. novel alternative methods or NAMs) like complex, 3-D cellular models, such as microphysiological systems, organoids, spheroids, and 3-D bioprinted structures derived from human cell lines and based in human biology have expanded in the past decade. Many of these models simulate human physiology and disease more accurately than traditional in vivo animal models do because they do not have to overcome the translational species hurdle. Currently, these tools are accessible to researchers working directly on their application and development. However, given their potential to improve preclinical and basic research as well as ongoing advances in their design, it is essential that investigators with knowledge or access gaps have the opportunity to take advantage of these cutting-edge in vitro methods. We cannot know how much progress might have been made if funding agencies had already made novel, non-animal methods a priority, but there is now a chance for them to catch up. It is both scientifically and ethically imperative that the NIH make the shifting of funding priorities toward non-animal methods and away from animal-based methods its agency-wide priority. There are many examples that demonstrate the scientific utility of non-animal methods over animal-based research for advancing progress into understanding specific biological processes or human states, including currently underserved areas of biomedical research. Here are just a few of the papers that demonstrate or describe their potential to

1. Please provide feedback on the use of novel alternative methods to study human biology, circuits, systems, and disease states, including how novel alternatives:	health: Adegbola A, Bury LA, Fu C, Zhang M, Wynshaw-Boris A. Concise review: Induced pluripotent stem cell models for neuropsychiatric diseases. Stem Cells Transl Med. 2017;6(12):2062-2070. Al-Hilal TA, Keshavarz A, Kadry H, et al. Pulmonary-arterial-hypertension (PAH)–on-a-chip: Fabrication, validation and application. Lab Chip. 2020;20(18):3334-3345. Allen A, Deshmukh H. All on “CHIP”: Using microfluidics to study neutrophil ontogeny. Transl Res. 2017;190:1-3. Arzua T, Yan Y, Jiang C, et al. Modeling alcohol-induced neurotoxicity using human induced pluripotent stem cell–derived three-dimensional cerebral organoids. Transl Psychiatry. 2020;10(1):347 Barrile R, van der Meer AD, Park H, et al. Organ-on-Chip Recapitulates Thrombosis Induced by an anti-CD154 Monoclonal Antibody: Translational Potential of Advanced Microengineered Systems. Clin Pharmacol Ther. 2018;104(6):1240-1248. Bergers LIJC, Reijnders CMA, van den Broek LJ, et al. Immune-competent human skin disease models. Drug Discov Today. 2016;21(9):1479-1488. Beydag-Tasöz BS, Yennek S, Grapin-Botton A. Towards a better understanding of diabetes mellitus using organoid models. Nat Rev Endocrinol. 2023;19(4):232-248. Blaurock-Möller N, Gröger M, Siwczak F, et al. CAAP48, a new sepsis biomarker, induces hepatic dysfunction in an in vitro liver-on-chip model. Front Immunol. 2019;10:273. Brown D, Namas RA, Almahmoud K, et al. Trauma in silico: Individual-specific mathematical models and virtual clinical populations. Sci Transl Med. 2015;7(285):285ra61. Brown JA, Codreanu SG, Shi M, et al. Metabolic consequences of inflammatory disruption of the blood-brain barrier in an organ-on-chip model of the human neurovascular unit. J Neuroinflammation. 2016;13(1):306. Cerchia C, Lavecchia A. New avenues in artificial-intelligence-assisted drug discovery. Drug Discov Today. 2023;28(4):103516. Cerneckis J, Bu G, Shi Y. Pushing the boundaries of brain organoids to study Alzheimer's disease. Trends Mol Med. 2023;29(8):659-672. Cohen A, Ioannidis K, Ehrlich A, et al. Mechanism and reversal of

drug-induced nephrotoxicity on a chip. Sci Transl Med. 2021;13(582):eabd6299.

Cuní-López C, Stewart R, White AR, Quek H. 3D in vitro modelling of human patient microglia: A focus on clinical translation and drug development in neurodegenerative diseases. J Neuroimmunol. 2023;375:578017.

Dauth S, Maoz BM, Sheehy SP, et al. Neurons derived from different brain regions are inherently different in vitro: A novel multiregional brain-on-a-chip. J

1. Please provide feedback on the use of novel alternative methods to study human biology, circuits, systems, and disease states, including how novel alternatives:	A, McGowan H, et al. Ethanol-mediated activation of the NLRP3 inflammasome in iPS cells and iPS cells-derived neural progenitor cells. Mol Brain. 2016;9(1):51. Diebel LN, Wheaton M, Liberati DM. The protective role of estrogen on endothelial and glycocalyx barriers after shock conditions: A microfluidic study. Surgery. 2021;169(3):678-685. Dirven H, Vist GE, Bandhakavi S, et al. Performance of preclinical models in predicting drug-induced liver injury in humans: a systematic review. Sci Rep. 2021;11(1):6403. Ehling P, Meuth P, Eichinger P, et al. Human T cells in silico: Modelling their electrophysiological behaviour in health and disease. J Theor Biol. 2016;404:236-250 Ethier SP, Guest ST, Garrett-Mayer E, et al. Development and implementation of the SUM breast cancer cell line functional genomics knowledge base. NPJ Breast Cancer. 2020;6:30. Ewart L, Apostolou A, Briggs SA, et al. Performance assessment and economic analysis of a human Liver-Chip for predictive toxicology. Commun Med (Lond). 2022;2(1):154. Fernández-Costa JM, Tejedera-Vilafranca A, Fernández-Garibay X, Ramón-Azcón J. Muscle-on-a-chip devices: a new era for in vitro modelling of muscular dystrophies. Dis Model Mech. 2023;16(6):dmm050107. Fosse V, Oldoni E, Bietrix F, et al. Recommendations for robust and reproducible preclinical research in personalised medicine. BMC Med. 2023;21(1):14. Haggarty SJ, Silva MC, Cross A, Brandon NJ, Perlis RH. Advancing drug discovery for neuropsychiatric disorders using patient-specific stem cell models. Mol Cell Neurosci. 2016;73:104-115. Hartung T. A call for a Human Exposome Project. ALTEX. 2023;40(1):4-33. Hoang P, Wang J, Conklin BR, Healy KE, Ma Z. Generation of spatial-patterned early-developing cardiac organoids using human pluripotent stem cells. Nat Protoc. 2018;13(4):723-737. Hockney S, Parker J, Turner JE, et al. Next generation organoid engineering to replace animals in cancer drug testing. Biochem Pharmacol. 2023;213:115586. Landhuis E. Deep learning takes on tumours. Nature. 2020;580(7804):551-553.

Lee CT, Chen J, Kindberg AA, et al. CYP3A5 mediates effects of cocaine on human neocorticogenesis: Studies using an in vitro 3D self-organized hPSC model with a single cortex-like unit. Neuropsychopharmacology. 2017;42(3):774-784.

Levy RJ, Paşca SP. What Have Organoids and Assembloids Taught Us About the Pathophysiology of Neuropsychiatric Disorders?. Biol Psychiatry. 2023;93(7):632-641.

Lieberman R, Kranzler HR, Levine ES, Covault J. Examining the effects of alcohol on GABAA receptor mRNA expression and function in neural cultures

1. Please provide feedback on the use of novel alternative methods to study human biology, circuits, systems, and disease states, including how novel alternatives:	pluripotent stem cells. Alcohol. 2018;66:45-53. Lim K, Donovan APA, Tang W, et al. Organoid modeling of human fetal lung alveolar development reveals mechanisms of cell fate patterning and neonatal respiratory disease. Cell Stem Cell. 2023;30(1):20-37.e9. Lyu Z, Park J, Kim KM, et al. A neurovascular-unit-on-a-chip for the evaluation of the restorative potential of stem cell therapies for ischaemic stroke. Nat Biomed Eng. 2021;5(8):847-863. Kim H, Park HJ, Choi H, et al. Modeling G2019S-LRRK2 Sporadic Parkinson's Disease in 3D Midbrain Organoids. Stem Cell Reports. 2019;12(3):518-531. Meigs L, Smirnova L, Rovida C, Leist M, Hartung T. Animal testing and its alternatives—the most important omics is economics. ALTEX. 2018;35(3):275-305. Meng F, Meyer CM, Joung D, Vallera DA, McAlpine MC, Panoskaltsis-Mortari A. 3D bioprinted in vitro metastatic models via reconstruction of tumor microenvironments. Adv Mater. 2019;31(10):1806899. Mobini S, Song YH, McCrary MW, Schmidt CE. Advances in ex vivo models and lab-on-a-chip devices for neural tissue engineering. Biomaterials. 2019;198:146-166. Mullen S, Movia D. The role of extracellular vesicles in non-small-cell lung cancer, the unknowns, and how new approach methodologies can support new knowledge generation in the field. Eur J Pharm Sci. 2023;188:106516. Muñiz AJ, Topal T, Brooks MD, et al. Engineered extracellular matrices facilitate brain organoids from human pluripotent stem cells. Ann Clin Transl Neurol. 2023;10(7):1239-1253. Neufeld L, Yeini E, Pozzi S, Satchi-Fainaro R. 3D bioprinted cancer models: from basic biology to drug development. Nat Rev Cancer. 2022;22(12):679-692. Nguyen VVT, Gkouzioti V, Maass C, Verhaar MC, Vernooij RWM, van Balkom BWM. A systematic review of kidney-on-a-chip-based models to study human renal (patho-)physiology. Dis Model Mech. 2023;16(6):dmm050113. Nzou G, Wicks RT, VanOstrand NR, et al. Author Correction: Multicellular 3D neurovascular unit model for assessing hypoxia and neuroinflammation induced blood-brain barrier dysfunction. Sci Rep. 2020;10(1):20384 Ochalek A, Mihalik B, Avci HX, et al. Neurons derived from

sporadic Alzheimer’s disease iPSCs reveal elevated TAU hyperphosphorylation, increased amyloid levels, and GSK3B activation. Alzheimers Res Ther. 2017;9(1):90.

Otero M, Canals J, Belio-Mairal P, et al. Advanced non-animal models in biomedical research: Autoimmune diseases. Publications Office of the European Union; 2022.

Park J, Wu Z, Steiner PR, Zhu B, Zhang JXJ. Heart-on-chip for combined cellular dynamics measurements and

1. Please provide feedback on the use of novel alternative methods to study human biology, circuits, systems, and disease states, including how novel alternatives:	Biomed Eng. 2022;50(2):111-137. Patel VS, Amin K, Wahab A, et al. Cryopreserved human precision-cut lung slices provide an immune competent pulmonary test system for "on-demand" use and long-term cultures. Toxicol Sci. 2023;191(2):253-265. Pičulin M, Smole T, Žunkovič B, et al. Disease progression of hypertrophic cardiomyopathy: Modeling using machine learning. JMIR Med Inform. 2022;10(2):e30483. Ramirez S, Mukherjee A, Sepulveda S, et al. Modeling traumatic brain injury in human cerebral organoids. Cells. 2021;10(10):2683. Richards DJ, Li Y, Kerr CM, et al. Human cardiac organoids for the modelling of myocardial infarction and drug cardiotoxicity. Nat Biomed Eng. 2020;4(4):446-462. Romania P, Folgiero V, Nic M, et al. Advanced Non-Animal Models in Biomedical Research: Immuno-Oncology. Publications Office of the European Union; 2021. Ronaldson-Bouchard K, Vunjak-Novakovic G. Organs-on-a-chip: A fast track for engineered human tissues in drug development. Cell Stem Cell. 2018;22(3):310-324. Rosenbluth JM, Schackmann RCJ, Gray GK, et al. Organoid cultures from normal and cancer-prone human breast tissues preserve complex epithelial lineages. Nat Commun. 2020;11(1):1711. Santhanam N, Kumanchik L, Guo X, et al. Stem cell derived phenotypic human neuromuscular junction model for dose response evaluation of therapeutics. Biomaterials. 2018;166:64-78 Sebastian R, Jin K, Pavon N, et al. Schizophrenia-associated NRXN1 deletions induce developmental-timing- and cell-type-specific vulnerabilities in human brain organoids. Nat Commun. 2023;14(1):3770. Scarnati MS, Halikere A, Pang ZP. Using human stem cells as a model system to understand the neural mechanisms of alcohol use disorders: Current status and outlook. Alcohol. 2019;74:83-93. Schiller AM, Howard JT, Convertino VA. The physiology of blood loss and shock: New insights from a human laboratory model of hemorrhage. Exp Biol Med (Maywood). 2017;242(8):874-883. Shrirao AB, Kung FH, Omelchenko A, et al. Microfluidic platforms for the study of neuronal injury in vitro. Biotechnol Bioeng. 2018;115(4):815-830. Siekmeier PJ. Computational modeling of psychiatric illnesses via

well-defined neurophysiological and neurocognitive biomarkers. Neurosci Biobehav Rev. 2015;57:365-380.

Sokolowska P, Zukowski K, Janikiewicz J, Jastrzebska E, Dobrzyn A, Brzozka Z. Islet-on-a-chip: Biomimetic micropillar-based microfluidic system for three-dimensional pancreatic islet cell culture. Biosens Bioelectron. 2021;183:113215.

Soscia D, Belle A, Fischer N, et al. Controlled placement of multiple CNS cell

1. Please provide feedback on the use of novel alternative methods to study human biology, circuits, systems, and disease states, including how novel alternatives:	2017;12(11):e0188146. Spijkers XM, Pasteuning-Vuhman S, Dorleijn JC, Vulto P, Wevers NR, Pasterkamp RJ. A directional 3D neurite outgrowth model for studying motor axon biology and disease. Sci Rep. 2021;11(1):2080. Strelez C, Jiang HY, Mumenthaler SM. Organs-on-chips: a decade of innovation. Trends Biotechnol. 2023;41(3):278-280. Tao T, Wang Y, Chen W, et al. Engineering human islet organoids from iPSCs using an organ-on-chip platform. Lab Chip. 2019;19(6):948-958. Tian L, Prasad N, Jang YY. In vitro modeling of alcohol-induced liver injury using human-induced pluripotent stem cells. Methods Mol Biol. 2016;1353:271-283. Urresti J, Zhang P, Moran-Losada P, et al. Correction: Cortical organoids model early brain development disrupted by 16p11.2 copy number variants in autism. Mol Psychiatry. 2021;26(12)7581 Venkat V, Abdelhalim H, DeGroat W, Zeeshan S, Ahmed Z. Investigating genes associated with heart failure, atrial fibrillation, and other cardiovascular diseases, and predicting disease using machine learning techniques for translational research and precision medicine. Genomics. 2023;115(2):110584. Vuorenpää H, Björninen M, Välimäki H, et al. Building blocks of microphysiological system to model physiology and pathophysiology of human heart. Front Physiol. 2023;14:1213959. Wei W, Cardes F, Hierlemann A, Modena MM. 3D In Vitro Blood-Brain-Barrier Model for Investigating Barrier Insults. Adv Sci (Weinh). 2023;10(11):e2205752. Wevers NR, Nair AL, Fowke TM, et al. Modeling ischemic stroke in a triculture neurovascular unit on-a-chip. Fluids Barriers CNS. 2021;18(1):59. Zamprogno P, Wüthrich S, Achenback S, et al. Second-generation lung-on-a-chip with an array of stretchable alveoli made with a biological membrane. Commun Biol. 2021;4(1):168. Zhong X, Harris G, Smirnova L, et al. Antidepressant paroxetine exerts developmental neurotoxicity in an iPSCderived 3D human brain model. Front Cell Neurosci. 2020;14:25. Zhuang P, Sun AX, An J, Chua CK, Chew SY. 3D neural tissue models: From spheroids to bioprinting. Biomaterials. 2018;154:113-133.

Ziraldo C, Solovyev A, Allegretti A, et al. A computational, tissue-realistic model of pressure ulcer formation in individuals with spinal cord injury. PLoS Comput Biol. 2015;11(6):e1004309.

Additional Supporting Resources:

Contopoulos-Ioannidis DG, Ntzani E, Ioannidis JP. Translation of highly promising basic science research into clinical applications. Am J Med. 2003;114(6):477-484.

Pound P, Ritskes-Hoitinga M. Is it possible to overcome issues of external validity in preclinical

1. Please provide feedback on the use of novel alternative methods to study human biology, circuits, systems, and disease states, including how novel alternatives:	Transl Med. 2018;16:304.
2. Please provide thoughts on approaches for catalyzing the development and validation of novel alternative method technologies, including:	If non-animal methods (a.k.a. novel alternative methods or NAMs) are to live up to their potential to transform biomedical research and catalyze discovery, their adoption must be commensurate with intense rigor. Otherwise, we risk abandoning critical methodologies and experiments not because they are fundamentally incorrect, but because they were improperly used. This would be a tragedy. Good laboratory and good cell culture practices are imperative. To aid in ensuring the robustness, replicability, reproducibility, and reliability of the technologies and the ensuing datasets, the NIH can provide dedicated funding for researchers in different laboratories to repeat experiments and fund accessible, public data repositories to promote transparency and data sharing. The NIH should also mandate that grantees adhere to high quality reporting standards, several of which have been recommended in the literature (see Supporting Resources). The UK's National Centre for the Replacement, Refinement, and Reduction of Animals in Research (NC3Rs) is currently undertaking a user testing study of its Reporting In Vitro Experiments Responsibly (RIVER) guidelines and have recently made a preprint available on these recommendations (The RIVER Working Group). These recommendations should ideally be in place for all research funded or undertaken by the NIH, but are increasingly important for non-animal methods so that their value is fully

2. Please provide thoughts on approaches for catalyzing the development and validation of novel alternative method technologies, including:	Resources: Emmerich CH, Harris CM. Minimum Information and Quality Standards for Conducting, Reporting, and Organizing In Vitro Research. Handb Exp Pharmacol. 2020;257:177-196. Hartung T, De Vries R, Hoffmann S, et al. Toward Good In Vitro Reporting Standards. ALTEX. 2019;36(1):3-17. OECD. Guidance Document on Good In Vitro Method Practices (GIVIMP), OECD Series on Testing and Assessment, No. 286, OECD Publishing, Paris. Published December 10, 2018. The River Working Group. Reporting in vitro experiments responsibly – The RIVER recommendations. MetaArXiv preprints. Updated June 21, 2023. Accessed August 15, 2023. https://osf.io/preprints/metaarxiv/x6aut/.
3. Please provide thoughts on strategies for maximizing the research value of novel alternative method technologies, including:	While there are research methods that can be used to study living humans (such as imaging), most methods are necessarily reductive. It will likely be the case that researchers or research groups need to use several non-animal methods (a.k.a. novel alternative methods or NAMs) in order understand a biological system or disease state. The benefit of non-animal, human biology-based methods is that, unlike animal-based

3. Please provide thoughts on strategies for maximizing the research value of novel alternative method technologies, including:	entirely different species. Many of these platforms can even be used to study systems and states in the individual patient of interest, using tissue and cell samples or genetic data, for example. A key strategy for bolstering technology readiness and the reliability of these technologies and ensuring their successful integration across research approaches and potential solutions is to increase funding for, access to, and training in these methodologies. This could be done by 1) making funding for non-animal research more readily available, 2) prioritizing non-animal research methods in training opportunities, and 3) establishing and expanding animal-free biomedical research resources. 1) Make funding for non-animal research more readily available: Decisions about grant funding must prioritize applicants who currently use non-animal methods, are making the transition from animal to non-animal methods, or are developing and/or validating non-animal methods. The NIH should offer Program Project Grants or Center Grants (P01/P30/P50) to investigators interested in establishing centers for non-animal methods at their institutions. The NIH should offer grant supplements to investigators who want to switch to non-animal methods mid-funding. 2) Training opportunities must prioritize non-animal research methods. The NIH should offer Institutional Training Grants to trainees at the undergraduate, graduate, and postdoctoral levels to receive training that would allow them to make the transition from animal to non-animal research methods. It should place particular emphasis on post-doctoral training fellowships that allow young scientists to receive training in non-animal methods. The NIH should offer Continuing Education Training Grants with the explicit purpose of establishing educational programs to train researchers on available non-animal methodologies. The NIH should offer awards to early stage investigators who are looking to switch from using animal models to conducting non-animal research. The NIH Director’s Early Independence Award should prioritize applicants who currently use non-animal, clinically-applicable methods; are making the transition from animal to non-animal methods; or are developing and/or validating non-animal methods. The NIH Bench-to-Bedside and Back Program should prioritize pairing basic science researchers using animal models with Intramural Research Program (IRP) clinical researchers. The goal should be to assist those researchers interested in permanently switching from animal-based research to clinical work. The NIH Graduate Partnership Program should prioritize those students who are hoping to use non-animal

3. Please provide thoughts on strategies for maximizing the research value of novel alternative method technologies, including:	at their home institution. These are just a few ideas. 3) Establish/Expand Animal-Free Biomedical Research Resources: The Office of Strategic Coordination—within the Office of the Director—should use the NIH Common Fund to establish multiple centers for non-animal methods across the U.S., as we suggested in a recent submission to an NIH Common Fund RFI. The NIH should establish Core Facilities at the NIH IRP that will provide investigators with access to resources and experts in the use of non-animal methods. Suggestions for such core facilities include a microphysiological systems core, an animal-free antibodies core, and a three-dimensional tissue printing core. The NIH should expand the current Human Tissue and Organ Research Resource. The NIH should require grant recipients to share their human bio samples with the "All of Us Research Program" biobank. As mentioned above, it is imperative that with increased funding for non-animal methods comes a mandate of rigorous practices, reporting, and data sharing.
File Upload	
Description	

If you would like to have a copy of your submission emailed to you, please check here	1
Email	emilyt@peta.org

Science Advancement & Outreach
A DIVISION OF PETA

1536 16th St. N.W., Washington, DC 20036

ADDITIONAL SUBMISSIONS MADE ON BEHALF OF PEOPLE FOR THE ETHICAL TREATMENT OF ANIMALS TO THE NATIONAL INSTITUTES OF HEALTH

November 2023: Request for Information (RFI): Inviting Comments and Suggestions on Updating the NIH Mission Statement

We propose replacing "living systems" with "humans" in the NIH's revised mission statement so that it reads: "To seek fundamental knowledge about the nature and behavior of humans and to apply that knowledge to optimize health and prevent or reduce illness for all people." For the NIH to achieve its goals in the latter part of the mission statement ("optimize health and prevent or reduce illness for all people"), the agency must move away from experiments on other animals, which do not provide the relevant, reliable, or translatable fundamental knowledge that is necessary to achieve these goals.

The last available estimate (2012) indicates NIH spends roughly 47% of its annual budget on experiments on animals (https://www.nap.edu/read/13322/chapter/4#23). In 2023, the agency is actively funding experiments on animals in areas where their use has led to no meaningful improvements in human health, such as sepsis and neurodegenerative disease. Across the board, experiments on animals have a low rate of translation to humans, with NCATS reporting that 95% of human clinical trials for new drugs fail (https://ncats.nih.gov/research/research-activities/ntu), despite having gone through safety and efficacy testing in animals.

While the NIH has increased its investment in human-relevant in vitro methods such as tissue chips, this investment remains paltry in comparison to its funding of animal-based experimentation. In fact, the agency appeared to double-down on its outdated support of animal models by shrouding what could be an innovative new program to replace animal use with a title that explicitly centers on the continued use of animals, and relegating human-relevant methods to a "complementary" status. (https://commonfund.nih.gov/complementarie/strategicplanning).

According to a November 2023 Pew Research poll, Americans' trust in science has declined in recent years (https://www.pewresearch.org/science/2023/11/14/americans-trust-in-scientists-positive-views-of-science-continue-to-decline/). Thirty-nine percent of respondents think that the U.S. is losing ground in scientific achievement, compared to the rest of the world (45% believe it is staying the same; only 14% think it is gaining ground). This could be attributed in part to the U.S.' inexplicable, unprogressive attitude toward more advanced, human-based methods. Compared to the U.S., other countries have made a more substantial push to move away from animal use toward human-relevant methods. For example, the Netherlands created the Transition Programme for Innovation without the use of animals (TPI), which aims to bring together stakeholders and offer a platform for identifying and developing

activities to increase the pace of the transition toward animal-free innovation (https://www.animalfreeinnovationtpi.nl/). In 2021, members of the European Parliament almost unanimously supported a motion for a resolution calling on the European Commission to develop an action plan—with a timeline and milestones—to phase out experiments on animals and accelerate the transition to innovation without the use of animals in research, regulatory testing, and education (https://www.europarl.europa.eu/doceo/document/TA-9-2021-0387_EN.html).

Scientists with People for the Ethical Treatment of Animals have developed a common-sense strategy that NIH can implement to phase out animal use and move toward superior, non-animal methods in an evidence-based way. The Research Modernization Deal (https://www.peta.org/wp-content/uploads/2023/01/peta-research-modernization-deal.pdf) calls on the agency to take the following steps:

1) End animal use in research areas in which animals have been demonstrated to be poor "models" of humans and their use has impeded scientific and medical progress. Multiple reviews have documented the overwhelming failure of animal use to benefit human health in specific areas, including neurodegenerative diseases, neuropsychiatric disorders, cardiovascular disease, strokes, cancer, diabetes, obesity, inflammation and immune responses, HIV/AIDS research, addiction studies, trauma research, and medical training as well as for regulatory testing. Experiments and tests on animals in these areas should be ended as soon as possible and replaced with non-animal methods.

2) Conduct systematic reviews of the efficacy of animal use to identify additional areas in which non-animal methods are available or the use of animals has failed to protect human or environmental health and can, therefore, be ended.

3) Redirect funds from animal studies to the use and development of reliable, non-animal methods. We have previously sent ideas for how this can be achieved within NIH's current structure.

4) Implement a harm-benefit analysis system for research involving animals that includes an ethical perspective and consideration of lifelong harm inflicted on animals, to be applied to all NIH intramural and extramural research.

5) Educate and train researchers in the benefits of and how to use non-animal testing approaches. Suggestions for how this could be achieved is also available in our previous correspondence.

By making NIH's mission explicit to seeking fundamental information about humans, the agency aligns itself with a more innovative, effective, and socially-acceptable research paradigm.

August 2023: Request for Information: NIH Common Fund is Soliciting Ideas for NIH-wide Challenges and Opportunities

Establish national centers for human-based complex cellular models. NIH Common Fund resources should be used to establish multiple national centers dedicated to advancing human-based *in vitro* research. These centers should accelerate the continued improvement and validation of complex

cellular models and provide *in vitro* resources as well as associated education and technical support to scientists throughout the U.S.

Critical challenge: Translating basic science and preclinical research into meaningful, affordable outcomes for patients

Despite decades of effort and billions of dollars invested in animal-based models of human biology and disease, effective treatments for the most common and deadly human diseases remain elusive. The "translation gap" between data emerging from basic science research and treatments for human disease is due in part to the limitations of animal models. Species differences in anatomy, physiology, gene expression, developmental trajectories, metabolism, immune responses, and disease susceptibility make translating data from an animal experiment into a human-relevant preventive measure, treatment, or cure extremely difficult. Additionally, animal models are often oversimplified and artificial versions of a complex human behavior, trait, or pathology, with targets that may be meaningful in an animal laboratory but are ultimately inadequate for humans. The failure of animal-based models and assays is contributing to the increased costs of drug development, and the public is declining to trust this type of science.

Emerging opportunity: Increase the availability of complex cellular models of human organs and systems to more researchers

Motivated by both the ethical concerns surrounding animal-based experimentation and testing as well as the limited translatability of animal-based data, advances in complex, 3-D cellular models, such as microphysiological systems, organoids, spheroids, and 3-D bioprinted structures derived from human cell lines and based in human biology have expanded in the past decade. Many of these models simulate human physiology and disease more accurately than traditional *in vivo* models using animals do. Currently, these tools are accessible to researchers working directly on their application and development. However, given their potential to improve preclinical and basic research as well as ongoing advances in their design, it's essential that investigators with knowledge or access gaps still have the opportunity to take advantage of these cutting-edge *in vitro* methods.

Establishing national centers for innovative and human-based *in vitro* models would help overcome the challenge of translational failure. They would allow many more researchers to have access to cutting-edge *in vitro* technology and increase the amount of human-applicable research being conducted in the U.S. Ideally, these centers would serve as concentrated hubs of technological advancement and expertise and as resources for researchers interested in using these tools. These centers could also serve as a biobank of cell lines, organoids, and microphysiological systems derived from humans of all ages, sexes, genders, SES status, and racial backgrounds as well as from different patient populations. These centers should be able to offer a full range of support for various types of "omic" technology and gene-editing tools for external researchers using the center's *in vitro* models.

Resources, tools, or knowledge that are needed to address the important challenge or opportunity

The relative novelty and rapid advancement of human-based cellular models make it challenging for researchers who aren't immersed in these tools to develop the needed expertise to use them independently. Numerous researchers at all levels lack access to the training or resources they need.

Additionally, many of the technological advances in human-derived 3-D cellular models are occurring outside traditional academic settings, which inherently limits data and technology sharing among investigators and institutes. NIH-funded *in vitro* models that are accessible to all investigators would help foster their advancement, validation, and applicability as well as collaborations among experts in this technology, clinical researchers, and researchers currently relying on inadequate animal models.

National advanced cell culture centers would ideally do the following:

- Bring together experts in the *in vitro* fields as research collaborators, rather than individual competitors.
- Help standardize, expand, and improve *in vitro* methods.
- Provide educational resources to external investigators.
- Accelerate the acceptance, familiarization, mastery, and use of these tools throughout the biomedical community.
- Reduce costs associated with the current fragmented development and validation of these models.
- Expedite the transition away from ethically problematic animal models.

Scientific advancements or other factors that make addressing the important challenge or opportunity particularly timely

The failure to translate data from "bench to beside" is well known within the science community and by the general public. Regulators, taxpayers, patients, and funding oversight committees are frustrated by the lack of meaningful progress in developing new treatments for prevalent diseases such as cancer, strokes, neuropsychiatric conditions, and neurodevelopment and neurodegenerative disorders. Additionally, as more details about the complexity of nonhuman animals emerge, society is becoming increasingly uncomfortable with using them in experiments—especially if they involve limited resources unlikely to result in health benefits for humans. Pressure for NIH to produce treatments and cures will continue to mount, as will discomfort with the use of sentient animals.

Innovations in complex, human-derived models have the potential to solve both the translational and ethical problems associated with animal-based research. It's critical to ensure that the entire scientific community can capitalize on the most innovative non-animal, human-relevant *in vitro* tools. This approach would help make NIH-funded research more accurate, relevant, and efficient—and, therefore, more acceptable to government oversight committees and the public.

Other comments or input you wish to provide.

Establishing cutting-edge *in vitro* centers to support researchers around the U.S. would achieve the goals that the Common Fund is expected to accomplish. These centers would be all of the following.

- **Transformative:** Improving the translatability of preclinical and basic science research by investing in human-derived cellular models and increasing their accessibility to all researchers would dramatically affect biomedical and behavioral research over the next decade.

- **Catalytic:** These centers should be part of the high-impact goal of reducing or replacing the use of ineffective animal models and replacing them with more effective and accurate human-based research.
- **Synergistic:** All NIH ICs and the research they fund would benefit from the increased use and availability of human cell-based models.
- **Cross-cutting:** These centers would help multiple NIH ICs achieve their goals, as these tools are being used to study multiple diseases and conditions. Establishing these centers and coordinating their subsequent resources would require a coordinated, trans-NIH approach.
- **Unique:** The NIH Common Fund is the agency's only funding source that could successfully establish and manage these centers.

Most importantly, these centers would help NIH achieve the last part of its mission, "to enhance health, lengthen life, and reduce illness and disability."

March 2023: **Request for Information (RFI) on Proposed Revised Simplified Review Framework for NIH Research Project Grant Applications**

The National Institutes of Health (NIH) Center for Scientific Review (CSR) is proposing to change its peer review instructions so that members of study sections will no longer provide an individual score for an applicant's "Environment" and "Resources" when assessing their study proposal. We are tentatively in favor of this change since, in theory, it could help to level the playing field, reduce the increasing favoritism that NIH has for awarding grants to older investigators,[1] and result in the benefit of increasing the awards disbursed to innovative, up-and-coming young minds who wish to improve the status quo.

Following PETA's extensive outreach, the agency is surely well aware that the current biomedical research paradigm is outdated and inefficient. It is estimated that 89% of preclinical experiments cannot be reproduced,[2] 90% of basic research fails to result in advances for human health,[3] and 95% of novel drugs (which, prior to the 2022 passing of the FDA Modernization Act 2.0, likely all went through animal testing) fail in human clinical trials.[4] U.S. citizens are getting a poor return on their significant investment into publicly funded human health research.

One reason for this persistent failure is this agency's inexplicable commitment to providing funding for animal research models that translate poorly to humans. Decades ago, when many of today's senior investigators were either starting their careers or in their prime, human-relevant methods were harder to come by. We did not yet have 3-dimensional cell culture capabilities, organs-on-chips, or artificial intelligence. These investigators learned what they could at the time—which was often many animal-based protocols and methods—and have since based their careers and the training of their students and post-doctoral researchers on these methods.

When grant reviewers score applicants based on their perceived expertise and resources, in reality, what they are often scoring is the investigator's age and lifelong achievement. Considering the existence and variety of different methods over time, favoring older and more established investigators inherently biases funding toward the use of animal-based research methods. This is problematic for many reasons, including the poor translatability of animal models, as described, and the ethical concerns. The most

recent Pew Research poll found that a majority of Americans did not approve of the use of animals in biomedical research.[5]

In addition to the changes suggested in the current RFI, we propose that CSR advise study section members to look favorably on an investigator's use of non-animal methods, particularly in areas of study where animal models are predominant, when assessing in the overall impact of a proposal. The use of poorly translatable animal models should reflect negatively on the adequacy of the approach and methodology proposed to carry out the research and is contrary to rigor, in that a proposal based on animal methods reduces likelihood that compelling, reproducible findings will result. Incentivizing non-animal methods is part of the plan proposed in PETA's Research Modernization Deal,[6] the world's first comprehensive plan to phase out ineffective experiments and divert funding to the most promising and clinically-relevant science.

We would also recommend each grant application be scored based on their Approximate Potential to Translate (APT), using existing publications associated with grants up for renewal or MeSH terms for new applications, similar to the calculations performed for published studies by the National Library of Medicine's iCite tool (https://icite.od.nih.gov/analysis).

References

1. Lauer MS, Roychowdhury D. Inequalities in the distribution of National Institutes of Health research project grant funding. Isales C, Zaidi M, Peifer M, eds. *eLife*. 2021;10:e71712. doi:10.7554/eLife.71712

2. Freedman LP, Cockburn IM, Simcoe TS. The Economics of Reproducibility in Preclinical Research. *PLOS Biology*. 2015;13(6):e1002165. doi:10.1371/journal.pbio.1002165

3. Contopoulos-Ioannidis DG, Ntzani EE, Ioannidis JPA. Translation of highly promising basic science research into clinical applications. *The American Journal of Medicine*. 2003;114(6):477-484. doi:10.1016/S0002-9343(03)00013-5

4. National Center for Advancing Translational Sciences. *Transforming Translational Science*.; 2019:2.

5. Strauss M. Americans are divided over the use of animals in scientific research. Pew Research Center. Accessed February 15, 2023. https://www.pewresearch.org/fact-tank/2018/08/16/americans-are-divided-over-the-use-of-animals-in-scientific-research/

6. People for the Ethical Treatment of Animals. *Research Modernization Deal*.; 2023:68. https://www.peta.org/wp-content/uploads/2023/01/peta-research-modernization-deal.pdf

Science Advancement & Outreach
A DIVISION OF PETA

1536 16th St. N.W., Washington, DC 20036

Request for Information: Accelerating Innovation through ARPA-H and FDA Collaboration

Response from People for the Ethical Treatment of Animals

May 2023

In order to accelerate "better health outcomes for everyone," ARPA-H and the FDA must work together to transition to robust non-animal testing strategies for safety and efficacy testing. Numerous scientific studies and reviews have demonstrated that an alarming number of animal tests fail to translate to humans.

The National Institutes of Health reports that **95 percent of novel drugs[1]—which have practically all gone through animal testing—fail in human clinical trials**. These failures feed into the enormous cost (>$2 billion per drug) and lengthy timeline (10-15 years) for bringing a new drug to market. Drug failure statistics are even more dire in certain disease areas (stroke,[2] sepsis,[3] Alzheimer's disease,[4] cancer,[5] and HIV vaccines,[6] for example), but the problem is largely disease agnostic.

The failure of preclinical animal tests to predict safety and efficacy in humans not only delays new treatments from getting to the clinic, drives up the costs of medications, and misuses funds, but can also directly lead to loss of life. Here are a few examples:

- In 2016, a Portuguese company developed a drug intended to help with mood, anxiety, and motor problems related to neurodegenerative disease. The six volunteers who participated in their phase I clinical trial experienced such adverse reactions after oral administration of this drug that they had to be hospitalized. One participant died.[7] These effects were not predicted by preclinical tests in animals, despite the fact that animals were given doses 400 times stronger than those given to the human volunteers.
- Preclinical animal tests also failed to predict the tragic outcome of the 2006 clinical trial for Theralizumab, an immunomodulatory drug, in which six human volunteers were given a dose 500 times smaller than that found safe in animal studies, but ended up facing life-threatening conditions involving multi-organ failure.[8]
- In the phase II study of fialuridine, an antiviral drug being tested against hepatitis B, almost half of the 15 patients experienced severe toxicity, which included liver failure, lactic acidosis, and pancreatitis, and resulted in the death of five of the patients.[9] Two additional patients required emergency liver transplants to survive.

This toxicity was not predicted by preclinical tests performed on dogs or monkeys and was not well replicated in post-trial studies in rats, who were administered a dosage that was 1000 times greater than what was given to humans.[10]

One study showed that animal tests fail to detect potential side effects of drugs in humans 81 percent of the time.[11] It is unfair to continue to burden U.S. taxpayers with the costs of ineffective research models and the subsequent elevated cost of drug development, all while putting their health at risk.

Advanced technologies that recapitulate human biology are increasingly shown to be more accurate at reflecting human outcomes when compared to animal tests. Here are a few examples:

- A human blood vessel-on-a-chip was able to predict human thrombosis caused by an antibody therapy.[12] This therapy had previously been determined to be safe following preclinical animal tests, but clinical trials had to be stopped after humans given the drug developed blood clots, which were not predicted by the experiments on animals.
- A computer algorithm was able to predict the human toxicity of new chemicals for nine hazard determinations with greater accuracy than animal tests.[13]
- *In vitro* tests using human cells predicted human liver injury caused by the diabetes drug troglitazone, which had not been detected in animal tests.[14] Troglitazone had been withdrawn from the market due to severe and fatal liver toxicity that killed at least 63 people.
- A human liver-on-a-chip was able to correctly identify 87% of drugs that passed animal testing but caused drug-induced liver injury in patients.[15] These drugs had caused nearly 250 human deaths and 10 liver transplants. Drug-induced liver injury is estimated to kill 7.6% of people who experience it.[16]

Reliance on animal models is diverting resources away from more promising research and development methods, delaying discoveries, increasing drug costs, compromising the testing of effective drugs and treatments, and limiting our ability to protect human health.

Critically, the recent passage of the FDA Modernization Act 2.0 has signaled that the public, the scientific community, and policymakers want to modernize the way biomedical research and testing are conducted, with greater focus on the importance of human-relevant methods and greater awareness of both the ethical and scientific issues that surround animal experimentation. The potential for this groundbreaking legislation to benefit animals and humans alike is why more than **200 organizations**—including biotech companies, medical associations, animal advocacy organizations, patient advocacy groups, and pharmaceutical companies—supported the bill.[17]

To this end, we recommend that ARPA-H and the FDA work together to do the following:

1. **Prohibit funding of animal use for drug discovery and preclinical testing in areas where it has been demonstrated that the animal tests and paradigms poorly predict human outcomes.** Replace animal use with more predictive non-animal systems based in human biology and prioritize validating these non-animal tests for regulatory acceptance. ARPA-H should implement a policy to fund promising human-relevant research methods, such as organs-on-chips, sophisticated uses of human stem cells, -omics technologies, imaging, and computer modeling instead of animal tests. A policy to fund these methods, which recapitulate human physiology and biology without using animals or their tissues, will benefit U.S. biomedical research as a whole, increase the safety of drugs approved by the FDA, and reduce the current length of time and failure rate associated with human drug development.
2. **Conduct systematic reviews on the predictive ability of animal use in drug discovery and preclinical testing to identify additional areas in which non-animal methods are available, could be available if provided increased resources, and/or where the use of animals has failed to protect human health.** In the latter case, animal studies must simply be stopped in order to prevent future adverse outcomes. ARPA-H could announce contracts to fund researchers to complete these systematic reviews, which would be then used by the FDA to make evidence-based decisions about regulatory acceptance.
3. **Work with other world leaders to harmonize and promote international acceptance of non-animal testing methods for regulatory toxicity testing requirements.** The regulatory acceptance of non-animal techniques in one region or country is an open door to international modernization of testing requirements. Likewise, a lack of international acceptance is a barrier to the use of a non-animal method. Therefore, we advocate that the FDA liaise with industry, research agencies, and relevant nongovernmental organizations worldwide to establish and promote clear paths to the validation and harmonization of non-animal techniques for regulatory testing requirements.

References

1. National Center for Advancing Translational Sciences. *Transforming Translational Science*. National Institutes of Health; 2019:2. Accessed May 25, 2023. https://ncats.nih.gov/files/NCATS-factsheet.pdf

2. Roth S, Liesz A. Stroke research at the crossroads – where are we heading? *Swiss Medical Weekly*. 2016;146(2728):w14329-w14329. doi:10.4414/smw.2016.14329

3. National Institute of General Medical Sciences. *NAGMSC Working Group on Sepsis*. National Institutes of Health; 2019:31. Accessed May 25, 2023. https://www.nigms.nih.gov/News/reports/Documents/nagmsc-working-group-on-sepsis-final-report.pdf

4. Cummings JL, Morstorf T, Zhong K. Alzheimer's disease drug-development pipeline: few candidates, frequent failures. *Alzheimer's Research & Therapy*. 2014;6(4):37. doi:10.1186/alzrt269

5. Wong CH, Siah KW, Lo AW. Estimation of clinical trial success rates and related parameters. *Biostatistics*. 2019;20(2):273-286. doi:10.1093/biostatistics/kxx069

6. Bailey J. An Assessment of the Role of Chimpanzees in AIDS Vaccine Research. *Altern Lab Anim*. 2008;36(4):381-428. doi:10.1177/026119290803600403

7. AFP. Man who died in French drug trial had 'unprecedented' reaction, say experts. *The Guardian*. https://www.theguardian.com/science/2016/mar/07/french-drug-trial-man-dead-expert-report-unprecidented-reaction. Published March 7, 2016. Accessed May 25, 2023.

8. Attarwala H. TGN1412: From discovery to disaster. *Journal of Young Pharmacists*. 2010;2(3):332-336. doi:10.4103/0975-1483.66810

9. McKenzie R, Fried MW, Sallie R, et al. Hepatic Failure and Lactic Acidosis Due to Fialuridine (FIAU), an Investigational Nucleoside Analogue for Chronic Hepatitis B. *N Engl J Med*. 1995;333(17):1099-1105. doi:10.1056/NEJM199510263331702

10. Institute of Medicine (US) Committee to Review the Fialuridine (FIAU/FIAC) Clinical Trials. *Review of the Fialuridine (FIAU) Clinical Trials*. (Manning FJ, Swartz M, eds.). National Academies Press (US); 1995. Accessed May 25, 2023. http://www.ncbi.nlm.nih.gov/books/NBK232098/

11. van Meer PJK, Kooijman M, Gispen-de Wied CC, Moors EHM, Schellekens H. The ability of animal studies to detect serious post marketing adverse events is limited. *Regulatory Toxicology and Pharmacology*. 2012;64(3):345-349. doi:10.1016/j.yrtph.2012.09.002

12. Barrile R, van der Meer AD, Park H, et al. Organ-on-Chip Recapitulates Thrombosis Induced by an anti-CD154 Monoclonal Antibody: Translational Potential of Advanced Microengineered Systems. *Clinical Pharmacology & Therapeutics*. 2018;104(6):1240-1248. doi:10.1002/cpt.1054

13. Luechtefeld T, Marsh D, Rowlands C, Hartung T. Machine Learning of Toxicological Big Data Enables Read-Across Structure Activity Relationships (RASAR) Outperforming Animal Test Reproducibility. *Toxicological Sciences*. 2018;165(1):198-212. doi:10.1093/toxsci/kfy152

14. Dirven H, Vist GE, Bandhakavi S, et al. Performance of preclinical models in predicting drug-induced liver injury in humans: a systematic review. *Sci Rep*. 2021;11(1):6403. doi:10.1038/s41598-021-85708-2

15. Ewart L, Apostolou A, Briggs SA, et al. Performance assessment and economic analysis of a human Liver-Chip for predictive toxicology. *Commun Med*. 2022;2(1):1-16. doi:10.1038/s43856-022-00209-1

16. Hayashi PH, Rockey D, Fontana RJ, et al. Death and Liver Transplantation within Two Years of Onset of Drug-Induced Liver Injury. *Hepatology*. 2017;66(4):1275-1285. doi:10.1002/hep.29283

17. Center for a Humane Economy. FDA Modernization Act of 2021 Endorsements and Cosponsors. Modernize Testing. Published 2023. Accessed May 25, 2023. https://modernizetesting.org/endorsements

In Response to:
NIH Request for Information: Soliciting ideas for new NIH Common Fund programs
Notice Number: NOT-RM-22-016

By People for the Ethical Treatment of Animals
September 2022

1. Transition-to-Translation program

Summary: This proposal would use the NIH Common Fund to create a Transition-to-Translation program, a vehicle for building confidence and competence in reliable and relevant non-animal methods that can best protect human health. This program would make human-relevant research accessible to investigators wishing to switch from animal to non-animal methods, prioritizing support for early-career researchers, but open to all. Built on existing NIH funding mechanisms, this program will help ensure a robust biomedical workforce that is able to compete with a rapidly changing scientific landscape and respond to increasing calls for improved translation of biomedical research findings into human health advancements.

Description: As animal-free research methods continue to expand, increased education and hands-on training will accelerate the transition to these methods. However, in deploying such initiatives, it is important to recognize that barriers can exist to adopting new approaches and technologies, and therefore, efforts to build confidence and provide additional support are needed. Early career researchers wishing to use non-animal methods, such as organs-on-chips or computational modeling, may have not had the training or opportunity to become familiar or adept at using these research tools. At the same time, mid-career investigators may find themselves using animal models they no longer feel to be relevant to their research question, but lacking the time and funding to re-train themselves and their team in non-animal methods, purchase new equipment, and support their students and staff during such a substantial transition.

A new Transition-to-Translation program, supported by the NIH Common Fund, would make animal-free research accessible to investigators wishing to switch from animal to non-animal methods, prioritizing support for early-career researchers, but open to those in all stages of their research career.

There is a need for education and hands-on training in non-animal methods. Students and early career scientists must be provided with opportunities to develop the skills necessary to contribute to the future of their own field, as well as the field of non-animal research, so that the U.S. can compete with international developments and does not fall behind in research advances. However, many study programs lack sufficient courses about animal-free methods. Some supplemental training programs have been developed to begin to fill this gap. For example, in the EU, the European Commission's Joint Research Centre hosts a summer school on non-animal approaches. In Canada, the University of British Columbia has accepted a new undergraduate module offered by the Society for Humane Science on "Non-Animal Methods in Biomedical Science", which focuses on training students in animal-free methods for research and testing. Many online resources by experts in the field also exist, including those offered by PETA Science Consortium International e.V. and the Physicians Committee for Responsible Medicine. The Dutch Transition Programme for Innovation created a series of "helpathons", action-orientated workshops built around a specific question that encourages researchers through a community forum to think creatively and harness the power of coincidence in the discovery of new opportunities with regard to non-animal approaches.

Thus, information about animal-free research and testing is available, but is rarely a component of biomedical education.

The UK's innovation agency, Innovate UK, has recognized that overcoming skepticism about the ability of non-animal methods to model biological processes will help remove a major barrier to the use of these methods. Furthermore, conservatism and inertia obstructing the move away from animal-based methods can be overcome by encouraging scientists "to think beyond their immediate research areas to how their skills, technology and 'know-how' can be leveraged and exploited to accelerate the development and adoption of" advanced non-animal methods. It is vital that such educational initiatives be adopted and given ample financial support from funders such as NIH, to benefit everyone from future scientists to established professionals.

The Transition-to-Translation program would provide early career and established intramural and extramural researchers using animal-based methods with retraining opportunities and encourage them to forge multidisciplinary collaborations to evolve their skills and establish new and innovative ways of asking research questions and methods for answering them.

There are a number of existing funding mechanisms NIH could employ within the Transition-to-Translation program:

- Institutional Training Grants can be provided to trainees at the undergraduate, graduate, and postdoctoral levels to receive training that would allow them to make the transition from animal to non-animal research methods.
- Continuing Education Grants can be offered with the explicit purpose of establishing educational programs to train researchers on available non-animal methodologies.
- The NIH Director's Early Independence Award could prioritize applicants who currently use non-animal, clinically-applicable methods; are making the transition from animal to non-animal methods; or are developing and/or validating non-animal methods.
- The NIH Bench-to-Bedside and Back Program could prioritize pairing basic science researchers using animal models with Intramural Research Program (IRP) clinical researchers. The goal would be to assist those researchers interested in permanently switching from animal-based research to clinical work.
- The NIH Graduate Partnership Program could prioritize those students who are hoping to use non-animal methods in their research but do not have access to those tools at their home institution.
- Program Project Grants or Center Grants can be offered to investigators interested in establishing centers for non-animal methods at their institutions.
- Grant supplements can be offered to investigators who wish to switch to non-animal methods mid-funding.

As the range of animal-free testing methods continues to expand, researchers must keep pace with these pivotal developments. Increased education and training initiatives are urgently required to build confidence and competence in reliable and relevant non-animal methods that can best protect human health. The Transition-to-Translation program would help ensure a robust biomedical workforce that is able to compete with a rapidly-changing scientific landscape and respond to increasing calls for improved translation of biomedical research findings into human health advancements.

Resources to support this proposal:
https://joint-research-centre.ec.europa.eu/events/jrc-summer-school-non-animal-approaches-science-3_en
https://www.forhumanescience.org/influencing-science-culture/university-education
https://www.thepsci.eu/our-work/training
https://www.pcrm.org/ethical-science/animal-testing-and-alternatives/nura
https://www.tpihelpathon.nl/
https://www.ukri.org/wp-content/uploads/2015/11/IUK-071221-RoadmapNonAnimalTech.pdf.

2. Systematic Review Collaboratory

Summary: This proposal would use the NIH Common Fund to create a Systematic Review Collaboratory (SRC) that would aid in the design and rapid execution of systematic reviews for translational and preclinical research. The SRC would develop and disseminate best practices and training on systematic reviews and provide funding for both intramural and extramural investigators wishing to conduct translational or preclinical systematic reviews. The SRC would work across NIH to perform systematic reviews that would assess the effectiveness of various translational and preclinical research models employed by NIH-supported researchers.

Description: The past two decades have brought to light many obstacles in scientific research, including both the "reproducibility crisis" and failures in the translation of research findings to the clinical setting. Depending on the metrics used, basic and preclinical research fail to lead to human benefit between 90 and 95 percent of the time, representing an enormous inefficiency of resources and a failure to meet the needs of patients and their families in a timely manner. Addressing this crisis requires funding agencies to step back and assess—with great care and accuracy—the sources of these inefficiencies. Systematic reviews (SRs) provide a method for doing this.

According to the Cochrane Library, SRs "identify, appraise and synthesize all the empirical evidence that meets pre-specified eligibility criteria to answer a specific research question. Researchers conducting SRs use explicit, systematic methods that are selected with a view aimed at minimizing bias, to produce more reliable findings to inform decision making." A new Systematic Review Collaboratory (SRC), supported by the NIH Common Fund, would provide the NIH and other federal funding agencies with clear evidence on which they could reliably base future policy and funding decisions and improve the agency's return on investment.

The SRC would support the execution of SRs at two levels. First, the SRC would convene or commission an unbiased team to conduct SRs to assess the effectiveness of the preclinical and translational research models—including both animal and non-animal methods—being used by NIH intramural and extramural researchers to provide evidence-based data on whether these models are fit-for-purpose. To assess these characteristics, SRs would include information on past translation of the research model and the return-on-investment received by the public for the results of experiments using such models, as well as the costs of the model, including the harms experienced by animals, where applicable. Examples of such SRs could include examinations of the use of the cecal ligation and puncture mouse models for understanding and treating human sepsis, the use of nonhuman primate models for developing HIV therapeutics, the use of human organs-on-chips for preclinical drug development in specific fields, and many more.

Second, the SRC would develop best practices and training modules to aid U.S. researchers in designing and performing their own SRs and provide funding for them to do so. According to a study on SR training conducted by the Netherlands Organisation for Health Research and Development (ZonMw), coaching researchers to conduct SRs "increased support for the 3Rs,

improved transparency, [increased] awareness of the need for better study quality, [resulted in] greater critical appraisal of the use of animals, and improved knowledge transfer."

Once resources and teams are in place, the SRC can also be utilized to conduct SRs of clinical and public health research.

NIH already supports the concept that SRs should be used to guide funding decisions. Several U.S. funding entities, including NIH, are members of the Ensuring Value in Research Funders' Forum (EViR), a collection of the most prominent international funding bodies formed to address waste in clinical and preclinical research. EViR states as its second guiding principle, "Research should only be funded if set in the context of one or more existing systematic reviews of what is already known or an otherwise robust demonstration of a research gap." It explains, "This is important because new research not set in the context of what is already known leads to unnecessary duplication, studies that cannot change decision making (e.g. will not change the meta analysis), or inappropriate design (e.g. inappropriate outcome measures, incorrect prevalence assumptions, failure to learn from past previous studies)." To apply this principle, EViR says that funders must "[r]outinely assess whether an adequate review has been done and whether the results of that review support the case for further clinical or preclinical research."

The recommendation to conduct scientific reviews of the efficacy of models is, therefore, already one that the largest funding bodies in the world agree is a necessary principle for guiding valuable research and reducing waste in research funding. Yet, NIH has yet to take concrete steps to implement this principle.

When established, the SRC will create valuable new data on model efficacy that will be accessible to all NIH institutes as well as the larger research community. SRC deliverables will guide funding decisions to improve efficiency and the translatability of NIH-supported research findings into prevention and therapies, helping NIH to realize its goals of protecting and improving health, ensuring a high return on the public's investment in research, and promoting the highest level of scientific integrity.

Resources to support this proposal:

https://www.cochrane.org/our-evidence/what-are-systematic-reviews
https://www.syrcle.network/
http://www.dcn.ed.ac.uk/camarades/default.htm
https://evir.org/our-principles/applying-the-principles/#principle2
https://www.elsevier.com/connect/authors-update/why-systematic-reviews-matter
Menon, et al. 2021: https://doi.org/10.1371/journal.pone.0260619
Ritskes-Hoitinga and Pound, 2022: https://doi.org/10.1177/01410768221093551
Russell, et al. 2022: http://dx.doi.org/10.1136/bmjos-2021-100219

SUGGESTED ACTION STEPS FOR PHASING OUT EXPERIMENTS ON ANIMALS

Science Advancement & Outreach
A DIVISION OF PETA

#1: Cease funding of new projects involving animals in areas of disease research in which there is ample evidence of poor applicability to humans.

The National Institutes of Health (NIH) must end its support of new research projects involving animals in fields in which the use of animals has been a well-documented failure. These areas should include cancer, cardiovascular disease, diabetes, neurodegenerative diseases, neuropsychiatric diseases, sepsis, and stroke.

#2: Conduct thorough systematic scientific reviews of the utility of animal-based research in all remaining disease and research areas in order to identify additional areas in which the use of animals can be immediately ended.

NIH must commission an unbiased committee to conduct a systematic review of the effectiveness of current animal models for each individual disease and health area that its institutes are studying. Such systematic reviews should include information on the return-on-investment received by the public for the results of animal-based research funded and conducted by NIH.

#3: Prioritize funding for research that uses non-animal, human-relevant research methods, including preventive and interventional research involving human participants.

Make Funding for Non-Animal Research More Readily Available

- Decisions about grant funding must prioritize applicants who currently use non-animal methods, are making the transition from animal to non-animal methods, or are developing and/or validating non-animal methods.
- NIH should offer Program Project Grants or Center Grants (P01/P30/P50) to investigators interested in establishing centers for non-animal methods at their institutions.
- NIH should offer grant supplements to investigators who want to switch to non-animal methods mid-funding.

(continued on page 2)

SUGGESTED ACTION STEPS TO PHASE OUT ANIMAL EXPERIMENTATION

Science Advancement & Outreach
A DIVISION OF PETA

- Training opportunities must prioritize non-animal research methods:
  - NIH should offer Institutional Training Grants to trainees at the undergraduate, graduate, and postdoctoral levels to receive training that would allow them to make the transition from animal to non-animal research methods. It should place particular emphasis on post-doctoral training fellowships that allow young scientists to receive training in non-animal methods.
  - NIH should offer Continuing Education Training Grants with the explicit purpose of establishing educational programs to train researchers on available non-animal methodologies.
  - NIH should offer awards to early stage investigators who are looking to switch from using animal models to conducting non-animal research.
  - The NIH Director's Early Independence Award should prioritize applicants who currently use non-animal, clinically-applicable methods; are making the transition from animal to non-animal methods; or are developing and/or validating non-animal methods.
  - The NIH Bench-to-Bedside and Back Program should prioritize pairing basic science researchers using animal models with Intramural Research Program (IRP) clinical researchers. The goal should be to assist those researchers interested in permanently switching from animal-based research to clinical work.
  - The NIH Graduate Partnership Program should prioritize those students who are hoping to use non-animal methods in their research but do not have access to those tools at their home institution.

Establish/Expand Animal-Free Biomedical Research Resources

- The Office of Strategic Coordination—within the Office of the Director—should use the NIH Common Fund to establish multiple centers for non-animal methods across the U.S.
- NIH should establish Core Facilities at the NIH IRP that will provide investigators with access to resources and experts in the use of non-animal methods. Suggestions for such core facilities include the following:
  - Microphysiological systems core
  - Animal-free antibodies core
  - Three-dimensional tissue printing core
- NIH should expand the current Human Tissue and Organ Research Resource.
- NIH should require grant recipients to share their human biosamples with the "All of Us Research Program" biobank

Request for Information (RFI): Request for Information (RFI) on the FY 2021-2025 National Institutes of Health (NIH) - Wide Strategic Plan Framework

Thank You - Your Comments Have Been Received. You may want to print this page with your comments for your records.

03/23/2020 at 05:44:35:832 PM

Title/Name

Dr. Emily R. Trunnell

Email Address

emilyt@peta.org

Name of Organization (if responding in professional capacity)

People for the Ethical Treatment of Animals

Type of Organization

Advocacy Group

Role in Organization

Research Associate

Please select yes/no if you are responding on behalf of your organization

Yes

Please include any comments on the following:

Cross-Cutting Themes articulated in the framework, and/or additional cross-cutting themes that may be considered.

There are opportunities within the following cross-cutting themes to reduce the use of animals and modernize biomedical research. •Optimizing Data Science and the Development of Technologies and Tools: NIH must increase funding in these areas only where the data, technologies, and tools are based in human biology, without the use of animals. •Promoting Collaborative Science: Where investigators lack the capabilities to conduct human-based research, NIH must help pair these individuals with others who have expertise to assist them in a transition away from experiments on animals. •Addressing Public Health Challenges Across the Lifespan: Diseases primarily affecting individuals in early and late life are often studied using crude experiments on animals, despite an abundance of evidence that these methods are failing. NIH must prioritize non-animal research for these conditions. An additional cross-cutting theme should be added: Eliminate Reliance on Non-Human Animals. NIH reports that novel drugs fail in 95 percent of human studies, even though they appeared safe and effective in preclinical experiments using animals (1). A 2014 analysis published in The BMJ found that—contrary to public perception—studies using animals largely have not furthered knowledge in the field of human health or led to the development of treatments for conditions

affecting humans (2). Experiments on animals lack both internal and external validity, meaning they are usually poorly executed, but even if the experimental methods were improved, the results would not translate to humans. The difficulties in applying data derived from animals to human patients are compounded by confinement and unnatural conditions of laboratory life, which thwart animals' ability to engage in natural behaviors. This deprivation contributes to their stress and alters their physiology and neurobiology, causing them to exhibit various psychopathologies. Importantly, the fact that animals in laboratories have altered physiology and neurobiology means that they will never be good "models," even for members of their own species who are free-roaming. Along with mounting evidence that experiments on animals do not reliably translate to humans and the increasing development and implementation of technologies that can supplant animal use in laboratories, our society has witnessed growing moral concern regarding animal experimentation. An August 2018 poll conducted by the Pew Research Center found that a majority of U.S. adults, the taxpayers who fund the NIH, oppose the use of animals in scientific research (3). If the public were fully aware of the mountain of evidence that studies on animals may very well be hampering the development of effective treatments, opposition would likely grow substantially. If our finite public funds are to be used responsibly, they must fund research, whether basic or applied, that leads to effective treatments for humans. The evidence that basic and applied research involving animals is impeding the development of treatment and cures for human ailments has not heretofore prompted NIH to rethink research and funding priorities sufficiently. However, such a paradigm shift is crucial. 1. https://ncats.nih.gov/files/NCATS-factsheet.pdf 2. https://doi.org/10.1136/bmj.g3387 3. https://www.pewresearch.org/fact-tank/2018/08/16/americans-are-divided-over-the-use-of-animals-in-scientific-research/

NIH's priorities across the three Objectives articulated in the framework, including potential benefits, drawbacks or challenges, and other priority areas for consideration.

Objective 1 can only be achieved by prioritizing human-based research and eliminating the use of animals. One of the efforts that NIH must take to fulfill this objective is to ensure that study sections are comprised of individuals with ample expertise in non-animal research and not dominated by those vested in the use of animals. Objective 2 can be achieved, in part, by providing additional financial assistance to investigators who wish to switch from animal-based to human-based methods; and by ceasing funding to train young investigators in animal methods. Regarding Objective 3, animal experiments lack internal and external validity and are in direct conflict with Scientific Integrity, Social Responsibility, and Good Scientific Stewardship. An additional objective should be added: Using Evidence-Based Methods to Improve Human Health Research. We propose a step-wise approach. 1. Immediately Eliminate Animal Use in Areas in Which Animals Have Been Shown to be Ineffective "Models" for Humans and Their Use has Impeded Progress: Multiple reviews have documented the failure of animal use to benefit human health in specific disease areas. Animal experiments in these areas should be ended as soon as possible and replaced with more effective and efficient non-animal research methods. 2. Increase Funds for Non-Animal Studies and Decrease Funds for Animal Studies: As long as part of the NIH budget goes to experiments on animals, the U.S. will be stalled in developing effective treatments for human disease. Forward-thinking scientists, some funded by NIH, are advancing and implementing methods for studying and treating diseases and testing products that do not entail the use of animals and are relevant to human health. Researchers have created human cell-derived skin models, "organs-on-chips," in silico models, and other methodologies that can replicate human physiology, diseases, and drug responses more accurately than experiments on animals do. Indeed, in its most recent five-year strategic plan, NIH announced that it would reduce and replace animal experiments. NIH must now take the next step and end the funding of experiments that have failed to provide effective treatments and cures. With greater investment in exciting and innovative non-animal methods and bold policy initiatives, far more promising cures and treatments for humans can be developed. 3. Conduct Critical Scientific Reviews of Previous Animal Studies to Identify the Areas in Which the Use of Animals Can Be Immediately Ended: For those areas of investigation where there is still some question as to whether the use of animals is beneficial, a thorough systematic review should be conducted to determine the efficacy of using animals. The National Academy of Medicine, formerly the Institute of Medicine, completed an examination of the scientific necessity of using chimpanzees in behavioral and biomedical research. That effort revealed that harmful studies had been approved, funded, and conducted for years, even though there were alternative methods in virtually every area in which chimpanzees were being used. Institutional oversight bodies and funding agencies had given their stamp of approval to these protocols. However, as we now know, the review processes in place were simply inadequate.

Future opportunities or emerging trans-NIH needs.

In addition to an overall paradigm change in NIH's reliance on the use of non-human animals, there are a number of areas of NIH intramural and extramural research which should be ended immediately. NIMH must end its support and conduct of psychological and other poorly-designed studies. Elisabeth Murray, an investigator at NIMH, carves out a section of a monkey's skull, injects toxins into the brain, suctions out portions of it or burns them, causing permanent and traumatic damage. She then repeatedly terrifies the monkeys with realistic-looking, animated artificial snakes and spiders. When Murray has finished with them, they may be killed or recycled into other experiments, to be further tormented. NIH has thrown $36 million to Murray's laboratory in the past 13 years, but not one treatment or cure for humans has come out of it in 30 years. NIMH Director Joshua Gordon has voiced his support for cruel experiments on animals that are notoriously poor models for studying human disease. Gordon has indicated he intends for the Institute to continue to fund the forced swim test, tail suspension test, foot shock, and social defeat experiments, where small animals are made to swim to keep from drowning, taped up by their sensitive tails, subjected to electric shock, and where experimenters incite some animals to attack and intimidate others, respectively. Nothing about these tests "models" complex human neuropsychiatric disorders and reliance on them is consistently cited as a leading

reason why so many neurobehavioral drugs fail in human trials. Another area of NIH funding that must end immediately is support for the use of non-human animals in sepsis experiments. Numerous peer-reviewed publications have described the inability of mice and other non-human animals to function as appropriate experimental models of human sepsis due to inherent genetic and physiological species differences, the disconnect between methods of experimental sepsis induction in non-human animals and the way that sepsis manifests in humans, and significant animal-welfare concerns that further confound study results. More than 60 clinical trials have been undertaken to test novel treatments for sepsis. However, all have failed to yield any benefit for humans. Clinicians cite unconstructive tests on animals as a primary reason for these failures and call for human-relevant methods to be adopted. For NIH to continue to spend taxpayers' dollars on experiments it has long known to lack translatability to sepsis in humans baselessly disregards the statutory and regulatory criteria that govern NIH's funding authority. Additionally, NIH must reverse its plans to support centralized infrastructure for experiments on marmosets, which have less to do with good science and everything to do with convenience. Marmosets are complex, unique, social individuals with the capacity to experience a wide range of emotions. In captivity, they are susceptible to many infectious pathogens—and they can also succumb to painful and potentially deadly marmoset wasting disease. Thus, the experimental use of marmosets introduces additional ethical concerns. By ramping up funding to increase the supply of marmosets for laboratories, NIH is doubling down on a failed enterprise.

PEOPLE FOR THE ETHICAL TREATMENT OF ANIMALS

Washington, D.C.
1536 16th St. N.W.
Washington, DC 20036
202-483-PETA

Los Angeles
2154 W. Sunset Blvd.
Los Angeles, CA 90026
323-644-PETA

Norfolk
501 Front St.
Norfolk, VA 23510
757-622-PETA

Berkeley
2855 Telegraph Ave.
Ste. 301
Berkeley, CA 94705
510-763-PETA

Info@peta.org
PETA.org

National Institutes of Health Request for Information on Enhancing Rigor, Transparency, and Translatability to Improve Biomedical Research Involving Animal Models

Response from
People for the Ethical Treatment of Animals (PETA)

As NIH has acknowledged in this Request for Information, the use of animals in biomedical research continues to face grave challenges in ensuring scientific rigor. Animals are being used in invasive and deadly experiments even when they poorly represent the human disease they are intended to model. Numerous scientific studies and reviews reveal that experiments on animals fail to lead to effective treatments and cures for human diseases, including the top killers in the U.S. The NIH's own National Center for Advancing Translational Sciences reports that new drugs (which are tested for safety and efficacy in other animals) fail in about 95% of human studies.[1] Reliance on animal models is diverting funds from more promising areas of research and delaying the development of effective drugs and treatments.

Though much could be done to address the poor quality of animal research, including the pervasive lack of research reproducibility, the confounding factors inherent in keeping animals in unnatural laboratory environments, and poorly-planned studies, no amount of improvement in these areas can address the *fundamental* inability of other species—even other primates—to serve as analogs for understanding human health and human biology. Poor rigor in animal experiments cannot be overcome by simply improving study design. This is because external validity, or the "extent to which research findings derived in one setting, population or species can be reliably applied to other settings, populations and species,"[2] can never be achieved. Intrinsic biological and genetic differences among species contribute significantly to inescapable problems in extrapolating results from nonhuman animals to humans, even in the best-controlled, best-executed study designs.

NIH must focus its efforts on redirecting funding from experiments on animals and instead towards providing greater support for non-animal, human-relevant research methods. To accomplish this goal, scientists with People for the Ethical Treatment of Animals have developed a robust blueprint titled, The Research Modernization Deal, which you can review by visiting www.peta.org/newdeal.

A paradigm shift in the current research culture is critical for this change. Several major problems exist, including the perverse incentive structure to publish above all else, the pressure on students and young investigators to engage in the antiquated animal-based research methods of their predecessors, and the lack of a diversity of expertise in the committees that review research proposals for funding consideration.

Affiliates:
- PETA Asia
- PETA India
- PETA France
- PETA Australia
- PETA Germany
- PETA Netherlands
- PETA Foundation (U.K.)

A paradigm shift in the current research culture is critical for this change. Several major problems exist, including the perverse incentive structure to publish above all else, the pressure on students and young investigators to engage in the antiquated animal-based research methods of their predecessors, and the lack of a diversity of expertise in the committees that review research proposals for funding consideration.

Unfortunately, success within the biomedical research community is often measured in terms of publication metrics. Publishing a greater number of papers in what are considered high-impact journals[3] improves a scientist's odds of receiving federal funding for research, which in turn generates more papers, which begets more funding, and so on, in an effort to advance a researcher's career--hence the common phrase in academia to "publish or perish." This emphasis on publishing leads to sloppy research practices, as scientists often rush to push out results at any cost, instead of being allowed the time and funding to learn superior human-relevant and animal-free techniques, invest in appropriate equipment, and ensure their methods are sound.[4]

Presently, and for much of human history, the biomedical research community has placed a bewildering amount of time, money, and effort manipulating the anatomies, physiologies, and genomes of other species. Older scientists who have been using the same archaic animal-based techniques that their own mentors used, and who have neither been pushed nor felt they had the time to learn more advanced non-animal methods, are the ones training younger scientists. Graduate students being trained in laboratories using animal-based techniques are rar elyexposed to the breadth of human-relevant research methods that exist and are pressured to design and perform experiments on animals in order to quickly publish papers.[5] To break this cycle, it is imperative that NIH robustly support, coordinate, and fund the training of young scientists in animal-free, human-relevant research methods.

In addition, NIH Center for Scientific Review must ensure a diversity of expertise within its Study Sections. Presently, Study Sections appear to be dominated by individuals with expertise only in animal-based methods, and who may have a vested interest in seeing animal experimentation persist as a dominant research paradigm. Or they may favor these proposals simply because this is the area with which they are most familiar. This means that scientists who submit proposals to address human health issues and answer important research questions using animal-free methods are likely being denied adequate consideration, as there are few reviewers who understand or support their strategies. NIH must ensure that the at least half of the membership of each Study Section is made up of scientists whose primary expertise is in safe and effective human-based practices.

To enhance rigor, reproducibility, and translation of research findings, NIH must now take the necessary steps to end the funding of experiments on animals that have repeatedly and overwhelmingly failed to provide effective treatments and cures for human conditions. With greater investment in exciting and innovative non-animal methods and bold policy initiatives, researchers can safely develop far more promising therapies for humans and also alleviate the immense suffering of tens of millions of animals who are currently used in experiments each year.

References

1. National Center for Advancing Translational Sciences. *Transforming Translational Science*.; 2019. https://ncats.nih.gov/files/NCATS-factsheet.pdf.

2. Pound P, Ritskes-Hoitinga M. Is it possible to overcome issues of external validity in preclinical animal research? Why most animal models are bound to fail. *J Transl Med*. 2018. doi:10.1186/s12967-018-1678-1
3. Paulus FM, Rademacher L, Schäfer TAJ, Müller-Pinzler L, Krach S. Journal Impact factor shapes scientists' reward signal in the prospect of publication. *PLoS One*. 2015. doi:10.1371/journal.pone.0142537
4. Higginson AD, Munafò MR. Current Incentives for Scientists Lead to Underpowered Studies with Erroneous Conclusions. *PLoS Biol*. 2016. doi:10.1371/journal.pbio.2000995
5. Lockwood A. *Test Subjects*. United States; 2019. https://www.testsubjectsfilm.com/.