ALTEX, accepted manuscript
published August 7, 2024
doi:10.14573/altex.2311141

Opinion Article

Potential Value of Animal Microphysiological Systems

Paul C. Brown[1], Barry H. Hooberman[2], Brianna L. Skinner[3], Claudia Wrzesinski[4], Dayton M. Petibone[5], Kevin A. Ford[1], Kristi L. Muldoon-Jacobs[6], Kyung E. Sung[4], Luis G. Valerio Jr.[7], Nakissa N. Sadrieh[1], Paul C. Howard[8], Peter L. Goering[9], Shelby A. Skoog[9], Suzanne C. Fitzpatrick[6], Tracy Chen[3], Tracy C. MacGill[3], Donna L. Mendrick[10]

[1]U.S. Food and Drug Administration, Center for Drug Evaluation and Research, Silver Spring, MD, USA; [2]U.S. Food and Drug Administration, Center for Veterinary Medicine, Rockville, MD, USA; [3]U.S. Food and Drug Administration, Office of the Chief Scientist, Silver Spring, MD, USA; [4]U.S. Food and Drug Administration, Center for Biologics Evaluation and Research, Silver Spring, MD, USA; [5]U.S. Food and Drug Administration, National Center for Toxicological Research, Jefferson, AR, USA; [6]U.S. Food and Drug Administration, Center for Food Safety and Applied Nutrition, Office of the Center Director, College Park, MD, USA; [7]U.S. Food and Drug Administration, Center for Tobacco Products, Office of Science, Division of Nonclinical Science, Beltsville, MD, USA; [8]U.S. Food and Drug Administration, Office of Regulatory Affairs, Rockville, MD, USA; [9]U.S. Food and Drug Administration, Center for Devices and Radiological Health, Silver Spring, MD, USA; [10]U.S. Food and Drug Administration, National Center for Toxicological Research, Silver Spring, MD, USA

All authors are members of FDA's Alternative Methods Working Group

Abstract

Microphysiological systems (MPS) are designed to recapitulate aspects of tissue/organ physiology *in vivo*, thereby providing potential value in safety and efficacy assessments of FDA-regulated products and regulatory decision-making. While there have been significant advances in the development, use, and proposals of qualification criteria for human organ MPS, there remains a gap in the development using animal tissues. Animal MPS may be of value in many areas including the study of zoonotic diseases, assessment of the safety and efficacy of animal therapeutics, and possibly reduction of the use of animals in regulatory submissions for animal therapeutics. In addition, the development of MPS from various animal species enables comparison to animal *in vivo* data. This comparison, while not always critical for all contexts of use, could help gain confidence in the use and application of human MPS data for regulatory decision-making and for the potential identification of species-specific effects. The use of animal MPS is consistent with the replacement, reduction, and refinement (3Rs) principles of animal use by identifying toxic compounds before conducting *in vivo* studies and identifying the appropriate species for testing.

Plain language summary

Microphysiological systems (MPS) mimic aspects of organs in humans or animals. These systems may provide information useful for FDA-regulated products. While there have been significant advances in the development of MPS made from human cells, there remains a gap in the development of MPS using animal cells. FDA believes animal MPS may be of value in many areas including the study of diseases transmitted from animals to humans, assessment of the safety and efficacy of animal drugs, and reduction of the use of animals in regulatory submissions. The development of animal MPS enables comparison to data from studies conducted in animals. This comparison provides confidence in the use of human MPS data for regulatory decision-making. The use of animal MPS is consistent with the 3Rs principles of animal use by allowing identification of toxic compounds before conducting animal studies and by helping select the appropriate species for further testing.

1 Introduction

This article describes the potential considerations for animal MPS from a regulatory viewpoint. It is expected that development of animal MPS will facilitate the routine incorporation of diverse MPS platforms using human tissues into product development by

Received November 14, 2023; Accepted July 18, 2024;
Epub August 7, 2024; © The Authors, 2024.

ALTEX 41(#), ###-###. doi:10.14573/altex.2311141

Correspondence: Paul C. Brown, Ph.D.,
U.S. Food and Drug Administration,
Center for Drug Evaluation and Research,
10903 New Hampshire Ave.,
Silver Spring, MD, 20993, USA
(paul.brown@fda.hhs.gov)

providing improved tools to conduct mechanistic investigations into both safety and efficacy. This is because animal MPS may build confidence in human MPS and enable the use of MPS studies for products that support human and animal health. In addition, some scientific and regulatory issues involve animal health and disease, so animal MPS have direct utility in these cases. It is anticipated that the use of animal MPS may address the 3Rs in the shorter term by reducing the number of animals in studies and, in the longer term, possibly by replacing some animal studies altogether. In some cases, cells for animal MPS may be primary cells obtained from animals but in many cases the animal cells can be obtained from tissue banks and commercial companies, thus minimizing the need to euthanize additional animals.

2 A decade in promoting MPS

The U.S. Food and Drug Administration (FDA) has had a long-standing commitment to promote the development and use of new technologies to evaluate and predict the potential risks, safety, and efficacy of FDA-regulated products. With the goal of promoting new predictive tools and technologies to advance regulatory science, FDA Office of the Chief Scientist (OCS) created the Alternative Methods Working Group in 2020 (AMWG) as the catalyst for the development of methods that could potentially replace, reduce and/or refine animal testing[1]. The AMWG developed an FDA working definition for what should be included under the definition of MPS and organ-on-a-chip, the latter being a subset of MPS and shared this definition on the website: Advancing Alternatives Methods at FDA[1]. The AMWG defined MPS as *in vitro* platforms composed of two-dimensional (2-D) or three-dimensional (3-D) tissue constructs that range in complexity and include multiple cell types and extracellular matrix components to mimic the structure, function, biochemical, electrical and/or mechanical properties of organs or tissues. Organ-on-a-chip models were defined as a miniaturized physiological environment engineered to yield and/or analyze functional tissue units capable of modeling specified/targeted organ-level responses. As noted, FDA considers this a working definition and recognizes that additional definitions exist[2].

FDA's experience with MPS has spanned more than a decade. This includes early funding for a heart-lung micromachine (Huh et al., 2010). The heart-lung device marked a new milestone by combining two different organ systems within a single microsystem for the first time.

In 2011, FDA began a collaboration with the Defense Advanced Research Projects Agency (DARPA) and the NIH to collaborate on developing MPS for drug safety assessment[3]. FDA's role was to help determine how this new technology could potentially be used to assess drug safety, and to provide advice on concepts to consider during MPS development that might facilitate eventual regulatory use of data generated by MPS. NIH continued to fund these efforts through the Tissue Chip Development Program. The goal of this program was to identify human drug toxicities and predict potential drug efficacy in a human population prior to clinical testing. These efforts focused on the development, qualification, and integration of robust human MPS.

In 2013, the FDA Office of Counterterrorism and Emerging Threats (OCET) initiated the research project "Organs-On-Chips for Radiation Countermeasures" [4] with the Wyss Institute at Harvard University via FDA's Medical Countermeasures Regulatory Science Initiative (MCMi)[5] grant program. Under this contract, scientists developed models of radiation damage in their lung, gut, and bone marrow organs-on-chips and applied these models to test candidate medical countermeasures to treat such damage (Torisawa et al. 2014; Jalili-Firoozinezhad et al., 2018).

The overarching objective of this project was to provide a capability to evaluate candidate medical countermeasures for Acute Radiation Syndrome (ARS) within the specific context of a target human organ system. In partnership with FDA Office of Women's Health, this contract was extended and expanded in 2018 to create male and female human bone marrow chips and to analyze differences in sex-specific responses to ionizing radiation and a chemotherapeutic drug.

FDA has continued to work with both NCATS and the IQ MPS Affiliate of the Innovation and Quality Consortium of Pharmaceutical and Biotechnology Companies (IQ Consortium) on influencing MPS development. The primary focus has been on MPS use for drug discovery and development, and ultimately, MPS qualification. A joint IQ MPS-FDA workshop publication reported on a consensus opinion that it is necessary to generate animal MPS to engender confidence in the translation of such data and confidence in human MPS (Baran et al., 2022). A similar opinion was expressed at a Berlin MPS meeting in 2020 (Marx et al., 2020).

FDA regulatory product centers and the National Center for Toxicological Research (NCTR) are performing research in their intramural laboratories and through collaborations with external MPS developers. These efforts involve the use of human and, in some cases, animal MPS. These efforts have highlighted the potential utility of these platforms to fill information gaps with applied research, to advance new policy and guidance development, and to provide opportunities to familiarize FDA scientific staff

[1] https://www.fda.gov/science-research/about-science-research-fda/advancing-alternative-methods-fda. Accessed June 4, 2023
[2] https://ntp.niehs.nih.gov/whatwestudy/niceatm/test-method-evaluations/mps/index.html, Accessed June 24, 2024.
[3] https://www.nih.gov/news-events/news-releases/nih-darpa-fda-collaborate-develop-cutting-edge-technologies-predict-drug-safety Accessed June 4, 2024
[4] Organs-On-Chips for Radiation Countermeasures | FDA, accessed June 4, 2024
[5] Medical Countermeasures Initiative (MCMi) | FDA, accessed June 4, 2024

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published August 7, 2024
doi:10.14573/altex.2311141

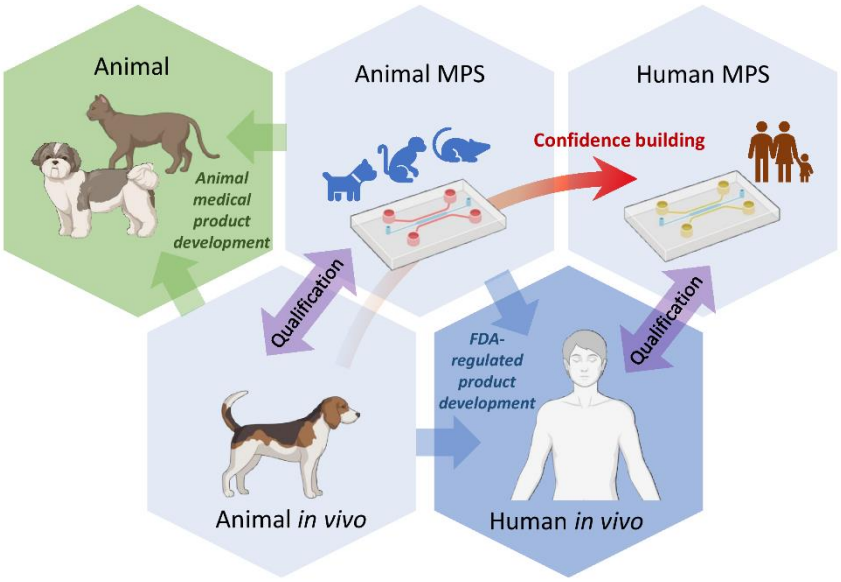

Fig. 1: Building confidence in MPS
Data generated from animal model microphysiological systems (MPS) can be compared with data from *in vivo* studies for specific treatment conditions and endpoints to evaluate and qualify the system. Once qualified, the animal MPS could be used to gain confidence in data generated using human MPS, and for conducting future studies that would have used conventional *in vivo* studies (3Rs). When used in combination, *in vivo* studies and MPS might be used to evaluate the toxicity and efficacy of new products for humans and animals.

with the technology prior to seeing MPS data in regulatory submissions. FDA's fiscal year 2023 budget included dedicated funding to support a new, FDA-wide New Alternative Methods (NAMs) Program to reduce animal testing through the development of qualified alternatives methods and spur the adoption of methods for regulatory use that can replace, reduce, and refine animal testing.

FDA developed the concept of "qualification[6]" as a conclusion that within the stated context of use, the results of an assessment using a model or assay can be relied upon to have a specific interpretation and application in product development and regulatory review. Part of the OCS charge to the AMWG was to outline the criteria/data needed to begin to qualify MPS data for use in regulatory assessments. Inextricable to qualification is the concept of "context of use" that is defined as a clearly articulated description delineating the manner and purpose of use for a particular approach. Criteria for validating new toxicology methods have been outlined in several documents from the Organisation for Economic Co-operation and Development (OECD)[7] and Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM)[8]. Many of the concepts outlined in these documents (e.g., biological relevance, technical characterization, data integrity, information transparency) are applicable to the qualification of a method for regulatory use, including MPS; however, specific guidelines addressing MPS are not currently available. One of the objectives of the FDA NAMs Program is to explore the possible development of such guidance.

3 Why are animal MPS needed and where may they be used?

Development of animal MPS models may be of value to many different FDA programs. Industry stakeholders and regulatory bodies have expressed the need to gain confidence in human MPS before they can be used to support regulatory decisions in lieu of other more traditional data sources such as in vivo animal studies. The study of animal MPS in concert with *in vivo* data from the same species may allow one to understand the ability of MPS to predict *in vivo* outcomes and thus gain confidence in the use of human MPS for predicting human *in vivo* outcomes (see Figure 1 and Steger-Hartmann and Raschke, 2020). These assessments may also support the use of animal MPS as surrogates for certain animal studies. Since human MPS data may potentially be submitted for product review in most FDA centers, there is a general need to increase understanding and utility of MPS across the Agency. In those cases in which a new method such as an MPS is to be used in lieu of a more traditional method, the Agency must be confident that the new approach is at least as good as the traditional method at providing information for assessing human and/or animal safety.

Historically, investigators assumed that results from animal data were translatable to humans. This assumption has been questioned and reproducibility issues in animal studies have been described (Weinhart et al, 2019; Berridge, 2021; Pound and Ritskes-Hoitinga, 2018). At this time, nonclinical safety testing still largely depends on animal studies; however, it is currently

[6] https://www.fda.gov/drugs/development-approval-process-drugs/drug-development-tool-ddt-qualification-programs. Accessed June 4, 2024
[7] OECD Guidance 34: OECD series on testing and assessment Number 34 guidance document on the validation and international acceptance of new or updated test methods for hazard assessment http://www.oecd.org/officialdocuments/displaydocument/?cote=ENV/JM/MONO(2005)14&doclanguage=en. Accessed June 4, 2024
[8] https://ntp.niehs.nih.gov/sites/default/files/2024-03/VWG_Report_27Feb2024_FD_508.pdf. Accessed June 4, 2024

being debated whether the scientific validity of new alternative methods (e.g., MPS) used for human safety assessment should be judged against these animal studies (Sewell et al., 2024). It may be difficult to replace general toxicology studies with MPS since a hallmark of these animal studies is the plethora of endpoints assessed such as the effects in a minimum of 40 organs and tissues (Bregman et al., 2003), which can be evaluated postmortem, including histology, from a single animal. For other nonclinical studies with more focused or fewer endpoints, MPS may be better able to address the relevant safety issues or provide insight into mechanistic questions. MPS may also provide information about the intended pharmacology of a drug and its potential for in vivo efficacy. MPS designed as disease models may, in some cases, be better able to represent human disease than other existing in vitro or animal models (Irrechukwu et al, 2023). For some endpoints, human data may be available from reference chemicals to evaluate the performance of the MPS in predicting *in vivo* outcomes. However, such human data are often difficult to obtain in sufficient quality and quantity to enable this evaluation of MPS performance. Generally, even in the presence of adverse events in humans, tissue biopsies that document the type of injury are rarely pursued, and the ability to see dose- and time-dependent toxicity (especially chronic effects) is limited in humans. For many compounds, data from *in vivo* animal studies may be more readily available or may be the only data available from compounds that were never administered to humans. These animal data can be leveraged together with available human data to evaluate the performance of MPS.

Like humans, animals are susceptible to infectious and non-infectious diseases. These diseases can be detrimental with outcomes that (1) pose a threat to human and animal health (e.g., zoonotic diseases and spillover events); (2) are disruptive to trade and economies (e.g., food animal disease outbreaks and losses); (3) are burdensome to social well-being (e.g., human-animal bonds and companionship); and 4) can cause unbalanced ecosystems (diminishing animal species can alter the environment). Additionally, infectious organisms may need multiple hosts for survival, and FDA would need to assess how this could impact FDA-regulated products. This is an example of where data from multiple species would be useful.

FDA partnered with the National Academies of Sciences, Engineering, and Medicine – Institute for Laboratory Animal Research (now known as the Board on Animal Health Sciences, Conservation, and Research) (NASEM/ILAR) to convene a workshop entitled "Microphysiological Systems (MPS): Bridging Human and Animal Research" on January 19-20, 2021. The workshop was attended by individuals from government agencies, academia, the public, and industry. The workshop proceedings have been published (NASEM, 2021). Several ideas were presented from this workshop and are summarized below.

- Interagency federal partners define One Health as "a collaborative, multisectoral, and transdisciplinary approach - working at the local, regional, national, and global levels - with the goal of achieving optimal health outcomes recognizing the interconnection between people, animals, plants, and their shared environment[9]." Since zoonotic diseases are a significant public health problem globally involving various domestic and wild animal reservoir hosts, FDA's One Health Initiative acknowledges that emerging zoonotic diseases threaten both human and animal health and can have devastating consequences[10].
- Although the typical epidemic link is from wildlife to livestock to humans, transmissibility can be bidirectional between humans and animals. The COVID-19 pandemic is one current example of zooanthroponosis (human-to-animal transmission) involving severe acute respiratory syndrome coronavirus 2 (SARS-CoV2) infection. There are increasing reports of zooanthroponotic transmission with organisms such as *Staphylococcus aureus, Cryptosporidium parvum, Ascaris lumbricoides, Mycobacterium tuberculosis*, measles, and others (Desta et al., 2022; Messenger et al., 2014; Willy et al., 1999). A global animal MPS tissue bank has the potential to offer a centralized resource for research efforts. For this reason, researchers have been challenged to develop a global MPS tissue bank for common zoonotic species that can assist in testing the predictive transmissibility of an organism from species to species (NASEM, 2021).
- The establishment of a global MPS tissue bank for common zoonotic species represents a forward-thinking approach to establish shared resources and standardize best practices for characterization of cross-species comparisons. Prioritization of which animal MPS to create will depend on the public health impact, transmission potential, geographical spread, severity, and treatment options. One suggestion could be to follow the World Health Organization and Centers for Disease Control and Prevention recommendations for prioritization of zoonotic diseases threats that may be of national or global significance[11,12].

  For example, Bats are known carriers of a multitude of viruses (e.g., rabies, coronaviruses) and are thought to be the original reservoir for SARS-CoV2. Intestinal organoids from this species have been used to study such infections (Zhou et al., 2020). Using bat-based MPS for identifying cellular immune mechanisms that protect bats from severe inflammatory responses to SARS-CoV-2 may point to novel treatment approaches in humans.

[9] https://www.cdc.gov/onehealth/index.html. Accessed June 4, 2024
[10] U.S. Food and Drug Administration (6 September 2022) Cross-cutting Topics: One Health Initiative. Retrieved from https://www.fda.gov/science-research/focus-areas-regulatory-science-report/cross-cutting-topics-one-health-initiative Accessed June 4, 2024
[11] World Health Organization. (16 June 2012). Research Priorities for Zoonoses and Marginalized Infections. World Health Organization Technical Report Series 971. Retrieved from https://www.who.int/publications/i/item/WHO-TRS-971 Accessed June 4, 2024
[12] Centers for Disease Control and Prevention. (1 November 2023). One Health Zoonotic Disease Prioritization (OHZDP). Retrieved from https://www.cdc.gov/one-health/php/prioritization/?CDC_AAref_Val=https://www.cdc.gov/onehealth/what-we-do/zoonotic-disease-prioritization/index.html Accessed June 4, 2024

- Animal drugs pose unique considerations for the development of animal MPS. The range of approved veterinary drugs encompass multiple species and multiple breeds for animals ranging in size from a two-pound chihuahua to a two-thousand-pound bull. Some areas of promise include animal MPS models that provide information to address breed differences within a species. Animal MPS models also could offer promise for insight on the impact and transmission of zoonotic diseases to other animal species and ultimately to humans. Animal MPS models may have the potential to be used in supporting the Center for Veterinary Medicine's (CVM) veterinary drug approvals and to understand intraspecies and interspecies differences.
- MPS technology may present opportunities to transform the way diseases are studied in humans and animals, as well as support the safety and efficacy assessments of human/animal pharmaceutical drugs to be evaluated, thereby potentially reducing the use of animals in traditional disease models and toxicology studies.

FDA's One Health Initiative is also interested in biotechnological advances that will assist in countering biological threats, thus aligning with national security strategies. The National Biodefense Strategy updated in 2022 calls for a multisectoral-coordinated One Health approach to respond to biological incidents and prevent pandemics[13]. Biotechnology is mentioned as one avenue to ensure these capabilities to reduce harm and improve quality of life for both animals and humans. According to the World Health Organization[14], approximately 75% of all emerging infectious diseases are zoonotic, with climate change fundamentally altering the nature and locations of these threats. The emergence and re-emergence of pathogenic diseases requiring multiple hosts can result in cross-species transmission that impact distribution and persistence of the disease. Therefore, ongoing discoveries with biotechnology, including MPS advances, could be of value in detecting, identifying, and acting on potential outbreaks in both animals and humans.

In addition to previous collaborations with the Wyss Institute, FDA OCET via the MCMi extended its partnership with the Wyss Institute to advance MPS models for MCM development through the "Human organ chips for radiation countermeasure development[15]" project awarded in 2019. The project was expanded in 2021 to develop MPS models of SARS-CoV-2 infection in partnership with NIH's National Institute of Allergy and Infectious Diseases. Furthermore, the Wyss Institute is collaborating with the UK Health Security Agency to develop MPS models of viral infection and pathogenicity[15]. The project specifically includes development of non-human primate (NHP) MPS models to provide a bridge from these models to clinical and nonclinical data.

FDA OCET via the MCMi is also supporting the Center for Food Safety and Applied Nutrition's (CFSAN) development of a neuromuscular junction (NMJ) MPS, both human and mouse, to test for bioactive *Clostridium botulinum* neurotoxin. CFSAN is working with NCATS to explore qualification of the model. If qualified as equivalent or better than the mouse lethality test, these NMJ MPS could potentially be used for testing other neurotoxins.

The Center for Biologics Evaluation and Research (CBER) continues to support the development and evaluation of innovative alternative approaches, such as human and animal MPS, to facilitate nonclinical evaluation of complex and heterogeneous biological products that are susceptible to manufacturing conditions. For some biological products, little is known about the mechanism of action, which makes it difficult to develop a good experimental model for nonclinical evaluation. CBER is actively engaged in research to advance and adopt MPS as a tool to address issues related to the manufacture and characterization of biological products, such as vaccines and cell and gene therapy products. Information can be found in CBER's 2021-2025 strategic plan[16].

The Center for Devices and Radiological Health (CDRH) supports the development and qualification of alternative methods for nonclinical assessment of medical devices; however, the application of animal MPS for medical device safety and effectiveness evaluation requires additional considerations specific to assessment of medical devices.

4 What potential contexts of use of animal MPS are envisioned?

Potential uses of animal MPS may include screening for hazard identification, identifying mechanisms of action, and assessing effects of complex mixtures all of which may inform regulatory decision-making, and will vary among the different FDA regulatory centers based on their mandates and products that each regulates. For example, the CVM might use animal MPS in ecotoxicity studies to replace fish in environmental assessments for animal feed and animal drugs, while human MPS could potentially be used to evaluate the impact of animal antimicrobial drug residues on human gut flora. Center for Drug Evaluation and Research (CDER) authors have reported on data gaps for which alternative methods (potentially including MPS) could provide value (Avila et al., 2020, 2023). For example, further development and qualification of NAMs that detect embryofetal malformations or lethality could provide information to enable regulatory decision-making in some drug development scenarios.

Multiple external authors have provided examples of how animal *in vitro* to *in vivo* correlations may assist in evaluating efficacy and safety in humans (Jardi et al., 2023; Kopper et al., 2021). The development of human, rat, and canine liver MPS has the potential to be used to detect species-differences for drug-induced liver injury, which remains a major cause of drug attrition

[13] https://www.whitehouse.gov/wp-content/uploads/2022/10/National-Biodefense-Strategy-and-Implementation-Plan-Final.pdf. Accessed June 4, 2024.
[14] WHO EMRO | Zoonotic disease: emerging public health threats in the Region | RC61 | À propos de l'OMS. Accessed June 4, 2024.
[15] Human organ chips for radiation countermeasure development | FDA. Accessed June 4, 2023
[16] https://www.fda.gov/media/81152/download. Accessed May 24, 2023.

(Jang et al., 2019). Combined in vitro liver-thyroid models have been derived from both rodent (Karwelat et al., 2023) and human (Kühnlenz et al., 2023) cells with the intent, in part, of understanding species-specific quantitative in vitro to in vivo extrapolation for human risk assessment while also contributing to the 3Rs. Cross species translation and understanding when an effect is species specific is of interest for both toxicity and efficacy assessment. Such translation could be facilitated by having the same animal and human *in vitro* model thus allowing direct comparisons.

Many of the current organ or tissue MPS have been developed with human cells. Culture conditions and other parameters have been optimized for human cell lines, primary cells or cells differentiated from human iPSCs. These conditions might not be optimal for using these models with cells from other species. It is recognized that it may take substantial effort in some cases to adapt models to use animal cells. The practicality and feasibility of creating animal versions will need to be considered for each tissue of interest and COU.

It is recognized that not all COUs for which the animal or human MPS are developed will meet a regulatory need, for example, those COUs that apply to early stages of product development may be outside the regulatory purview of the Agency.

Even those regulatory applications for MPS with COUs that might be submitted to the Agency may be qualified to different degrees depending on the COU. For example, those COUs that seek to fully replace an existing method may be more rigorously assessed and may require supporting data from more reference compounds or may need to have a higher accuracy in their predictive capacity. Whereas methods submitted as supporting information, perhaps regarding a mechanism of toxicity, may not need as broad a set of supporting validation data. In addition, while the use of animal cell-based MPS may provide opportunities to increase confidence in the use of human MPS in general, not all human MPS will need to be recapitulated in animal models. This is especially the case, for example, when exquisitely human-specific targets are being investigated for which there is no animal analog.

5 What species and tissue types might be explored for MPS development?

The answer to this question will depend on the Center(s), what aspect of regulatory science is being evaluated, and on what animal species would be the most appropriate. Some examples include:

- There is broad interest in animal MPS that could be used for comparison in areas such as carcinogenicity assessments, human and animal medical countermeasures, and reproductive studies (Baran et al., 2022; Marx et al., 2020). The animal MPS may be used to bridge to existing animal studies and to gain confidence with human MPS. Extensive data in animals exists in many of these areas and so understanding how well animal MPS predict in vivo outcomes can be assessed by comparing the MPS predictions with actual in vivo outcomes. While one goal of generating animal MPS is to reduce the number of animals in product development and testing, topics such as carcinogenicity, medical countermeasures and reproductive toxicity are so highly complex that even in cases where animal studies may not be fully replaced, animal and human MPS may provide supplemental information to support assessments in these areas.
- FDA's agency-wide One Health Initiative is interested in models of zoonotic importance, such as agricultural, laboratory animal (e.g., canine, rat), aquaculture and wildlife (e.g., bats) species.
- CDER is interested in building confidence in the ability of both animal and human MPS to predict *in vivo* pharmacology and toxicology; such *in vivo*/*in vitro* correlation should be seen in commonly used nonclinical species such as rodents, canine and NHPs.
- CVM is interested in studying canine MPS models of multiple organs as an alternative method to assess the safety of a product to support animal drug approvals. One example is building a canine on a chip series of MPS models.
- Both CDER and the Center for Tobacco Products (CTP) are interested in pulmonary MPS models that can recapitulate, corroborate, and interrogate animal and human lung biology and physiological responses. Such models would help to understand potential respiratory effects resulting from repeated long-term exposures to aerosolized mixtures in animals and humans and enable cross-species extrapolation. Animal pulmonary MPS may also benefit animal health by reducing the need for animal inhalation studies and by addressing animal pulmonary diseases.
- CTP is also interested in MPS models to study cardiovascular effects of tobacco products.
- CFSAN and CDER are interested in animal and human MPS barrier models such as the gastrointestinal tract and the skin.

6 How can the development of these models be facilitated?

There could be benefits to both animal and public health from the development and adoption of animal MPS. There is broad interest in the scientific community for establishing partnerships to develop and eventually qualify both human and animal MPS to ensure usefulness for potential regulatory decision-making. Engagements with DARPA and NCATS initiatives that funded development of human MPS have proven to be valuable. Congress, through the NIH Omnibus bill has requested that NIH provide funds to small businesses to develop alternative assays, including those that look at animal versions of organotypic models "with a focus on

comparisons between *in vivo* and *in vitro* toxicity endpoints[17]." Other sources of funding may become available to develop animal MPS as the field progresses and there is a more broadly recognized need for these models.

7 Conclusions

Currently, it is challenging to incorporate human MPS into regulatory decision-making as such tools are not mature yet and need further investigation. The application of animal MPS to identify species-specific mechanistic issues and to determine which species should be tested are possible uses of MPS that will likely be adopted far earlier than the total replacement of animal studies. These early applications of MPS may be more easily supported because the COU is relatively narrowly focused, and the decisions being made are less likely to be pivotal. The information needed to show that these uses are scientifically valid may be easier to obtain than broader applications such as replacement of general *in vivo* toxicology studies. The early use of animal MPS for these purposes will also serve to enhance the maturity of MPS in general.

One challenge in trying to understand whether human MPS reflects *in vivo* responses is that extensive correlative research cannot be conducted in humans. Nonclinical toxicology data are used to avoid adverse effects in humans as much as possible. In addition, while one might see an adverse reaction in humans, the exact nature may not be understood as histopathological assessment of the target organ is seldom conducted. Postmortem evaluations such as histopathology are performed in nonclinical animal studies and can include examination of over 40 tissues and organs (Bregman et al., 2003) to fully evaluate adverse events in multiple organs. These broad assessments cannot be conducted in humans. Therefore, *in vivo* animal data provide robust information with which the predictivity of animal MPS can be assessed. In some cases, microscopic examination of MPS can provide a basis to compare morphological changes in animal and human MPS with the in vivo animal data which may contribute to the assessment of MPS predictivity.

The use of animal MPS in candidate selection during the human drug discovery phase is being explored by industry stakeholders and may lead to reductions in animal use (Baran et al., 2022; Marx et al., 2020). The use of animal MPS is also likely to further impact the integration of such complex *in vitro* systems (human or animal) into investigational toxicology and ultimately drug development. It remains to be seen whether MPS will be able to detect all types of toxicity and tissue damage. However, the use of animal MPS together with human MPS to "de-risk" findings from in vivo animal studies may help enable moving potentially beneficial drugs into human clinical trials. In addition, animal MPS may help advance animal medicine and reduce use of live animals in this process. Other uses of animal MPS include the study of zoonotic diseases and therapies to combat them. Two concerns to note moving forward include: 1) there does not seem to be a concerted effort to create animal versions of MPS; and 2) while most of the work has been done with human cells, even these MPS platforms are still evolving. However, evaluating and understanding the potential of human and animal MPS may improve safety and efficacy assessment for therapeutic products and harm reduction in the case of tobacco products. This could be beneficial for regulatory decision-making and protecting and promoting public health. Finally, the use of new approaches, including animal MPS, can help accomplish the objectives of the 3Rs of animal use.

References

Avila, A. M., Bebenek, I., Bonzo, J. A. et al. (2020). An FDA/CDER Perspective on Nonclinical Testing Strategies: Classical Toxicology Approaches and New Approach Methodologies (NAMs). *Regul Toxicol Pharmacol 114*, 104662. doi:10.1016/j.yrtph.2020.104662

Avila, A. M., Bebenek, I., Mendrick, D. L. et al. (2023). Gaps and challenges in nonclinical assessments of pharmaceuticals: An FDA/CDER perspective on considerations for development of new approach methodologies. *Regul Toxicol Pharmacol 139*, 105345. doi:10.1016/j.yrtph.2023.105345

Baran, S. W., Brown, P. C., Baudy, A. R. et al. (2022). Perspectives on the evaluation and adoption of complex *in vitro* models in drug development: Workshop with the FDA and the pharmaceutical industry (IQ MPS Affiliate). *ALTEX 39*, 297-314. doi:10.14573/altex.2112203

Berridge, B. R. (2021). Animal study translation: The other reproducibility challenge. *ILAR J 62*, 1-6. doi:10.1093/ilar/ilac005

Bregman, C. L., Adler R. R., Morton D. G. et al. (2003). Recommended Tissue List for Histopathologic Examination in Repeat-Dose Toxicity and Carcinogenicity Studies: A Proposal of the Society of Toxicologic Pathology (STP). *Toxicologic Pathology 31*, 252-253. doi:10.1080/01926230390183751

Desta, G. B., Jena, P. K., Hassen, H. M. et al. (2022). Factors associated with zoonosis and reverse zoonosis of mycobacterium tuberculosis and mycobacterium bovis in Ethiopia: A systematic review and meta-analysis. *Int. J. Health Sci 6*, 1630-1653. doi:10.53730/ijhs.v6nS5.8922

Huh, D., Matthews, B. D., Mammoto, A. et al. (2010). Reconstituting organ-level lung functions on a chip. *Science 328*, 662-8. doi:10.1126/science.1188302

Irrechukwu, O., Yeager, R., David, R. et al. (2023). Applications of microphysiological systems to disease models in the biopharmaceutical industry: Opportunities and challenges. *ALTEX 40*, 485–518. doi:10.14573/altex.2204071

[17] https://ntp.niehs.nih.gov/sites/default/files/iccvam/suppdocs/funding/2022-2_sbir-sttr-topics-niehs-508.pdf Accessed June 4, 2024

Jalili-Firoozinezhad, S., Prantil-Baun, R., Jiang, A. et al. (2018). Modeling radiation injury-induced cell death and countermeasure drug responses in a human Gut-on-a-Chip. *Cell Death Dis 14*, 223. doi:10.1038/s41419-018-0304-8

Jang, K. J., Otieno, M. A., Ronxhi, J. et al. (2019). Reproducing human and cross-species drug toxicities using a Liver-Chip. *Sci Transl Med 11*, 517. doi:10.1126/scitranslmed.aax5516

Jardi, F., Kelly, C., Teague, C. et al (2023). Mouse organoids as an *in vitro* tool to study the *in vivo* intestinal response to cytotoxicants. *Arch Toxicol 97*, 235-254. doi:10.1007/s00204-022-03374-3

Karwelat, D., Kühnlenz, J., Steger-Hartmann, T. et al. (2023). A rodent thyroid-liver chip to capture thyroid toxicity on organ function level. *ALTEX* 40, 83-102. doi:10.14573/altex.2108262

Kopper, J. J., Iennarella-Servantez, C., Jergens, A. E. et al. (2021). Harnessing the Biology of Canine Intestinal Organoids to Heighten Understanding of Inflammatory Bowel Disease Pathogenesis and Accelerate Drug Discovery: A One Health Approach. *Front Toxicol 10*, 773953. doi:10.3389/ftox.2021.773953

Kühnlenz, J., Karwelat, D., Steger-Hartmann, T. et al. (2023). A microfluidic thyroid-liver platform to assess chemical safety in humans. *ALTEX 40*, 61-82. doi:10.14573/altex.2108261

Marx, U., Akabane, T., Andersson, T. B. et al. (2020). Biology-inspired microphysiological systems to advance patient benefit and animal welfare in drug development. *ALTEX 37*, 365-394. doi:10.14573/altex.2001241

Messenger, A. M., Barnes, A. N., and Gray, G. C. (2014). Reverse zoonotic disease transmission (zooanthroponosis): a systematic review of seldom-documented human biological threats to animals. *PloS One 9*, e89055. doi:10.1371/journal.pone.0089055

NASEM – National Academies of Science Engineering and Medicine (2021). Microphysiological Systems: Bridging Human and Animal Research: Proceedings of a Workshop—in Brief. National Academies Press (US). doi:10.17226/26124

Pound, P. and Ritskes-Hoitinga, M. (2018). Is it possible to overcome issues of external validity in preclinical animal research? Why most animal models are bound to fail. *J Transl Med 16*, 304. doi:10.1186/s12967-018-1678-1

Sewell, F., Alexander-White C., Brescia S., et al. (2024). New approach methodologies (NAMs): identifying and overcoming hurdles to accelerated adoption. *Toxicol Res (Camb) 13*, 1-9. doi:10.1093/toxres/tfae044

Steger-Hartmann, T. and Raschke, M. (2020) Translating in vitro to in vivo and animal to human. *Current Opinion in Toxicology 23-24*, 6-10. doi:10.1016/j.cotox.2020.02.003

Torisawa, Y. S., Spina, C. S. Mammoto, T. et al. (2014). Bone marrow-on-a-chip replicates hematopoietic niche physiology *in vitro*. *Nat Methods 11*, 663-669. doi:10.1038/nmeth.2938

Weinhart, M., Hocke, A., Hippenstiel, S. et al. (2019). 3D organ models-Revolution in pharmacological research? *Pharmacol Res 139*, 446-451. doi:10.1016/j.phrs.2018.11.002

Willy, M. E., Woodward, R. A., Thornton, V. B. et al. (1999). Management of a measles outbreak among Old World nonhuman primates. *Lab Anim Sci 49*, 42-48. doi:10.1016/j.phrs.2018.11.002

Zhou, J., Cun, L., Ziu, X., et al. (2020). Infection of bat and human intestinal organoids by SARS-CoV-2. *Nature Medicine 26*, 1077-1083. doi:10.1038/s41591-020-0912-6

Conflict of interest

The authors have no conflict of interest.

Data availability

No datasets were generated or analyzed for this manuscript.

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