IN DEFENSE OF ANIMALS

The American Recovery and Reinvestment Act:

A Grand Opportunity to Support 21st Century Science

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Executive Summary

The 2009 American Recovery and Reinvestment Act is a timely opportunity to reinvigorate our economy, and the National Institutes of Health (NIH) appropriations it includes are necessary to ensure that our nation maintains its position as a world leader in medicine, science, and technology.

The Recovery Act is a major opportunity for medical progress as well as economic and political progress. Thanks to projects like the Challenge Grants and the Grand Opportunity Grants, the NIH has the power to bypass a fundamentally flawed grant award system that incentivizes incremental research in order to directly fund transformational proposals which might ordinarily be rejected. In light of this unique opportunity, we recommend that the Institutes and Centers of the NIH explore the potential of promising non-animal methods, which have been systematically underestimated, underutilized, and underfunded. This Report is an elaboration of this request, supported by peer-reviewed critiques of animal models culled from the scientific literature and evaluations of extremely promising non-animal research methods that have not received the recognition or the funding they deserve.

In the body of our Report, we describe some leading non-animal technologies with extraordinary potential which remain unexplored by the NIH. It is organized into three categories of non-animal research – *in silico*, *in vitro*, and human-based – with one exemplary technology featured in each.

In the *in silico* section, we describe the Virtual Human Project, a proposed simulation of all physiological processes in the human body. The goal of this project is to integrate all existing physiological models and international physiome projects into one common model. The task of synthesizing, organizing, and visualizing this massive amount of data has been undertaken by two major efforts, the Virtual Physiological Human in Europe and the Virtual Human Project in the United States.

The Virtual Physiological Human is an international collaborative project supported by the member states of the European Union, as well as nations across the globe, most notably Australia and Japan. By contrast, the Virtual Human Project is primarily the work of one research institution, Oak Ridge National Laboratory (ORNL), with some help from collaborating universities and corporations. Ideally, these two efforts would be complementary, as ORNL has focused its energies on modeling the human respiratory system, while past European projects have modeled the human heart, kidneys, epithelial tissues, and musculoskeletal system. If this project is to be fully realized, the U.S. research community needs to participate in this international endeavor. In the meantime, however, we can support research in computational physiology, which will advance the Physiome Project simply by adding realistic simulations to our growing knowledge base.

In the *in vitro* section, we review the cell culture analog, also known the animal-on-a-chip or the human-on-a-chip, which is an approximation of the physiology of the whole human body –

complete with reservoirs seeded with different cell types and canals that act as vasculature. Cell culture analogs (CCAs) can be used to test the mechanisms, efficacy, and toxicity of drugs and prodrugs, which are metabolized within the chip itself. CCAs have a liver compartment which has already been tested using naphthalene (a toxicant) and Tegafur (a prodrug), each of which only produces an effect when metabolized.

The applications of CCAs go beyond simple toxicology – they can be used to study drug-drug and organ-organ interactions, as well as to predict disease progression and treatment outcomes. The scientist credited with inventing the CCA concept, Michael Shuler at Cornell University, is currently pursuing a three-dimensional model as well as a CCA that uses human stem cells. These could be the first steps towards a personalized CCA - a replica of a patient's unique physiological profile – which would be a crucial tool for personalized medicine.

The work of Shuler and others has steadily progressed towards this goal, in spite of a lack of support from the NIH, with funding coming from multiple other sources including the National Science Foundation and venture capitalists. The absence of NIH funding for such cutting-edge research is conspicuous, particularly when NIH has a responsibility to invest in research that is immediately relevant to public health.

In the human-based research section, we examine multi-modal scanners, which combine multiple imaging methods into one scanner, allowing for real-time physiological recordings and precise anatomical readings to be performed simultaneously. Also known as hybrid imaging, this combination of two or more complementary imaging techniques allows the strengths of one technique to compensate for the weaknesses of another, presenting a more complete picture of physiological phenomena.

Magnetoencephalography (MEG) and electroencephalography (EEG) can offer data with high temporal resolution (milliseconds) but low spatial resolution, whereas MRI techniques can give pharmacological and physiological information with low temporal resolution, but high spatial resolution (millimeters). Structural MRI provides precise anatomical maps, while functional MRI captures local blood flow correlated with behavior in real time. An integrated PET/CT scanner combines the visualization of metabolic reactions with an accurate identification of anatomical regions. Originally created for tumor detection, the combined PET/CT scanner has become so essential for oncology that over 80% of all PET scanners sold today are hybrid scanners.[1]

The National Institute of Biomedical Imaging and Bioengineering has issued a challenge topic titled "**05-EB-101 – Comparative Effectiveness of Advanced Imaging Procedures**" which stated in its description: "Evaluation of hybrid imaging such as combined PET-CT is particularly encouraged." This is definitely a step in the right direction. Given the prevalence of PET-CT scanners in oncology, future investigations should examine how hybrid imaging might be expanded into other areas of research.

Based on the documented failure of animal methods and the vast promise of non-animal methods, we offer a number of recommendations for the NIH which are summarized here in brief.

In order to support the invention, validation, and integration of non-animal methods, the NIH should:

- Fund greater numbers of mentorship and training grants which allow clinical and non-clinical investigators to advance their skills using non-animal methods;

- Track research projects and funding involving animals, have independent reviewers periodically evaluate the projects' effectiveness, and comply with the results of such reviews to better oversee reallocation and outcomes of resource dollars;

- Appoint individuals to grant review boards who are experienced with non-animal methods.

These goals are all in line with the objectives of the 1993 NIH Revitalization Act, which mandated that the NIH prepare a plan to "conduct or support research into – (A) methods of biomedical research and experimentation that do not require the use of animals….".

Above all, we recommend that the NIH, and all its Institutes and Centers, use the entirety of the Recovery Act funds to support researchers using non-animal methods to study human patient populations.

I. Introduction

In the National Institutes of Health (NIH) mission statement, the agency claims that one of its major goals is to "ensure a continued high return on the public investment in research."[2] However, in practice, the NIH has a long record of funding animal research that produces unreliable data and has little or no relevance to human health. As reported in a recent *New York Times* article, the NIH grant system has become "a sort of jobs program, a way to keep research laboratories going year after year with the understanding that the focus will be on small projects unlikely to take significant steps toward curing cancer" or achieving the other lofty goals that are the main objectives of the NIH.[3] The population of critics is much larger than a single reporter, and it includes NIH-funded scientists themselves. As Dr. Richard Klausner, former director of the National Cancer Institute, states: "There is no conversation that I have ever had about the grant system that doesn't have an incredible sense of consensus that it is not working. That is a terrible wasted opportunity for the scientists, patients, the nation and the world."[4] A particularly damning statement comes from the acting NIH director, Dr. Raynard S. Kingston, who says "[W]e also recognize that the system probably provides disincentives to funding really transformative research." [5]

The 2009 American Recovery and Reinvestment Act provides an invaluable opportunity for economic progress, and the NIH budget increase included in the Act provides a similar opportunity for medical progress. However, with all significant expenditures there is also the risk for massive waste – which is why oversight is so critical. It is the task of the NIH Institutes'

Advisory Councils to ensure that these funds are being distributed in a manner that creates not just jobs, but also the treatments and cures that have evaded us in the past. The current broken funding system has sustained outmoded animal methods for many years, often on the grounds that previous animal experiments serve as precedents - however inaccurate and irrelevant with regard to human health. The Recovery Act grants are an opportunity to create a new standard, to reform the present grant system and to support effective research in the process. With this in mind, we justifiably request that all funds in the NIH stimulus package be allocated to promising non-animal methods that have been historically underfunded and as a result, relatively unexplored.

We acknowledge that our organization, In Defense of Animals, is an international non-profit whose priority is to protect the welfare of non-human animals (hereafter 'animals'). However, as humans, we are also concerned with our own health, and as tax-payers, we are concerned about our investment in the National Institutes of Health. In this Report, we present an argument to decrease the use of animal methods within biomedical research and to fund non-animal methods that re-focus research on the human body, human medicine, and human health. The evidence for this argument comes from economic analyses, animal research literature reviews, and prototypes of emerging techniques that have the potential to make animal methods obsolete.

The argument for replacing animal models with non-animal methods such as human cell cultures, computer models, and human-based research is already well demonstrated.[6] [7] [8] The aim of the next three sections is to present three categories of non-animal research – *in silico*, *in vitro*, and human-based research – and offer instances of underfunded and overlooked techniques within each category. Each category will include a detailed evaluation of one particularly promising method that is exemplary in its potential to advance science and save lives, although there are many more such methods which we have not reviewed for this Report. It will also contain a selection of current animal methods, a description of their strengths and weaknesses, and a comparison of these methods to the non-animal method featured. The categories are structured so that they follow the flow of research itself: potential solutions are proposed *in silico*, tested extensively *in vitro*, and finally administered to humans in a clinical or experimental setting.

II. *In Silico* Research

In silico research is an emerging part of biomedical research that primarily uses information technologies to collect, organize and generate information about biological systems. Of course, much of this information is derived using standard research methods, but the simulation programs produced with this information can generate new scenarios, hypotheses, and predictions. One *in silico* project that aims to catalog the human physiome, the encyclopedia of human physiology, is the Virtual Human Project.

i. Virtual Human Project: Description

The first glimpse of what is now called "The Physiome Project" was given in a 1993 report from the Commission on Bioengineering in Physiology to the International Union of Physiological

Sciences (IUPS).[9] The IUPS has since developed a Physiome Commission responsible for coordinating the Human Physiome Project, which is supported by interdisciplinary research in labs all over the world. These researchers and their institutions are united in the pursuit of the human physiome, or "the quantitative description of the functioning organism in normal and pathophysiological states."[10] What would the compilation and organization of this massive data set look like? According to Jim Bassingthwaighte, past Chair of the IUPS Commission on Bioengineering in Physiology, "The human physiome can be regarded as the virtual human."[11]

The idea of actually visualizing the human physiome has grown into a number of collaborative efforts. On January 1, 2006, the European Commission launched a coordination action entitled STEP: a Strategy for The EuroPhysiome (Coordination Action #027642).[12] The objective of STEP was to unite the European contributions to the Physiome Project under a single banner, EuroPhysiome[13], and to establish a roadmap for achieving their ultimate goal, the Virtual Physiological Human (VPH). Past EuroPhysiome projects have been completed by organ or organ system, for instance, the GIOME Project models the gastrointestinal tract, the Cardiome Project models the heart, the Renal Physiome Project models the kidney, the Epitheliome Project models the epithelial physiome, and the Living Human Project models the musculoskeletal system.[14] Ultimately, all of these organ and organ system projects will be integrated into a model of the whole human organism – the Virtual Physiological Human. Although the STEP coordination action expired in 2007 after the publication of their roadmap to the VPH, the Virtual Physiological Human Network of Excellence (VPH NoE) was launched in 2008 as the new infrastructure for NPH collaboration.

In the U.S., a similar project called "The Virtual Human Project" is underway at Oak Ridge National Laboratory (ORNL). The project began around 1996 with an attempt to modify the National Library of Medicine's Visible Human Project for the purpose of guiding radiation therapy. The Visible Human Project, a 3-D anatomical model of male and female bodies based on MRI scans, CT scans, and post-mortem photographs, was an ambitious challenge in its own time and served as the conceptual ancestor of the Virtual Human Project. Unfortunately, the Virtual Human Project (VHP) in the U.S. has not received nearly as much government support as the EU has given to the Virtual Physiological Human. As a result, VHP research has progressed slowly and focused on much smaller goals. However, ORNL's concentration on their Virtual Human Respiratory System (VHRS) and Virtual Human Thorax (VHT) could be complementary to the efforts of the EuroPhysiome projects.[15] In an interview for the ORNL Review in 2000, one of the VHP principal investigators described the project as "an idea whose time has come."[16] If its time was the turn of the millennium, then the Virtual Human Project is long past due.

Over one decade after the birth of the Physiome Project and the Virtual Human Project, the real work is just beginning. It is clear the Physiome Project is a truly awesome task, as its subject spans a 10^9 spatial range (nanometers to meters) and a 10^{15} temporal range (microseconds to a human lifespan).[17] To account for concurrent feedback loops between genes and cells and organs, all operating on vastly different scales, researchers are encouraged to take a "middle-out" approach, in which top-down and bottom-up effects occur simultaneously, with no "privileged" level of causation.[18] In order to describe and organize this massively complex data set, researchers have understandably started with simplification and standardization. Much recent work has focused on hierarchy and modularity in multi-scale systems,[19] [20] [21] however, other notable efforts have been made to organize researchers[22] and to standardize modeling tools using shared markup languages.[23]

ii. Virtual Human Project: Comparisons

Drawing a parallel to the relationship between structure and function, some researchers have identified the Visible Human Project and the Human Genome Project as the foundations of a much larger quest: the Human Physiome Project.[24] The Physiome Project integrates information from not only anatomy, physiology, and pathology, but also genomics, proteomics, interactomics, biochemistry, biophysics, fluid dynamics, and many more disciplines. The very nature of the undertaking encourages the generation and dissemination of information through computation, collaboration and open-source online distribution. Perhaps the most important factor for public health, however, is its focus on the physiology, normal or pathological, of *humans*. As Clay Easterly, one of the principal investigators of the Virtual Human Project, says, "It's an opportunity for biomedical researchers to put their results in the context of the human body."[25]

This project should not only remind researchers of the ultimate objective of their work, but it should also steer them away from the inaccurate results that come from using animal models to predict human responses. When faced with an undeniable disparity between clinical and preclinical results, many researchers have spoken out about how and why animal models do not work.[26] A review of the relevant literature reveals three major explanations of why animal tests are not predictive of human results, one which blames methodological difficulties, one which blames environmental discrepancies, and one which blames physiological differences. Methodological inadequacies endemic to animal research that are rare in clinical research include a lack of randomization, a lack of blinding, a lack of standardization, high publication bias, small sample sizes, and therefore, poor statistical power.[27] [28] [29] Experimental environments are unable to mimic the complexity of human environments, and immeasurable inconsistencies between laboratory environments can create different results from identical strains within the same procedure.[30] [31] [32]

Perhaps the most damning argument for why animal research is a poor predictor of human responses is the argument from physiology, which one cannot change in the same way that one could refine one's methodology or environment. This argument is concisely summarized by David Hackam, a clinical pharmacologist, when he says, "animal models may not adequately mimic human pathophysiology."[33] He goes on to explain: "Test animals are often young, rarely have comorbidities, and are not exposed to the range of competing (and interacting) interventions that humans often receive. The timing, route, and formulation of the intervention may also introduce problems."[34] A more fundamental criticism is that differences arise between human and animal results due to "disparate animal species and strains, with a variety of metabolic pathways and drug metabolites, leading to variation in efficacy and toxicity," "different models for inducing illness or injury with varying similarity to the human condition," and "selection of a variety of outcome measures, which may be disease surrogates or precursors and which are of uncertain relevance to the human clinical condition."[35] The best evidence for this claim is primarily selected from systematic reviews, which compare clinical results to preclinical results for any given treatment.

In recent years, there has been a significant increase in the number of systematic reviews undertaken to examine the validity of animal models. In 2002, a call for evidence-based research was issued in response to a decision by the House of Lords that animal research was necessary for medical advancement.[36] The authors clearly stated the importance of systematic reviews in evidence-based research:

> Hardly any systematic reviews, meta-analyses, or retrospective, historical evaluations either support or refute the practice of using animals as models of human disease. The Lords' assertion of the value of animal experimentation rests on the increase in effective human treatments that have arisen at the same time as the expansion of animal experimentation. This correlation does not mean that animals were necessary for the development of these treatments.[37]

Since then, systematic reviews of several treatments have arisen, each explicitly examining the predictive utility of animal models based on their similarity or dissimilarity to human responses. A systematic review of six interventions with well-known effects in humans found that animal data predicted the effects in three cases and did not in three cases, noting in each summary of the animal studies that "the quality of the experiments was poor." [38] Another systematic review of nimodipine, an alleged neuroprotective agent which failed to produce a beneficial effect in clinical trials, found that 50% of the studies showed a beneficial effect in animals, and also noted that "the methodological quality of the studies was poor."[39] A review of all 1,026 neuroprotective treatments tested in humans or animals found that 62-74% of the 1,009 tested in preclinical trials were successful (depending upon the test method) and not one of the 114 treatments tested in clinical trials was successful.[40] This long line of failures led some to question "whether these animal models are financially and ethically viable."[41] Systematic reviews of animal models are available in a growing number of research areas, including traumatic coagulopathy[42] and fluid resuscitation for uncontrolled hemorrhage.[43] In a 2004 summary of all six systematic reviews comparing preclinical and clinical results in the research literature, three of the treatments reviewed went on to fail in clinical trials, even though preclinical trials demonstrated potentially harmful effects of each, and another two treatments failed in clinical trials which were run simultaneously with preclinical trials rather than sequentially.[44] This strongly indicates that animal results were perceived as irrelevant to human responses and were ignored. The final systematic review examined primate models of the interaction between stress, social status, and coronary heart disease, which the initial reviewers determined were skewed by publication bias and were contradicted by an established corpus of epidemiological studies.[45]

A systematic review of the most cited articles in the seven leading scientific journals testing interventions (preventive or therapeutic) in animal research found that their results were consistent with human trials in 37% of the cases, contradicted by human trials in 18% of the cases, and untested in the other 45% of cases.[46] This is a surprising statistic because these studies are considered the most promising in their respective areas. Systematic examinations of specific examples like these, in addition to methodological and environmental limitations, point to the compelling conclusion that there are "serious difficulties for those seeking to show that the value of animal models is supported by quantitative evidence rather than anecdote."[47] Indeed, many scientists, including P.J. Hunter, the current Chair of the IUPS Physiome Commission, express their hope that progress in the Physiome Project "will result in a more rational basis for medical diagnostics and drug discovery."[48]

The reduction and replacement of animal research is explicitly listed among the aims of both the Virtual Human Project and the Virtual Physiological Human. The description of the Virtual Human Project at ORNL clearly states that "[u]se of the Virtual Human will minimize the need for human subjects being involved in testing and also reduce the need for animal studies."[49] EuroPhysiome's public statement is a bit more cautious, using the word 'could' instead or 'will':

> The VPH hopes that in the future personalized care solutions with new modelling environments for predictive, individualized healthcare will create better patient safetly and efficacy. The use of *in silico* (by computer simulation) modelling and testing of drugs could reduce the need for experiments on animals. There will be a more holistic approach to medicine with the body treated as a single multi organ system rather than as a collection of individual organs.[50]

This last point is an important response to one common defense of animal models – that a cell culture or a computer model is no replacement for a *whole organism*. For the moment, we can disregard the fact that the *wholeness* (i.e. net result of many interactions of many independent variables) of non-human animals is precisely the reason they are not suitable as models of human anatomy, physiology, or pathology. In this case, it is only necessary to recognize that the many interactions within a whole organism can be modeled *and* the whole organism modeled can be a human organism.

One example of the physiome's potential is Monica Spiteri's research on pulmonary fibrosis. There is currently no treatment that has any effect on the clinical progression of pulmonary fibrosis, which kills approximately two-thirds of patients within 2 to 4 years of diagnosis.[51] Stem cell therapy could be the key, however, the extracellular fibrotic factors associated with the damaged tissue may interfere with the differentiation of the grafted stem cells. In order to test this possibility, Spiteri turned to computational modeling: "As there are no animal models that fully capture PF-disease processes, information obtained from well-designed mathematical models could be critical for development of strategies that would beneficially enhance stem cell engraftment in *in vivo* implants."[52]

In principle, once the model reaches a sufficient level of complexity, scientists can simulate almost any test or treatment on the Virtual Human that they could apply to a real human. In fact, the Virtual Human could be used to simulate experiments and treatments that might not be ethical or cost-effective to perform on real humans. There are dozens of different projects within the 2006 (FP6) and 2007 (FP7) VPH Initiatives, and they are all united by a common objective:

> patient-specific computer models for personalized and predictive healthcare and ICT – based tools for modeling and simulation of human physiology. Collaborative projects within this call will meet specific research objectives related to computational modeling of human physiology. VPH-I projects range from those developing advanced computational models of the heart, predictive models for dementia, osteoporosis and drug safety, through to projects furthering a common health information infrastructure to facilitate easier access to European supercomputing power.[53]

Given what we now know from the Human Genome Project, namely, that slight genetic differences can cause major functional discrepancies, it appears that personalized medicine is the next necessary step in making our tests as predictive as possible. Many scientists believe that this next step is the next "major revolution in biology - one in which mathematical modeling of proteins, cells, tissues, organs and organ systems allow the linking of genomic and proteomic information to integrated whole-organism behaviour, and the quantitative understanding of human pathologies in terms of the altered model parameters of normal physiology."[54] When asked how long this kind of revolution might take, Dr. Kohl, Oxford University physiologist and principal investigator on the VPH initiative, replied: "50 years - no 10 years - maybe even 5 years. I am getting ahead of myself, but I am very optimistic."[55]

iii. Virtual Human Project: Leaders

The IUPS Physiome Project is supported by a few labs within the U.S., most notably the National Simulation Resource (NSR) in the Department of Bioengineering at the University of Washington. The NSR, managed by Jim Bassingthwaighte, past Chair of the IUPS Commission on Bioengineering in Physiology, conducts summer courses on physiological modeling, shares models and software through its wiki, and encourages the submission of new physiome projects to their database. Another University of Washington project with exemplary collaboration is Respiratory Tract 3D, which involves eight institutions across the nation including the University of Iowa, the University of Utah, UC Davis, and the Hamner Institutes for Health Sciences. Additionally, Washington University has at least three different labs devoted to physiological modeling: the Sauro Systems Biology lab, the National Simulation Resource for Circulatory Transport, and the Cardiac Bioelectricity Research and Training Center.

There are a number of U.S. labs and centers that are currently making small- or medium-scale physiological models, including Caltech's Biological Network Modeling Center, UC San Diego's Cardiac Mechanics Research Group and San Diego Supercomputer Center, University of Connecticut's Center for Cell Analysis and Modeling, University of Washington's National Simulation Resource for Circulatory Transport, and Case Western's Database and Bioinformatics Lab. There are also large-scale physiome projects and databases at The Center for Cardiovascular Bioinformatics and Modeling at Johns Hopkins University, The National Center for Cell Analysis and Modeling at the University of Connecticut Health Center, The NIH Center for Bioelectric Field Modeling, Simulation and Visualization at the University of Utah, and The Hamner Institutes for Health Sciences in Research Triangle Park, NC. On a federal level, the Interagency Modeling and Analysis Group (IMAG), an interagency coalition born out of the National Institute of Biomedical Imaging and Bioengineering, claims that it now "represents 17 NIH components, four NSF directorates, two Department of Energy (DOE) components, five Department of Defense (DOD) components, the National Aeronautics and Space Administration (NASA), the United States Department of Agriculture (USDA), and the United States Department of Veterans Administration(USDVA)."[56]

The Virtual Human Project needs this kind of collaboration if it is to survive. In the past, the Virtual Human Project investigators at ORNL have collaborated with James Bassingthwaighte and the National Simulation Resource at the University of Washington. They have also collaborated with the Biomedical Engineering Department at Vanderbilt University, the Department of Resuscitative Medicine at the Walter Reed Army Institute for Research, the Innovative Computing Laboratory at the University of Tennessee – Knoxville, and the Center for

Scientific Computing and Imaging and the Center for Bioelectric Field Modeling, Simulation and Visualization at the University of Utah. Given its enormous size and complexity, the completion of the Virtual Human Project will require a collaborative effort at least as large as that of the EuroPhysiome, and perhaps even in tandem with the EuroPhysiome. Although cooperation between investigators and institutions is crucial, any funding that goes to physiological modeling will add to the compilation of physiome data and eventually aid in the completion of the Physiome Project.

III. *In Vitro* Research

In vitro research is the study of molecules, cells, tissues, or organs - usually those that have been isolated from a larger organism. *In vitro* research is the most well-known source of alternatives to animal methods, primarily due to the recent EU ban on seven cosmetic toxicology tests involving animals. The rising use of human cell and tissue cultures signals a significant shift towards more predictive procedures, which should eventually reach its full potential in the field of personalized medicine and tools like personal cell culture analogs.

i. Personal Cell Culture Analogs: Description

The major criticism of *in vitro* models, especially in toxicology but elsewhere as well, is that they are oversimplifications. Cells in culture act differently from cells in an organism, where they are surrounded by and connected to other cells, tissues, and organs. Even drugs *in vitro* are often different from their counterparts in an organism, where they are administered, transported, circulated, converted and excreted. In fact, these pharmacokinetic changes probably have profound effects on the results of *in vitro* toxicity tests – by one estimate, about 40 percent of drugs become toxic after metabolic conversion within the body.[57]

That's where the cell culture analog comes in. A cell culture analog, also known as an animal-on-a-chip or a human-on-a-chip, is a silicon wafer with reservoirs that represent organs and canals that act as vasculature. Once the reservoirs and canals are lined with cells of the appropriate type, then fluid is pumped through the system so that it flows through the organs in the proper physiological sequence. In 2004, Shuler and his associates at Cornell University published a proof-concept study in which they tested the systemic toxicity of naphthalene, a toxicant that is known to cause lung damage. Naphthalene does not directly have a negative effect on lung tissue, rather, it is broken down by the liver into two reactive metabolites, 1,2-naphthalenediol and 1,2-naphthoquinone, which damage lung tissue by depleting glutathione. Because Shuler's cell culture analog included a 'liver compartment' which metabolized naphthalene, it succeeded in demonstrating naphthalene's toxicity where traditional cell cultures have failed.[58] Additionally, the study also showed that the majority of the toxicants were sequestered in fat cells, presumably, until they could be slowly excreted.

The potential for cell culture analogs doesn't end there. The next step beyond the standard cell culture analog is a personalized cell culture analog, also known as "You-on-a-chip."[59] Personalized cell culture analogs (PCCAs) would essentially be miniature replicas of a person's unique physiology. PCCAs could precisely model the effect of a drug or other treatment on one individual, as all the cells that compose a PCCA would be supplied by that individual. To date, PCCAs do not exist; however, Shuler and others are exploring the idea. One proposed application would be pretesting chemotherapy on a patient's healthy cells as well as their tumor biopsy, in order to determine which drugs would be most appropriate for that patient – ideally, they would attack the tumor while sparing the patient's healthy tissues. In fact, Shuler is already working on developing new drug combinations and treatment strategies for combating 'normal' and multidrug resistant tumors.[60] Possibilities for future research go far beyond toxicology, as PCCAs could be used to study "drug protein binding under flow conditions, drug-drug interactions, and interactions between different organs, as well as varying metabolic disposition of pharmaceutical compounds depending upon the underlying genetic makeup of the populations to which they are exposed."[61]

Personalized cell culture analogs would only require the synthesis of three technologies which already exist in various stages of research: the transformation of human skin cell samples into pluripotent stem cells, the differentiation of pluripotent stem cells into any and all relevant cell types, and the assimilation of these cells into miniature organs, which could then be assembled into an integrated cell culture analog. The first feat was accomplished by Yu and colleagues at the University of Wisconsin – Madison in the groundbreaking study described the *Science* article aptly named "Induced Pluripotent Stem Cell Lines Derived from Human Somatic Cells."[62] The second obstacle, controlling the differentiation of pluripotent stem cells, is really an ongoing series of obstacles. However, there is hope for continuing collaboration between stem cell researchers and cell culture analog researchers: at least two separate parties have differentiated muscle cells on microfluidic chips similar to those used by Shuler *et al.* in their toxicity experiments.[63] [64] The third challenge, assimilating cells of various types into cell culture analogs, is exactly what Shuler and others are working on now.

Although we never know what effect a treatment will have on humans until we test it on humans, human cell culture analogs are the next best thing. They are the best representation of the effect of a drug on a whole human organism, and they are a critical link between *in vitro* and human research. As *in vitro* and human research shift closer together, through the use of more realistic cell cultures and less risky human testing like microdosing, animal research will increasingly become obsolete.

ii. Personal Cell Culture Analogs: Comparisons

Within the scientific community, acknowledgement of the limitations of animal methods and research into alternative techniques has emerged primarily from toxicology and pharmacology. One major reason for this phenomenon is that drug development is aligned with major commercial interests. As such, there is a diminishing tolerance for the expensive, slow, and inaccurate science that characterizes animal research. Even those in favor of continuing the use of animal models admit publicly that "[s]pecies differences frequently lead to large errors in such predictions."[65] Many scientists are acutely aware of these difficulties, stating:

> While *in vivo* animal testing can replicate some of the complex inter-cellular and inter-tissue effects [in humans], animal studies are expensive, labor-intensive, and time consuming; they also bear the ethical burden of requiring the sacrifice of large numbers of living creatures in the course of their effectuation. One of the most significant drawbacks of *in vivo* animal testing, due to the pharmacokinetic limitations inherent in the allometric

scale-up and extrapolation of assay results from one species to another, is that animal studies are frequently of extremely limited predictive correlation when evaluating human risk.[66]

Toxicity data from animal models was never meant to be directly extrapolated to human populations, but that is often the case. Toxicologists Afshari, Nuwaysir, and Barrett explain this unfortunate paradox:

> Traditionally, toxicologists have used rodent bioassays to identify potentially hazardous substances, including carcinogens, reproductive toxins, immunotoxins, and neurotoxins. These assays require high doses, often take years to complete, and are expensive. It was originally intended that such assays would be the first step in carcinogen hazard identification and that further studies on mechanisms of action, species extrapolation, and effects at low doses would be subsequently performed to determine the risk of chemicals to humans. Unfortunately, because the task of performing all of these subsequent studies is large, in most cases, the information gained from rodent bioassays is used to regulate chemicals to which humans are exposed.[67]

This is unfortunate because of the high risk that these results will make an incorrect prediction about the toxicity of a substance in human populations. In particular, rodent carcinogenicity assays have created a number of critics, including Bruce Ames, inventor of the famous Ames Mutagenicity Test and Director of the National Institute of Environmental Health Sciences Center at U.C. Berkeley. As of July 1997, approximately half of all chemicals tested in the rodent carcinogenicity assay were determined to be carcinogens in rats.[68] Ames explained this misleading phenomenon as a result of the very high doses typically used in these tests - an idiosyncrasy of the method itself:

> In standard cancer tests rodents are given chronic, near-toxic doses, the maximum tolerated dose (MTD). Evidence is accumulating that cell division caused by the high dose itself, rather than the chemical per se, is increasing the positivity rate. High doses can cause chronic wounding of tissues, cell death, and consequent chronic cell division of neighboring cells, which is a risk factor for cancer (50). Each time a cell divides the probability increases that a mutation will occur, thereby increasing the risk for cancer. At the low levels to which humans are usually exposed, such increased cell division does not occur.[69]

On top of its inaccuracy, which alone is unacceptable, "(t)he rodent cancer bioassay is a particularly expensive and time-consuming assay, as it requires almost 4 yr (sic), 1200 animals, and millions of dollars to execute and analyze."[70] All of this time, money, and life is wasted on a safety procedure that does not make us any safer. Liver damage is the most commonly reported reason for recalling a drug from the market, and about 50% of drugs that caused liver damage in clinical trials did not cause liver damage in preclinical animal experiments.[71] The explanation is simple: as Hμrel Corporation chief executive Robert Freedman says, "a rat liver is not a human liver."[72]

In contrast, a toxicity test in a cell culture analog takes only hours to perform, has a projected cost of $50 per chip, and requires no live animals, which can cost hundreds or thousands of dollars apiece to feed and house.[73] Thus, this technology could save time and money in drug development, on top of the thousands of lives lost to adverse drug effects every year. "We're talking about running a test in one or two days that would take months with animals," says Schuler.[74] Although Shuler himself is hesitant to call the cell culture analog a replacement for animal tests, he does recognize its potential for reduction, saying, "one can find the compounds and conditions most likely to give interesting results in animals…we look at this as an animal-sparing technology."[75]

Other scientists are bolder in their commendation of cell cultures and condemnation of animal testing. As Kelly BéruBé, a Cardiff University cell biologist, says, "These models have the ability to be far more accurate. I sometimes think it is just tradition — that feeling that if it's safe in an animal it's safe in a human — which means so many animal tests are still carried out…By recreating tissue environments, we will improve understanding of many aspects of cell behaviour including wound healing and responses to therapeutic drugs without the use of animal models."[76] For the majority of interested researchers, however, the first priority is to refocus medical research on studying and serving human physiology. "It may be ethically more palatable than using lab animals, and most importantly, you could use real human cells to run these tests," says Shuichi Takayama of the University of Michigan Biomedical Engineering Department.[77] Alfred Tonelli, the vice-president of Preclinical Development at Johnson and Johnson echoes this sentiment, stating the cell culture analog "actually could replace early animal screening studies for pharmacokinetics" and even become "a model that's better than animals, more human-relevant."[78]

iii. Personal Cell Culture Analogs: Leaders

The two pioneers in this field are collaborators Michael Shuler, Chair of the Biomedical Engineering Department at Cornell University, and Gregory Baxter, previous Senior Scientist at the Cornell Nanofabrication Facility and associate-turned-adjunct professor in the School of Chemical Engineering at Cornell University. The two met in 1997, the year Baxter joined the Cornell faculty and suggested that Shuler miniaturize his bench-top systemic toxicity tests.[79] Baxter has since left Cornell to work full-time as the Chief Scientific Officer at the Hµrel Corporation, which he helped found. Shuler's work at Cornell continues – conspicuously without current NIH funding – and his current cell culture analog projects include one with three-dimensional structure, one that screens environmental chemicals for endocrine disruption, one that screens drug combinations for colon cancer chemotherapy, one that screens drug combinations for multidrug resistant tumors, and one that models the gastrointestinal tract.[80] A recently published paper described how physiologically realistic this GI tract model is – in terms of drug absorption - and why it is such a major advance: it allows for the testing of orally administered medications.[81]

Other researchers have begun working on cell culture analogs as well. Shuler is collaborating with USDA physiologist Raymond P. Glahn, on the aforementioned gastrointestinal tract project.[82] Researchers Linda G. Griffith at MIT and Martin L. Yarmush at Harvard-MIT are attempting to make the *in vitro* liver more structurally realistic. Griffith has constructed a perforated silicon chip in which cells assembled themselves around hollow spaces in a physiologically accurate way, and Yarmush has created a technique to arrange alternating cell types in a striated fashion.[83] Shuichi Takayama and one of his students at the University of Michigan, Wei Gu, have built a cell culture analog out of a Braille display device that uses pins to pump fluids through the entire system. In a 2004 study, Gu *et al.* pumped serum to cultured cells (C2C12 myoblasts) at varying flow rates and saw varying differentiation patterns in the cell populations. Their system is remarkable not only because it is a cheap and readily available solution to a complex engineering problem, but also because it will allow for controlled changes in local flow rates, which could mimic a number of physiological phenomena.[84]

A major force in the research and development behind cell culture analogs has been the pharmaceutical industry, which annually spends hundreds of millions of dollars testing tens of thousands of drugs in the hopes that one of them will demonstrate the desired effect without too many side effects. In 2004, Pfizer's Head of Safety Services expressed a great deal of interest in cell culture analogs: "Everyone in the industry hopes to have surrogates for animals and humans when it comes to testing compounds…This is the sort of technology we'd want in our toolbox."[85] Some, like Johnson and Johnson's Head of Mechanistic Toxicology in Preclinical Development, have gone even further, saying "The holy grail of the industry is to be able to predict toxicity from a cell culture."[86]

Various companies are simultaneously solving different pieces of the wider puzzle. When Dawn Applegate's company, RegeneMed of San Diego, was founded in 2004, it received a crucial $8 million Small Business Innovation Research NIH grant. Since then, RegeneMed has announced the development of a 3-D liver model that can survive for longer periods of time in order to test the effects of acute and chronic toxicity..[87]

William Wang's Pharmacom Corporation is allegedly adding brain cells to their ADMET model, while Tissue Genesis is using cell culture analogs to develop their adipose tissue therapies.[88] GeSim and other European biotech companies contributed to a team of 27 researchers from 12 countries to work on *CellProm*: an EC Integrated Project studying the nanoscale reprogramming of individual cells.[89] [90]

The industry leader is Shuler and Baxter's company, Hμrel, which stands for 'Human-Relevant.' This name is better explained by a statement in which they claim that their cell culture analogs "produce unrivaled levels of physiologically relevant predictive sensitivity - 'human relevance'- that define the current state of the art of hepatic and multi-tissue cell-based assays." [91] There is good evidence to back up their optimism. In May 2009, Shuler and his Cornell University colleagues published a study in which they found that the therapeutic effects of Tegafur, an anti-cancer drug, only manifested after it was metabolized into 5'-fluorouracil (5-FU) by the liver.[92] In another paper published May 2009, Chao *et al.* at the Hurel Corporation validated their CCA by testing six model compounds (carbamazepine, caffeine, timolol, sildenafil, imipramine, and buspirone) on human hepatocytes and cross-checking the metabolic clearance data with the *in vivo* research literature.[93]

Recently, the Hμrel Corporation has been focusing on large-scale production of their 'biochips' and the other needs of its partners - pharmaceutical companies. Estimates of attrition rates indicate that 89% of drugs that enter clinical trials "fail to produce the desired results in humans, confirming that human disease mechanisms and the general metabolism of humans are quite different from those of animals."[94] Baxter estimates that a CCA screening before animal testing would identify 20% of the early compounds as toxic to non-human animals, saving about $50 million for every one drug that gets FDA approval.[95] If the screening eliminated another 20% of the drug candidates before human trials, it would save another $88 million – not insignificant considering that a drug company spends an average of $467 million in human trials for every one drug released on the market.[96] If Shuler and Baxter are right, the effect of cell culture analogs on personalized medicine could be equally enormous.

IV. Human Research

Human research is the study of human anatomy, physiology, and psychology through the study of humans. The oldest form of human research, clinical observation, has been central to medicine since at least the time of Hippocrates.[97] Human research can be roughly divided into biomedical and behavioral research, and it includes clinical research, autopsy, epidemiology, post-market drug surveillance, microdosing, personalized medicine, prevention/treatment program oversight, as well as sociological, psychological, and neurological research. Human research is critically important to any organization conducts scientific research for the sake of human health.

i. Hybrid Imaging: Description

There is no question that imaging methods have revolutionized modern medicine.[98] What structural magnetic resonance imaging (MRI) and computed tomography (CT) have done for the study of anatomy, functional magnetic resonance imaging (fMRI) and positron emission tomography (PET) have done for physiology and pharmacology. Pathology has moved beyond using CT scans to determine gross anatomical damage to using PET scans to predict Alzheimer's Disease susceptibility and severity based on changes in local glucose metabolism.[99] [100] [101] The use of PET scans to track tumors began in 1953 and is now a universal standard in oncology.[102] Imaging techniques are routinely used for basic research as well as diagnostics; in fact, they have been central to recent advances in neuroscience, psychology, and pharmacology, in addition to orthopedics, psychiatry, and internal medicine.

Although these imaging methods have come a long way and continue to be refined, they still have some significant limitations. Just as each imaging technique has its own unique strengths, each imaging technique also has its own idiosyncratic drawbacks. Luckily, the strengths of one technique can compensate for the weaknesses of another. Structural MRI provides general anatomical maps, while functional MRI captures local brain activity correlated with behavior in real time. Magnetoencephalography (MEG) and electroencephalography (EEG) can offer data on population potentials with high temporal resolution (milliseconds) but low spatial resolution, whereas MRI techniques can give radiotracer and local cerebral blood flow data with low temporal resolution, but high spatial resolution (millimeters).[103] [104] [105] A PET scan has a low spatial resolution and therefore cannot precisely identify anatomical regions, whereas a CT has a high spatial resolution, but cannot track metabolic reactions occurring prior to, or in spite of, gross anatomical changes.[106] So it should come as no surprise that researchers have decided to combine these complementary techniques.

Originally, researchers were forced to use scans from separate scanners, but recently, they have turned to simultaneous multimodal imaging using combined scanners. At this time, there are MEG/MRI scanners,[107] EEG/MRI scanners,[108] PET/MRI scanners,[109] PET/CT scanners,[110] and SPECT/CT scanners.[111] The original implementation of a joint scanner took place around 1990: it was a SPECT/CT system designed by Hasegawa *et al.*[112] That scanner was followed by a PET/CT scanner created by Townsend, Beyer, and their colleagues.[113] These innovations were initiated as a response to a common challenge in both radiotracer techniques - difficulty orienting the data with respect to organs and other anatomical features. As Townsend and Beyer's team stated in their hybrid scanner proposal: "In regions such as the thorax and abdomen, the demonstration of increased FDG uptake is limited in value without an unambiguous localization of tracer uptake to a specific structure, for example, a tumor seen on a corresponding CT image."[114] They also voiced their concerns regarding sequential scanning on separate scanners, saying, "Post hoc PET-CT alignment, however, is difficult due to shifting of the position of organs, which can occur without patient motion and may even depend upon the relative curvature of the patient beds of the separate scanners."[115] Today, over 80% of all PET scanners sold annually are joint PET/CT scanners.[116]

The greatest strength of joint scanners is their ability to co-localize, or synthesize, scans of different types, say, PET scans and CT scans. A PET/CT joint scanner can coordinate the two images while avoiding insensitive algorithms and experimenter errors, so that the data from each can be combined and compared. This is called co-localization. In the past, co-localization of images from separate scanners has been achieved through image registration processes. Image registration was originally performed manually, by matching anatomical or external markers, but has since been surpassed by automated registration programs.[117] However, the registration process is still a substantial source of error.[118]

Joint scanners can also be used to clarify other methodological inconsistencies or confounding variables. For example, a joint PET/CT scanner was used to determine the efficacy of a technique for reducing the uptake of FDG (the most common PET radiotracer) by Brown Adipose Tissue (BAT), which systematically limits PET studies by absorbing FDG that could otherwise be used to identify clinically-relevant structures like tumors. The experimental group adhered to a high-fat, low-carbohydrate loading period rather than the standard fasting period, and as a result, because fatty acid presence suppresses glucose metabolism, less FDG was taken up by BAT areas and more was available for target areas.[119] Given that nuclear medicine has revolutionized the disciplines of oncology, pharmacology, and internal medicine, to name only a few, it is an interesting fact that researchers are saying: "The development of dual-modality imaging systems has revolutionized the practice of nuclear medicine."[120]

ii. Hybrid Imaging: Comparisons

Imaging techniques have become so powerful that even animal researchers are adopting them.[121] Unfortunately, most of these researchers are co-opting imaging technology in order to study non-human animals, rather than using it to study the clinically relevant population it was intended for: humans. Nevertheless, their critiques of classic animal experiments in anatomy, physiology, and pharmacology are valid and worthy of note. A review of their admissions will also help reveal the limitations of animal models that persist across experimental techniques, from dissection studies to electrophysiology to non-invasive imaging.

One major discipline in which imaging techniques will replace wasteful animal use within academia and industry is pharmacology. In addition to toxicology studies, which were discussed in the previous section, pharmacologists use animals in binding and efficacy studies. Historically, binding studies require the postmortem dissection and visualization of an animal's target tissue, whereas efficacy studies often evaluate behavior and therefore require a live, behaving animal. Both binding and efficacy studies have their own unique problems which can be easily resolved using noninvasive imaging techniques.

Binding studies that require the dissection of an animal or their organs, including tissue staining, *in situ* hybridization, and immunohistochemistry, are primarily limited by temporal issues, as the animal must be deceased before it can produce any data. The anatomical information given in a discrete form by slices can be provided by scans in a more valuable continuous form over the entire time course of the treatment. The use of a live subject allows multiple scans to be compared at different time points within a single session and across sequential sessions. Animal researchers have promoted scans over slices for the same reason, saying, "a dedicated animal scanner permits the entire time course of a tracer to be measures in a single animal and provides the means for repeated studies of that same subject over an arbitrary period of time, instead of pooling and averaging the data from a larger animal population sample."[122] Ideally, scans are taken before, during, and after treatment. This allows the subject to serve as their own control, reducing the error due to between-subjects variation as well as reducing the number of subjects necessary.[123] [124] Additionally, multi-modal imaging further reduces the amount of subjects and scanner time required, with the use of paired statistics "significantly increasing the statistical power of the study."[125] In fact, the between-subjects data produced by dozens of animals, in control and experimental conditions, sacrificed at different intervals post-treatment can be effectively replaced by the within-subject data set of one subject.

Behavioral studies, including those used to evaluate the efficacy of a drug or other treatment, produce spatially imprecise data, due to fundamental limits of their observational techniques. Studies that involve only the behavioral observation of an animal provide no direct data on the internal interactions of a drug or treatment. Studies that utilize invasive recording techniques, like electrophysiology experiments or cannulation experiments, introduce other confounding variables including anesthesia administration, stereotaxic discrepancies, tissue damage, gliosis, and potentially infection. The insertion of an electrode or cannula can cause many kinds of damage to brain tissue, from local inflammation to superior ablation, which can result in profound physiological alterations in the area of interest and beyond. Researchers conducting push-pull perfusions, for instance, admit: "it is evident that the inflammatory reaction to the local injury induced by the cannula may have different consequences in the function of a particular brain area."[126] Non-invasiveness is a key property of imaging; in fact, some researchers have even claimed: "The principal goal of PET imaging is to characterize biological processes in tissues and organs with minimally invasive procedures."[127] Still other researchers disagree, defining PET first and foremost as a replacement for histological techniques: "PET is an in vivo analog to tissue dissection, autoradiography, and other techniques that involve either sectioning and imaging or counting excised tissue samples taken from animals into which a radioactively labeled probe has been introduced."[128] It is safe to say that it serves both purposes.

To their credit, many animal researchers have been quick to recognize imaging as a superior alternative to both dissection studies and invasive experiments. In a review of small animal imaging challenges, Balaban and Hampshire state: "The major advantage of noninvasive studies is the ability to conduct studies on living animals without significant consequence to the animal or its physiology."[129] They also criticize dissection studies, saying: "Noninvasive techniques are also advantageous compared with conventional gross pathology procedures, which require sectioning the animal and organs. This advantage is realized in cost, speed of acquiring data, and the nondestructive technology."[130] Beyond simply replacing inferior procedures, imaging techniques can offer results that previous procedures were incapable of producing. Researchers have recognized the role of imaging in replacement and revolutionary science, with some sorting current PET experiments into two categories: "those in which tracers serve as surrogates for invasive or histological measurements of biological processes (such as perfusion, metabolism, or reporter probe expression) and those in which tracers target specific aspects of a biological process (such as enzyme activity, receptor concentration, uptake transporter concentration, or protein synthesis)."[131]

The solution that most animal researchers suggest, small animal imaging, is simply another Pandora's Box of methodological problems. First of all, anesthesia is necessary in order to keep an animal motionless inside the scanner.[132] As many animal researchers have pointed out, a particular method of anesthesia may amplify, diminish, or negate the properties of a given substance, depending upon the mechanism. Or it may have no effect at all. In fact, animal researchers continually use this as a justification for denying analgesics and anesthetics to experimental animals undergoing invasive and potentially painful procedures.[133] Needless to say, this is a massive act of hypocrisy which betrays carelessness with regard to the fundamental incongruities in animal models. The administration of anesthesia also requires an attending animal technician at all times which, as researchers note, "could limit the practical use of these systems."[134] The alternative to anesthesia is active restraint, which is rarely chosen, as "the physiological effects or reproducibility of the physical and mental stress imposed on the animal is unclear, especially in cardiovascular studies."[135]

The usual problems with animal research also apply here: significant differences in anatomy, physiology, metabolism, and size (relevant to dosage and scaling procedures). Of course, the major benefit of using a human subject rather than an animal is the invaluable chance to study human conditions in human beings, rather than analogous approximations in other species. It hardly needs to be said, but if we want to study human anatomy and physiology, we need more than just a live subject, we need a human subject.

iii. Hybrid Imaging: Leaders

The labs currently using SPECT/CT and PET/CT scanners are too numerous to list here. Less popular technologies, like the PET/MRI and the MEG/MRI scanners, show a great deal of potential, but are not well-funded or widely used. Combined MEG/MRI scanners are slightly more prevalent than PET/MRI scanners. The first joint MEG/MRI machine was constructed by the Biological and Quantum Physics Group at Los Alamos National Laboratory.[136] The same group recently published the first results of MEG readings simultaneous with a low-field MRI scan, which operated at 46 microT.[137] Investment in MEG/MRI systems is also being made internationally. A team of researchers at the University of Helsinki are building a microtesla MEG/MRI scanner using novel magnetometers as part of a 4-year Seventh Framework Programme project funded by the European Union.[138] The project, which began on May 1st, 2008, is expected to take two years and cost 6.85 million euro.[139] If the predictions of the Los

Alamos group are correct, the low cost of microtesla magnets should lead to a higher demand for stand-alone and dual-modality MRI scanners.[140]

The largest PET/MRI scanner in the world, a 9.4-tesla magnet, is currently under construction at the Jülich Institute of Neurosciences and Biophysics.[141] To our knowledge, this may be the largest PET/MRI scanner by virtue of being the first human-sized PET/MRI scanner, as every previously published PET/MRI study we reviewed was conducted on either mice or rats.[142] [143] [144] [145] The first successful PET/MRI feasibility study was published in September of 2008 by a German team of researchers at the University Hospital Tübingen.[146] This study used a modified 3-T clinical scanner. In 2002, group of British researchers stated: "There is no reason in principle why the technology could not be scaled up to a human imaging system, although the problems associated with NMR compatibility (in particular the effect of the PET scanner materials on the MR) may become more serious."[147] Crucially, they note that the technical difficulties are not the greatest obstacle: "The main issue for the construction of human system at this stage is probably the motivation to undertake such a project."[148]

V. Recommendations

IDA makes the following recommendations to the NIH, the Advisory Council of each NIH Institute, and the President's Council of Advisors on Science and Technology, to consider for adoption in the future in order to better focus on human-centered medicine:

NIH should track funding for research projects that use animals, have independent reviewers periodically evaluate the projects' effectiveness, and comply with the results of such reviews

To the best of our knowledge, the NIH does not now, nor has it ever, kept records on the amount of funding that goes towards biomedical research and experimentation that uses animals. We believe this is a necessary first step to understanding how federal funding is appropriated for research projects. There can be no realistic evaluation of the cost-effective contribution of research using animals without tracking its funding, especially in light of the increasing number of peer-reviewed scientific publications questioning the validity of animal models cited in this Report. Such independent reviews of the effectiveness of NIH-funded research using animals must be conducted on a regular basis, and if such research is found to be ineffective, the NIH must comply with the independent review findings and terminate the funding and/or area of research.

As well, if the NIH is to demonstrate compliance with the NIH Revitalization Act of 1993, [149] there must be some means whereby the NIH can demonstrate that it has conducted or supported non-animal methods of biomedical research and experimentation that reduce the number of animals used in such research, as stipulated by the Act.

NIH should support a greater number of mentorship and training grants which allow investigators to develop expertise in non-animal methods, as well as mentorship grants which allow clinical practitioners to develop skills to further clinical research.

The NIH Revitalization Act of 1993 also mandated that "scientists be trained in the use of such [non-animal] methods that have been found to be valid and reliable."

Examples of such grants include current NIH grant 5K01DA016746-03, [150] which is a Mentored Research Scientist Development Award (K01) that will offer training to the investigator in magnetic resonance imaging for use in human research of adolescent nicotine exposure, and NIH grant 5K23HL080231-03, [151] which funds a training program to develop a board certified pediatrician with a subspecialty in neonatology into an independent clinician-scientist specializing in investigations of pulmonary function testing in neonates and infants.

NIH should appoint individuals to its review boards who have expertise in the range of *in silico*, *in vitro* and human clinical non-animal methods discussed in Parts II, III and IV of this Report.

Without peer reviewers who are trained in and appreciative of the value of non-animal methods, NIH will continue down the same road of funding questionable animal experiments. As part of the peer review process, the feasibility of potential research projects is judged against precedents in the research literature, and because animal experimentation is the status quo, all projects are judged against a corpus largely composed of animal models. As a result, non-animal methods are marginalized by the sheer quantity of animal methods and the central place they occupy in biomedical research. In order to give non-animal models the equal consideration they deserve – especially in light of the advanced non-animal methods cited in this Report – each review board should include experts on *in silico*, *in vitro* and human-based research who have the necessary experience to judge what non-animal research is realistic and what is not.

VI. Conclusion

In the spirit of the Challenge Grants, and in light of the evidence presented here, we strongly urge you to exclusively fund non-animal methods, especially those which are currently underfunded and those which have the greatest potential impact. As an animal protection organization, In Defense of Animals is not fundamentally opposed to the objectives of the NIH. We are completely in favor of science that serves human health. However, based on decades of evidence from science and medicine, we do not believe that animal research does so. The Advisory Council has the power to end the tradition of favoring inaccurate animal research that has consistently failed to advance human medicine. Indeed, your decisions could change the course of our country's future health care, advancing it enormously. Please remember that it is the responsibility of the NIH to invest in promising technologies that will guide the future of science and medicine, even if the potential of these technologies is still unknown.

In this Report, we have cited the NIH Revitalization Act of 1993, which mandated that the NIH prepare a plan to "conduct or support research into – (A) methods of biomedical research and experimentation that do not require the use of animals…." In 1994, the NIH concluded its response to Congress mandated by the Act with the following statement, a full-throated defense of the status quo – the use of animals in research:

Our second recommendation is as much to our colleagues -- scientists, physicians, administrators -- as to the agencies of government. It is that we join in responding with the truth about animals in research to the misinformation and disinformation that has been so widely distributed and has been given currency in the media. We hold the truth to be that:

- *Research mammals are indispensable for the progress of human and veterinary medicine and the maintenance of human and animal health. Although the numbers of such animals needed in the near term for research may not rise, neither are they likely to fall significantly.*
- *The advance of modeling and model systems enhances science; it will not substitute for animal research and testing.* [152]

Less than ten years later, despite the technological explosion that had occurred since 1993, Congress once again felt it necessary to step in and advance science against the will of the apparently hidebound NIH. Dr. Ruth Kirschstein made a timely statement when she, as NIH Director, guided the creation of the National Institute of Biomedical Imaging and Bioengineering (NIBIB) - the result of a Congressional mandate initially opposed by the NIH. She stated: "While dedicating an institute to medical technologies… may seem novel for the NIH, it is truly a reflection of what science is today – and where science will be taking us tomorrow."[153] The Recovery Act should indeed move America towards recovery, away from the mistakes of the past and towards the science of the 21st century and beyond.

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