U.S. FOOD & DRUG ADMINISTRATION

Advancing New Alternative Methodologies at FDA

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Commissioner of Food and Drugs
Stephen M. Hahn, MD

A Message from the FDA Commissioner

This is an unprecedented time to be part of the U.S. Food and Drug Administration (FDA). The Coronavirus Disease 2019 (COVID-19) pandemic has revealed as almost no other event in a century the transformative effect on our lives of emerging technologies, new areas of science, and globalization. The seismic shift created by the convergence of these elements in our regulatory science landscape has produced both unparalleled challenges for the global scientific community and exciting opportunities for improving public health.

FDA has a long history of fostering innovation, most recently to meet 21st century public health challenges. Our rigorous scientific activities and collaborations with stakeholders, both domestic and international, have proved pivotal to our ability to identify needs, maintain momentum, and establish a community that can facilitate the development of new regulatory methods and methodologies. Keeping pace with advances in basic and applied science and technology is critical to FDA's ability to reach sound regulatory decisions and retain the public's trust.

A key part of this effort has been our robust support for the development of new regulatory tools that can help improve predictivity and potentially replace, reduce, and/or refine animal testing. I am proud to highlight in this report some of the activities in which FDA is engaged that are moving us closer to the goal of replacing, reducing, and refining the use of animals in medical product development while continuing to advance disease modeling, toxicology, and pharmacology in support of FDA's mission.

Stephen M. Hahn, M.D.
Commissioner of Food and Drugs

Chief Scientist
RADM Denise Hinton

A Message from the Chief Scientist

More than a decade ago, FDA's Office of the Chief Scientist (OCS) was established[1] to forge the cross-agency efforts needed to address the rapid changes in vital areas of science, technology, and our globalized economy.[2] FDA recognized the need for more expansive engagement with our stakeholders to modernize the scientific and technical tools required to ensure food safety; evaluate and predict the safety, effectiveness, and reliable manufacture of medical products; and make tobacco-related death and disease part of America's past, and not its future.

Delivering on the promise of new science has demanded a global response and a new way of working—across FDA centers and offices and with *all* stakeholders, domestic and international. To that end, OCS created intramural collaborations to tackle regulatory science problems and gaps that require the expertise of multiple product centers. OCS formed extramural collaborations with academic centers to advance regulatory science through leading-edge research, training, and scientific exchanges. Nimble funding mechanisms were launched, like the Advancing Regulatory Science Broad Agency Announcement, to support novel approaches to evaluating FDA-regulated products and help advance new technologies, like human organs on chips.

With the goal of advancing new tools as well as new areas of science, OCS created cross-agency scientific working groups. FDA's Alternative Methods Working Group, which has spearheaded this report, is a good example. This Working Group is the catalyst for the further development and potential application of alternative technologies like microphysiological systems to inform FDA decision-making.

[1] See section 910 of the Federal Food, Drug, & Cosmetic Act (21 U.S.C. 399a) (added by section 602 of the Food and Drug Administration Amendments Act of 2007 (FDAAA)).
[2] *FDA Science and Mission at Risk: Report of the Subcommittee on Science and Technology* https://advance.uncc.edu/sites/advance.uncc.edu/files/media/dr-gail-cassell.pdf

Ultimately, these new regulatory tools have the potential to achieve a goal we have long been working toward—to reduce, refine—and even replace animal testing. They may also help bring novel FDA-regulated products to market faster while preventing products with increased toxicological risk from even entering the development pipeline.

OCS is providing FDA leadership and support toward achieving this goal. We remain deeply engaged in identifying and fostering strategies that can bring alternative testing methods to FDA for integration into the marketing review process. As a first step, in 2017, OCS launched FDA's Predictive Toxicology Roadmap. The roadmap provides a formal framework to spur the development and evaluation of emerging toxicological methods—as well as new technologies—and to incorporate them into FDA regulatory review. The roadmap ensures that the end users of this new technology—FDA regulators—are involved up front as these technologies evolve from design to testing to qualified context of use.

FDA's efforts to promote the development of alternative test methods can be viewed on the Alternative Methods Working Group website. The website hosts related FDA co-authored publications and presentations, and recently a webinar series was launched by the Working Group that enables developers to showcase their pioneering technologies to FDA scientists. This kind of synergy has proved critical to developers as they test out these new technologies and methodologies and to FDA scientists who must remain up-to-date on new approaches they may one day be encountering in medical product marketing submissions.

OCS looks forward to our continued work with all of our partners. We share the same goals—advancing science both to improve public health and ensure our nation's access to affordable, innovative treatments. We welcome your input and comments at alternatives@FDA.hhs.gov.

Introduction

The U.S. Food and Drug Administration (FDA or the Agency) is responsible for protecting the public health by ensuring the safety, efficacy, and security of human and veterinary drugs, biological products, and medical devices; and by ensuring the safety of our nation's food supply, cosmetics, and products that emit radiation. FDA also has the responsibility of regulating the manufacturing, marketing, and distribution of tobacco products to make tobacco-related death and disease part of America's past, and, by doing so, ensure a healthier life for every family.

Moreover, FDA plays a significant role in the nation's counterterrorism capability, ensuring the security of the food supply and fostering development of medical products to respond to deliberate and naturally emerging public health threats, such as the COVID-19 pandemic.[3]

An increasingly critical FDA role involves advancing public health by helping to speed innovations that make FDA-regulated therapeutic products more effective and safer. The Agency also helps the public get the accurate, science-based information it needs to use medical products and foods to maintain and improve their health. One foundational area in which FDA has been enabling innovation is in the science of new predictive methods. If validated, such methods may enable FDA to decrease reliance on animal studies for safety and toxicity by using alternative predictive methods within the regulatory review process.

Background

Toxicological testing is a critical tool for studying the safety and biological effects of drugs, chemicals, agents, and other substances. Testing methods are used to identify harmful effects, dosages that cause those effects, and safe exposure limits of these products.

Historically, scientists have relied heavily on animal studies to determine if a drug is toxic before testing it in humans. For example, animal studies have shown great accuracy in predicting safe doses used in clinical trials, identifying potential target organs of toxicity and helping to determine appropriate monitoring for adverse effects. The development of safe and effective medical products would not have occurred without animal studies. Nevertheless, while animal testing is still necessary in many situations, it is also expensive and time-consuming. Further, animal studies do not always detect toxic effects specific to humans and do not usually provide information about the role that genetic differences within human populations play in toxicity.

Thus, toxicity has been a major challenge in medical product development and in assessing environmental hazards. Advances over recent decades in toxicology testing (e.g. the use of stem cells, engineered tissues, mathematical modeling) hold the promise to improve the predictive ability of toxicology in conjunction with more traditional approaches. Most current alternative methods cannot predict effects that occur in highly complex interacting systems. This is especially true for animal tests that include repeated dosing, long-term implantation

[3] https://www.fda.gov/about-fda/what-we-do#mission

(e.g. medical devices), and evaluation of multiple endpoints. Additional research is necessary before alternative methods are able to be used routinely for addressing such complex issues.

Worldwide research continues to create alternative technologies that could replace—or at least reduce—animal testing. In its 2007 report *Toxicity Testing in the 21st Century: A Vision and a Strategy,* the National Research Council of the National Academies of Science Engineering and Medicine advocated for a shift away from traditional animal testing towards the use of mechanisms of action as the organizing principle for risk assessment.

In 2011, FDA's Advancing Regulatory Science Plan identified transforming toxicology as one of its key priorities. The focus was—and continues to be—on promoting a better understanding of toxicity mechanisms by evaluating safety and risk assessment data at multiple biological levels, including genes, proteins, biochemical pathways and cell/organ function.

During the past two decades, FDA has launched multiple Agency initiatives to advance predictive toxicology and reduce animal testing.

- In 2010, FDA was invited by the U.S. Environmental Protection Agency (EPA), National Institute of Environmental Health Sciences (NIEHS), and the National Center for Advancing Translational Science (NCATS) to join the Tox21 Consortium, a federal research collaboration aimed at driving the evolution of toxicology for safety testing by developing rapid alternative methods in lieu of animal testing.
- In 2012, FDA partnered with the Defense Advanced Research Projects Agency (DARPA) to support work that could spur the development of physiologically and pathologically accurate human models, using tissue engineering platforms—new tools known as *organs on chips*. These models could contribute to developing medical countermeasures since efficacy testing in humans is often neither feasible nor ethical for diseases and conditions that we might need to treat in a public health emergency—like anthrax, smallpox, pandemic influenza, and radiation and toxin exposure. This remains a key FDA priority.
- In 2017, under the aegis of its Office of the Chief Scientist, the Agency published its Predictive Toxicology Roadmap, FDA's current thoughts on viable ways to foster the development and evaluation of emerging toxicological methods and new technologies and incorporate them into FDA regulatory review.
- In 2019, FDA recognized that it needed to create a platform available to stakeholders and the public that would inform them of FDA's progress in promoting the development of alternative test methods. To that end, FDA established an Alternative Methods Working Group, which is developing a targeted strategy for moving towards the use of alternative methods for regulatory testing. The Working Group's website provides the latest updates to our stakeholders on FDA's progress.

FDA's collaborations with stakeholders are critical to advancing alternative methods that could reduce the time it takes for new treatments to move to human testing and approval. These new tools may help identify toxicity, efficacy, and disease susceptibility earlier in product

development, thus protecting patients, lowering development costs, and speeding new treatments to patients in need.

About this Report

This report was developed by the Alternative Methods Working Group to highlight the significant progress FDA scientists have made in our product centers and offices in laying the groundwork for integrating alternative approaches into FDA regulatory programs. It demonstrates FDA's strong commitment to reducing animal testing and to encouraging stakeholders to continue partnering with FDA to achieve this important goal.

FDA scientists have vast expertise and experience using available tools to make tough scientific decisions about the safety and effectiveness of the multitude of therapeutic products in our regulatory portfolio. They've seen what works and what does not; therefore, they can provide unique insights and help solve challenges in defining how best to develop and evaluate new tools. Once FDA accepts a scientific tool, industry can more easily use it for its qualified purpose during product development.

Many of the important research activities on alternative methods described in this report are being conducted in FDA laboratories, and many of these include collaborations with outside stakeholders. Results from these ground-breaking research activities add to the body of knowledge that FDA can draw upon as it evaluates regulatory acceptance criteria for new alternative methods.

FDA believes partnerships between our diverse programs and outside stakeholders are essential to furthering progress in developing strong alternative approaches. FDA is open to dialogue from all sectors of the population because we believe it will strengthen our scientific and regulatory thinking. Some of these partnerships are described in this report.

Cross-agency working groups on high-priority scientific and regulatory issues have also been critical in developing FDA policies on emerging science issues. Partnerships with other agencies both in the U.S. and international sphere strengthen FDA's continuing knowledge base in this vital area.

Transformative change requires robust and continuing support at the highest level of a federal agency. That is why FDA's Office of the Chief Scientist in the Office of the Commissioner serves as the engine to drive this program forward.

Communicating and collaborating with our stakeholders is essential for moving toward and adopting new methodologies. This is evidenced throughout this report in many of the activities described and since communication with our stakeholders is essential for FDA progress on

alternatives, we have established a website to keep the public current on our progress on alternative methods.

FDA invites all our stakeholders to join us by visiting our alternatives website, by sending comments to FDA through the following e-mail address: alternatives@fda.hhs.gov and by proposing innovative collaborations that will advance the science of alternative methods.

Alternative Methodologies: FDA Office and Center Achievements

Office of the Chief Scientist

FDA's Office of the Chief Scientist (OCS) in the Office of the Commissioner fosters innovation and scientific excellence through its regulatory science activities, communication, and management of intramural and extramural collaborations with stakeholders in academia, industry, and government. Its scope encompasses interdisciplinary toxicology research, health informatics, technology transfer, laboratory safety, scientific training and education, minority health and health equity, as well as leadership, coordination, and oversight for FDA's national and global health security, counterterrorism, and emerging threats portfolios.

Human organ on a chip for radiation countermeasure development

Acute radiation syndrome (ARS) is an illness affecting a combination of organs. It occurs when the body receives a high dose of radiation over a short period of time, as could happen after a nuclear or radiological incident. The U.S. government has identified a need for safe and effective radiation medical countermeasures for military and civilian applications, as well as to mitigate toxicities associated with medical radiotherapies (e.g. chemotherapy).

FDA's Medical Countermeasure's Initiative, which is managed out of OCS's Office of Counterterrorism and Emerging Threats, is researching human organ-on-chip to replicate the body's response to radiation exposure. FDA and collaborators are investigating this technology as part of a computational discovery pipeline to develop new medical countermeasures to treat ARS.

The development of medical countermeasures to treat ARS presents complex scientific challenges. For example, ARS may involve many organ systems, which make it difficult to study candidate medical countermeasures that target the radiation effects on one specific organ system in animal models. Thus, there is a crucial need for more predictive in-vitro models of the effects of ionizing radiation on human tissue and organ function. Furthermore, a key goal of the OCS's Medical Countermeasures Initiative Regulatory Science Program is closing such knowledge gaps to further medical countermeasure development and availability.

Along with other academic labs and commercial companies, the Ingber Laboratory at the Wyss Institute has developed human organs-on-chips microfluidic culture devices that replicate human organ-level pathophysiology. This project builds on past FDA-funded work (through FDA's Advancing Regulatory Science Broad Agency Announcement) at the Wyss Institute that

led to the development of human organ chip models of ARS in bone marrow, intestine, and lung organ-on-chips and the application of these tools to evaluate ARS medical countermeasures.

Through a partnership with FDA's Office of Women's Health, the project also explored differences in sex-specific responses to ionizing radiation and chemotherapeutic drugs. Under the current project, the Wyss team is advancing the use of human organs-on-chips for medical countermeasure development by demonstrating biomimicry and defining critical qualification criteria, helping to reduce the reliance on testing in small animals and nonhuman primates (NHPs). Some of the outcomes from this project include the following:

- Assessing the effects of medical countermeasures (FDA-approved and in development) on reducing radiation damage in male versus female bone marrow-derived chips to determine differences in radiation response by sex
- Analyzing the interplay between human bone marrow organs-on-chips and intestine organs-on-chips that include a complex human gut microbiome in response to radiation exposure
- Recapitulating radiation-induced pneumonitis and response to existing ARS medical countermeasures in the human lung alveolus chip
- Using the Wyss computational discovery pipeline with transcriptomics data to support repurposing existing drugs as radiation medical countermeasures

Read More:

Human organ chips for radiation countermeasure development

Human organ chips for the development of countermeasures against Coronavirus (COVID-19)

The emergence of SARS-CoV-2 in 2019 and the associated COVID-19 pandemic, and prior SARS-CoV and MERS-CoV outbreaks, demonstrate the significant threat posed by coronaviruses.

In this new Medical Countermeasures Initiative project, the University of Liverpool (ULIV) in partnership with Public Health England (UK), University of Bristol (UK), King Fahad Medical City (Saudi Arabia), Agency for Science, Technology, and Research (A*STAR, Singapore), and University of Oxford (UK) will analyze SARS-CoV-2, SARS-CoV, and MERS-CoV clinical samples to better understand coronavirus evolution and virulence, characterize host–pathogen interactions and immunity, and identify biomarkers of disease progression and severity.

During this project, ULIV will conduct RNA sequencing and immunological analysis of samples from patients who were infected with SARS-CoV-2, SARS-CoV and MERS-CoV during the 2019-2020 pandemic and outbreaks that began in 2003 (SARS-CoV) and 2012 (MERS-CoV). The project will use a biobank of diverse samples from humans with these infections and compare their response to relevant animal and in vitro models being used to develop medical countermeasures. This includes developing and accessing organs-on-chips technology for investigating coronavirus infection and their application to medical countermeasures evaluation and apply data generated from this project to both human infections and animal models.

Center for Food Safety and Applied Nutrition

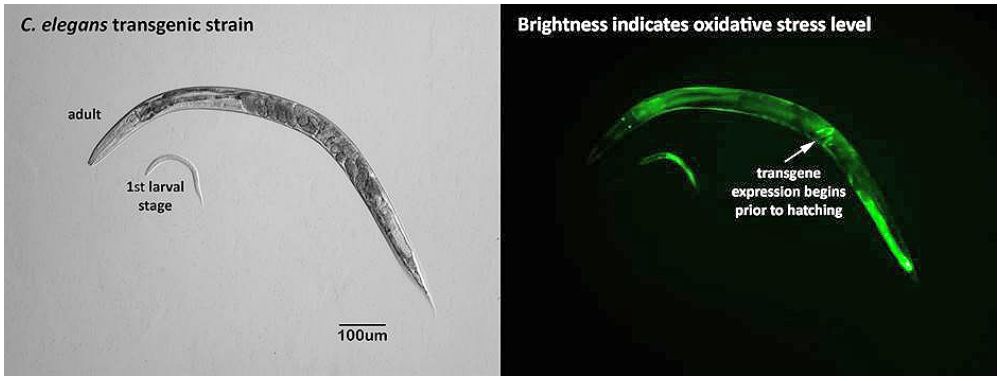
Toxicity tests using *C. elegans*: assessment of usefulness for regulatory purposes

Caenorhabditis elegans (*C. elegans*) are microscopic, non-pathogenic roundworms with specialized cells and tissues that function in ways that correspond to vertebrate organs. Given that many genetic and cellular pathways involved in organismal development, neuronal architecture and function, and toxic mode of action are conserved from worms and humans, this tiny invertebrate species may prove useful for predictive toxicity testing.

The three-day lifecycle of *C. elegans* and ease of maintenance indicate that the model could provide fast and inexpensive data to inform safety assessments, but only if specific assays can be demonstrated to provide results that correspond to human toxic responses. Using chemicals with clearly defined mammalian toxic effects, FDA is currently evaluating previously developed *C. elegans* toxicity assays for their capacity to produce correlative responses to developmental and reproductive toxins.

FDA is also developing higher-throughput test methods for other endpoints, such as neurotoxicity, epigenetic toxicity, and oxidative stress. These efforts will contribute to our understanding of the accuracy and fit-for-use categories for *C. elegans* toxicity testing and its usefulness to prioritize those compounds that may not need extensive animal testing.

Figure 1: Rapid testing for oxidative stress response using *C. elegans*



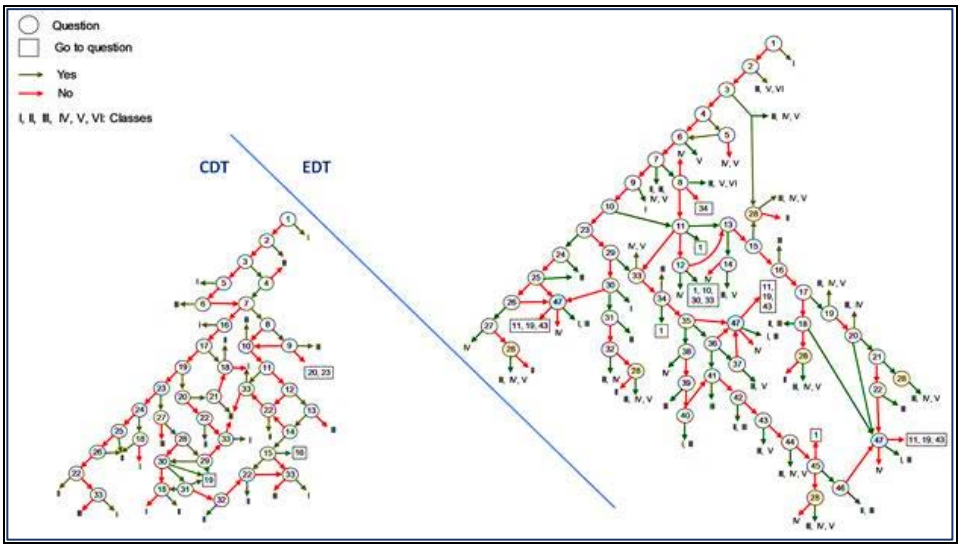
Expanded Decision Tree Software for classifying compounds according to their relative toxic potential

During the last seven decades, scientific advancements have led to an exponential increase in the number and types of chemicals to which humans are known to be exposed, leading to an ever increasing need to screen and prioritize these substances according to their relative toxicity.

The Cramer Decision Tree (CDT)[4] is a screening and prioritization tool that sorts chemicals into three classes of relative toxicity. FDA has updated and expanded the CDT to reflect the current state of the science and to make it applicable to a much broader scope of substances present in food, food contact materials, cosmetics, dietary supplements, and other materials (Fig. 2). FDA increased the number of classes of relative toxicity from three to six (non-toxic, low, medium, high, very high, and extreme toxicity) and quantified the toxic potential of each class by calculating a Threshold of Toxicological Concern level for each of the six Expanded Decision Tree (EDT) classes.

The EDT has been created but the software development is still under discussion. By screening and prioritizing chemically defined substances, the EDT and its software will help focus resources on the safety assessments of substances with greater potential for public health risk and help reduce the use of animals for safety testing.

Figure 2: Expanded Decision Tree



***Read More**:*

FDA's Predictive Toxicology Roadmap 2018 Annual Report

The European Food Safety Authority (EFSA) Scientific Committee (2019). Guidance on the use of the Threshold of Toxicological Concern approach in food safety assessment. EFSA Journal, 17(6), e05708.

[4] Cramer et al. (1978). The Cramer decision tree is an approach for classifying and ranking chemicals on the basis of their expected level of oral toxicity that was proposed by Cramer, Ford and Hall in 1978. It is a priority-setting tool used in assessing the safety of indirect food additives to make expert judgments more reproducible and trustworthy.

Center for Tobacco Products

FDA's Center for Tobacco Products (CTP) has pursued several applied research projects using in vitro and in silico techniques to broaden our understanding of toxicities from tobacco products. Studies similar to inhalation products are conducted for oral tobacco products, although the route of administration for these products is different, requiring different sample preparation and exposure systems.

CTP has performed in vitro studies for assessing the genotoxic and cytotoxic properties of emissions and individual ingredients from combustible and electronic nicotine delivery system (ENDS) tobacco products.

In addition, physiologically relevant in vitro models cultured at air-liquid interfaces will be used to assess potential lung toxicity following exposure to tobacco smoke or aerosols. In silico computational approaches include prediction models and cheminformatic techniques to help evaluate the genotoxic, mutagenic, carcinogenic, respiratory hazards, and possible penetration of the central nervous system of chemicals found in tobacco products such as flavors and byproducts.

Current research activities also encompass computational fluid dynamic modeling of aerosol deposition across species and respiratory dosimetry models for relating in vitro responses to realistic human exposure scenarios.

Coupled with these in vitro and in silico strategies, CTP is using cutting-edge approaches, such as the 'breathing-smoking human Lung-on-a-Chip' system for assessing lung toxicity, although the novelty of these methods requires careful assessment of research results.

CTP encourages the use of alternative methods for testing toxicity when it is appropriate and has sought to use in vitro and in silico methods synergistically to expand on the results of studies that use research animals in addition to answering unique questions that cannot be addressed using non-human systems.

Center for Biologics Evaluation and Research

National Institute of Standards and Technology (NIST) Workshop

FDA's Center for Biologics Evaluation and Research (CBER) participated in a NIST–FDA workshop on standards for next generation sequencing detection of viral adventitious agents in biologics and biomanufacturing.[5]

The application of "next generation sequencing" to detect adventitious agents in manufacturing intermediates, such as cell substrates for manufacture of vaccines, in lieu of currently recommended evaluation assays is an important contribution to working to replace the use of animals in characterization and lot release testing of many biological products, including vaccines.

Notably, the standard battery of assays to detect adventitious agents in intermediates and final products still involves use of multiple animal-dependent assays (embryonated chicken eggs by two routes; adult mice; suckling mice; and guinea pigs).[6]

The National Toxicology Program Interagency Center for the Evaluation of Alternative Toxicological Methods Workshop

CBER participated in a 2-day workshop co-sponsored by the National Toxicology Program (NTP) Interagency Center for the Evaluation of Alternative Toxicological Methods and the International Alliance for Biological Standardization North America to discuss non-animal alternative methods for potency testing of human and veterinary Rabies Virus Vaccine (RVV).[7] The traditional method relies on the use of mice. The animal assay is highly variable, time-consuming (up to 6 weeks), and requires use of 200 mice per test. Therefore, moving to a non-animal replacement test is considered a high priority.

Participants agreed that work should proceed to validate the use of highly specific monoclonal antibodies in an ELISA-based assay (enzyme-linked immunosorbent assay)[8] to quantitate the rabies virus vaccine G glycoprotein compared to the use of the animal assay.

Initial Targeted Engagement for Regulatory Advice on CBER Products (INTERACT)

CBER initiated a new regulatory meeting pathway, INTERACT to support individualized discussions about product development, including types of nonclinical studies required to start first-in-human trials.

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[5] *See* Report of the 2019 NIST-FDA workshop on standards for next generation sequencing detection of viral adventitious agents in biologics and biomanufacturing: Biologicals 64 (2020) 76–82.

[6] https://www.fda.gov/media/78428/download

[7] Biologicals 60 (2019) 8–14

[8] **ELISA** (enzyme-linked immunosorbent assay) is a plate-based assay technique designed for detecting and quantifying soluble substances such as peptides, proteins, antibodies, and hormones. Other names, such as enzyme immunoassay (EIA), are also used to describe the same technology.

Predicting the potential of multipotent stromal cells to develop cartilage-forming activity

CBER scientists developed an alternative approach using 3D organoids that identifies multipotent stromal cells (MSCs) that are more likely to differentiate (specialize) into cartilage forming cells after being stimulated with certain growth factors. This approach is important because cartilage is a very complex tissue, making its medical repair or restoration difficult.

Consequently, there is much interest among scientists and physicians to use donated human MSCs to enable patients to regain full use of their joints. However, the potential for stimulated MSCs to form cartilage can vary, depending on which donor they are obtained from and the conditions in which they are prepared for therapeutic use.

***Read More**:*

FDA's Predictive Toxicology Roadmap 2018 Annual Report

EFSA Scientific Committee (2019). Guidance on the use of the Threshold of Toxicological Concern approach in food safety assessment. EFSA Journal, 17(6), e05708.

A quantitative approach for predicting which MSCs will successfully differentiate into cartilage-forming cells would improve the ability of manufacturers to prepare safe and effective treatments for repairing or restoring cartilage in patients.

Center for Drug Evaluation and Research

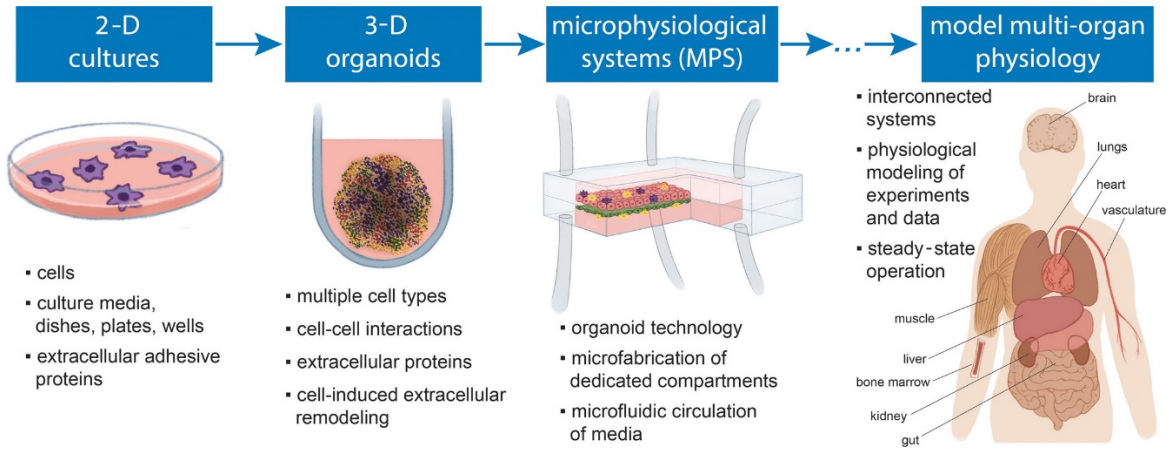
In Vitro Cellular Systems

Microphysiological Systems

Researchers in the Center for Drug Evaluation and Research (CDER) in the Division of Applied Regulatory Science are evaluating microphysiological systems—or organ tissue on-a-chip systems—as cell culture platforms to model human-specific physiology of tissues or organs to improve the efficiency of drug development. Initial work involved applications of liver and cardiac systems because side effects in these organs represent the primary causes for drug attrition.

This effort is now being expanded to heart–liver interconnected systems and other cellular systems that represent other organs or tissue types, such as kidney, lung, and gut. In addition to potentially predicting human-specific side effects, these platforms will also be useful when clinical trials are limited or not feasible. Studies will be initiated in applications that may benefit the development of both generic and new drugs.

Figure 3: Different types of culture platforms to enhance the physiology of cells in culture



Ribeiro et al. Clin Pharmacol Ther 2019, 106(1):139-47.

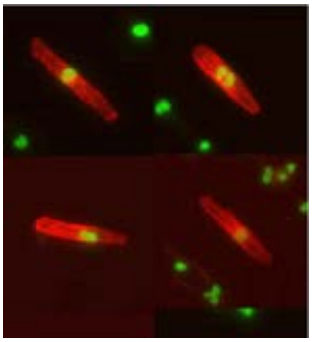
Cellular materials for engineered cellular systems

The quality of cells or tissues cultured in microphysiological systems strongly affects the performance of these platforms in modeling drug effects. CDER is exploring quality control functional parameters for cells to be used in microphysiological systems. For human-specific models, cells can be isolated from human tissues (primary cells) or differentiated from induced pluripotent stem cells (iPSCs) that can be reprogrammed from unlimited numbers of human donors.

Primary cells are not available from all tissue types and it is usually difficult to test effects of inter-donor variability within diverse populations with primary cells due to limits in the number of donors. These challenges of primary cells may be better addressed with iPSCs that are easily reprogrammed from blood samples or skin biopsies of donors and are then differentiated into different types of tissue cells.

However, cells differentiated from iPSCs are known to have fetal-like properties and can fail to replicate the physiology of mature human tissues. To establish quality control properties of cells for using functional systems in drug development, CDER scientists are comparing primary cells with iPSC-differentiated cells and discovering criteria that define mature tissues.

Figure 4: Single cells differentiated from iPSCs and isolated for genetic profiling



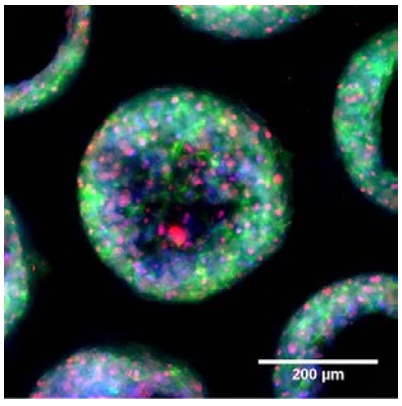
Liver microphysiological systems for applications in drug toxicity and pharmacology

Liver microphysiological systems culture different kinds of hepatic cells (hepatocytes, Kupffer cells, stellate cells, endothelial cells, cholangiocytes, and others) in three dimensions and in medium that is pumped to flow through the microtissue formed by these cells.

These conditions prolong liver-specific cellular functions (including enzymatic activities, transport functions, and inflammatory response) and may in the future allow for the testing of chronic drug effects, drug-drug interaction effects, and drug-activated mechanisms involving multiple types of differentiated cells.

CDER researchers have characterized liver microphysiological systems to test their usefulness for the study of drugs with hepatotoxic effects and for studying drug metabolism, transport, and intracellular accumulation. Cells in these systems show prolonged enzyme activities and albumin production and respond differently to hepatotoxic compounds relative to traditional culture methods. While this is a promising technology, there is no forthcoming data suggesting that these platforms can replace animal studies currently.

Figure 5: Fixed and fluorescently labeled primary human hepatocytes after being cultured in a liver system



Green is actin, blue is nucleus, and red is cytoplasmic membrane.

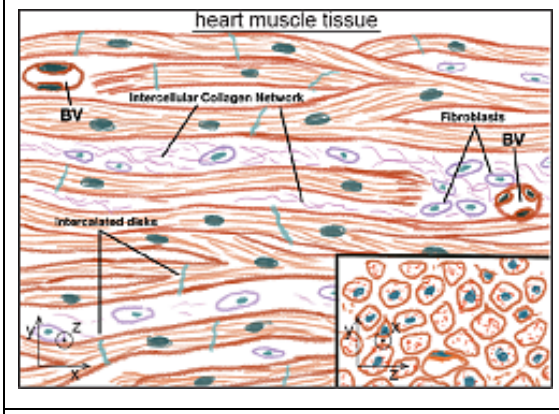
Cardiac engineered tissues to predict toxic drug effects

Micro-engineered cellular systems with human cardiac cells can help to predict drug effects in the heart. These micro-engineered systems involve devices and instrumentation that expose cells to cues that induce the cellular alignment that is observed in the myocardium, that regulate tissue mechanics, and that lead to a three-dimensional architecture.

To further enhance their physiological relevance, different cell types found in the heart are often co-cultured in cardiac cellular systems and exposed to electrical stimulation.

To test and characterize the use of these cardiac cellular systems as preclinical tools, CDER researchers are comparing how drug effects can be detected on different platforms with distinct characteristics that influence the physiology of cultured cells. It should be noted that, to date, such systems cannot replace animal studies examining all types of cardiac injury.

Figure 6: Cytoarchitecture of the myocardium that is aimed to be reproduced in cellular systems



Ribeiro et al. Front Pharmacol 2019, 10:934.

Evaluation of Human iPSC-Derived Hepatocytes as Models of Hepatic Function and Toxicity

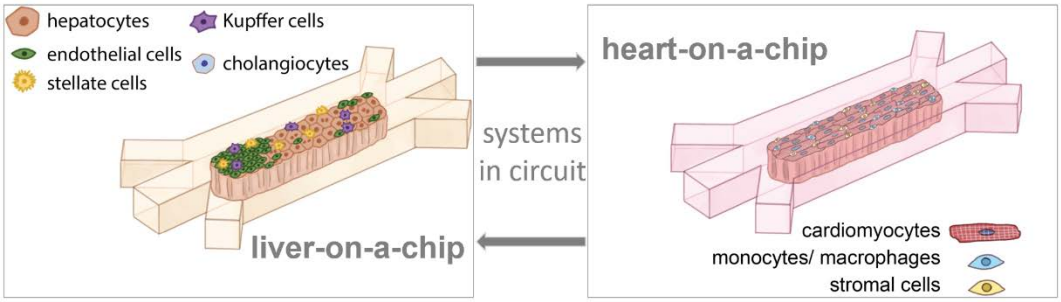
This investigation compares iPSC-derived hepatocytes in planar and spheroid form to primary human hepatocytes in assays of hepatocyte function (drug metabolism and transport) and drug toxicity, to assess their usefulness in drug development. The study focuses on phase II metabolism in these systems, specifically sulfonation and glucuronidation.

Heart-liver Interconnected microphysiological systems and future applications

A given drug may have toxic effects in multiple organs and drug toxicity may depend on liver metabolism. CDER scientists are testing liver-heart interconnected microphysiological systems and how they can be used to predict cardiac drug effects that depend on liver metabolism or cause dual-organ toxicity.

By initially studying drugs with known clinical effects in these interconnected systems, the researchers will evaluate how different chip-based technologies can be combined for specific uses. In addition to these platforms, CDER scientists are investigating the use of lung, kidney, and other organ systems.

Figure 7: Liver and heart microphysiological systems interconnected to facilitate experiments involving both organ systems



Dame et al. Exp Biol Med (Maywood) 2020 doi: 10.1177/1535370220959598.

Center for Devices and Radiological Health

In 2019, the Center for Devices and Radiological Health (CDRH) continued to successfully deliver several in vitro and in silico methods to predict and assess health risks from exposure to medical device extractables and leachables. These achievements highlight CDRH's commitment to develop clinically relevant, non-animal test methods to support biocompatibility evaluations of medical devices.

Strategies for Rapid Risk Assessment of Color Additives Used in Medical Devices

Many polymeric medical devices contain color additives for differentiation or labeling. Although some additives can be toxic under certain conditions, the risk associated with the use of these additives in medical device applications is not well established and evaluating their impact on device biocompatibility can be expensive and time consuming.

CDRH scientists developed a conservative physics-based model, described in terms of parameters, and validated to predict patient exposure to color additives found in medical device polymers. Such health-protective models based on conservative assumptions can be routinely used for estimating a maximum exposure dose for toxicological risk assessment of color additives in medical devices.

When combined with a database of tolerable intake values, the model enables screening-level risk assessments to be conducted instantaneously. Thus, the framework has the potential to not only reduce reliance on new animal testing but also reduce the time, expense, and uncertainty associated with the current toxicological risk assessment paradigm for color additive-containing medical devices.

In terms of regulatory science benefit, adopting a physics-based transport model(s) to estimate a clinically relevant maximum exposure dose that replaces the current non-predictive approach is a priority for FDA.[9] The framework described in the manuscript is the basis for the Color Hazard and RISk calculator (CHRIS), which is a web-based tool[10] that conducts rapid-screening-level risk assessments to aid in the biocompatibility evaluation of polymeric medical device components containing color additives.

These assessments can assist device manufacturers by providing instantaneous feedback on whether the presence of color additives in a device would require additional justification and/or testing to demonstrate acceptable risk for specific biocompatibility endpoints. Use of a simulation-based exposure model is not limited to color additives and can potentially be applied to any leachable chemical found in polymeric medical devices.

Read More:

Toxicological Sciences 172(1), 2019, 201–212

An In Vitro Blood Flow Loop System for Evaluating the Thrombogenicity of Medical Devices and Biomaterials

Blood-contacting medical devices may cause thrombosis and thromboembolic-related complications in patients because of the induction of non-physiologic blood flow patterns and the exposure of blood to foreign materials. Both long-term and short-term use devices can cause thrombosis, resulting in device failure and patient morbidity or death.

Preclinical thrombogenicity evaluation is important for ensuring the safety of blood-contacting devices and biomaterials and is generally needed for regulatory approval/clearance of new devices. One commonly used thrombogenicity evaluation method is an in vivo nonanticoagulated venous implant (NAVI) assay conducted in dogs or other large animals. A reliable in vitro dynamic test method to evaluate device thrombogenicity was needed to improve the design and safety of blood-contacting medical devices, while reducing the use of animal studies. Therefore, CDRH developed a recirculating, in vitro flow loop system for thrombogenicity testing that reduced variabilities in existing tests by standardizing parameters, such as test duration, blood temperature, and inherent inter- and intra-species differences in blood. The dynamic test loop system was able to effectively differentiate medical device materials with different thrombogenic potentials.

[9] National Academies of Sciences, 2017; U.S. FDA, 2019
[10] https://dsaylor.github.io/CHRIS/

***Read More*:**

ASAIO Journal 2020, 66(2):183-189.

Coauthors and affiliation**:** Megan A. Jamiolkowski, Matthew C. Hartung, Richard A. Malinauskas, and Qijin Lu; Center for Devices and Radiological Health (CDRH), Office of Science and Engineering Laboratories (OSEL).

Medical Device Development Tools (MDDT) Qualification Program

CDRH is continuing to expand acceptance of alternative information and non-animal testing to support biocompatibility evaluations of medical devices. The Center's guidance on Qualification of Medical Device Development Tools explains how new tools can be developed and qualified for a specific context of use, so that qualified tools can be used to support regulatory submissions to the Center. The policy outlined in the guidance is applicable to in vitro models to replace animal testing, where appropriate. Slides, an audio presentation, and a transcript of a webinar presented to answer questions on the guidance can be found on FDA's website. To date, biocompatibility or toxicology tools are not yet qualified under the Center's Medical Device Development Tool program. Once qualified, these tools will be published in the Medical Devices section of FDA's website.

Center for Veterinary Medicine

Previously, clinical endpoint bioequivalence (BE) trials were the only mechanism for assessing the comparability of formulations containing drugs that are not systemically absorbed. In many situations, this translated to the need to conduct a clinical endpoint trial for every indication on the pioneer label,[11] rendering it frequently untenable for generic animal drug sponsors.

Types of formulations involved included ophthalmic products, intramammary dosage forms used in treating bovine mastitis, many Type A medicated articles, and those products containing drugs that are intended to treat canine intestinal parasites.

A Type A medicated article is an FDA-approved product with standardized potency that contains one or more new animal drugs intended for use in the manufacture of another medicated article or a medicated feed. Each of these trials typically required many animals, often necessitated creating artificial infections, and were both time-consuming and expensive.

Moreover, those studies involving the treatment of canine GI parasites required the sacrifice of each of the study animals to generate the necessary clinical data.

Currently, through an understanding of drug physicochemical properties, formulation-critical quality attributes, and, in some cases, the use of physiologically based pharmacokinetic (in silico) models, the Center for Veterinary Medicine (CVM) is developing roadmaps for alternative approaches for the BE evaluation of these various types of products. These alternatives will

[11] The original label on the first product.

enable a highly efficient and reliable drug product evaluation that avoids the need for artificial infections and animal sacrifice and is both time and cost effective.

For the Type A medicated articles, the three-pronged approach has been established (guidance is under development) that combines SUPAC[12]-like concepts, in conjunction with biowaiver concepts described in several CDER product-specific guidance documents, to enable the assessment of Type A medicated articles containing non-systemically absorbed low solubility compounds (Abbreviated New Animal Drug Application ANADA 200-639). This approach is the subject of a guidance under development.

For ophthalmic products, CVM collaborated with CDER on research supporting an in vitro BE approach for comparing canine cyclosporine ophthalmic preparations that is now considered the CVM approach for determining the BE of these kinds of formulations.[13]

CVM is also working with CDER experts to identify an in vitro roadmap for comparing intramammary products. Lastly, we are involved in an extensive research project that will test the appropriateness of an in vitro BE approach for assessing products containing both systemically available and locally acting (within the canine GI tract) drugs. Successful confirmation of the in vitro approach's ability to accurately assess the BE of formulations acting within the canine GI tract will provide the basis for future product approvals and CVM guidance.

National Center for Toxicological Research

Evaluation of Patient-Specific Induced Pluripotent Stem-Cell Derived Cardiomyocytes (iPSC-CMs) to Improve Oncology Drug-Induced Cardiotoxicity Detection and Prediction

The rapid development of novel oncology drugs has revolutionized cancer treatment. However, some anticancer agents are associated with severe cardiotoxicity that was not revealed by conventional animal toxicity studies. In some cases, these toxicities are not observed until late-stage clinical trials or even after drug approval. Therefore, there is a need to develop translational and sensitive preclinical approaches to improve drug-induced cardiotoxicity detection in humans early in the drug development process and well before clinical trials are initiated for some drug classes.

Patient-specific iPSC-CMs are generated from individuals with diverse genetic backgrounds and represent a promising alternative, non-animal model to evaluate drug-induced cardiotoxicity at the population level and may enable personalized cardiac safety prediction.

To explore the potential of iPSC-CMs for cardiotoxicity detection and prediction, in collaboration with researchers from the Medical College of Wisconsin, scientists at the National Center for Toxicological Research (NCTR) are employing a large panel of patient-specific iPSC-CMs to evaluate the molecular and genetic mechanisms underlying oncology drug-induced cardiotoxicity.

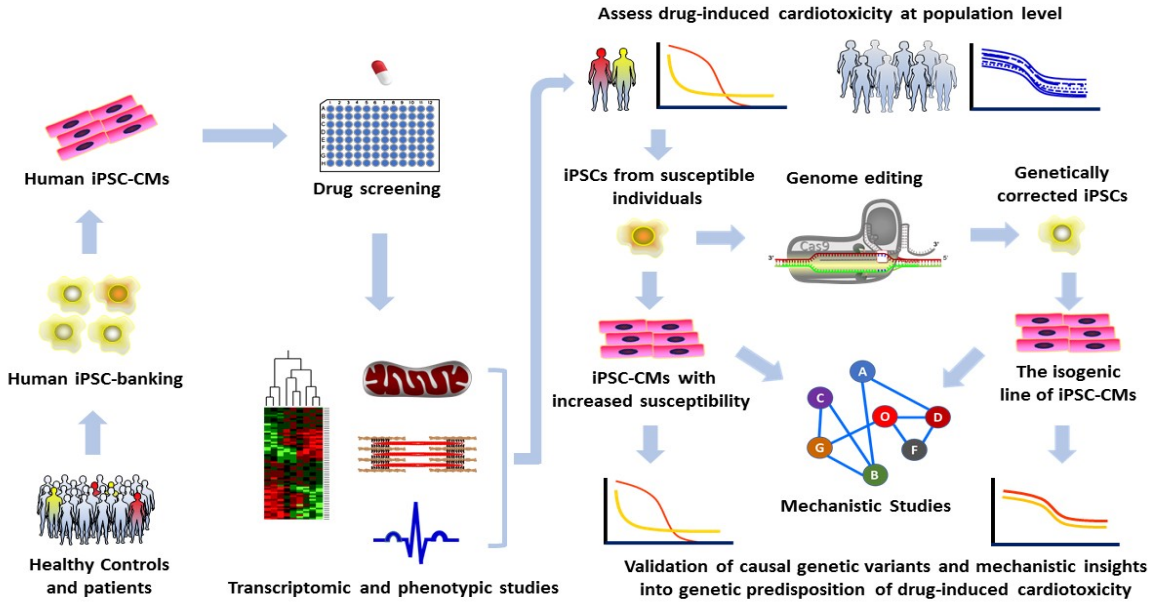
[12] Scale-up and Post-Approval Changes

[13] Dong Y, Qu H, Pavurala N, et al. Formulation characteristics and in vitro release testing of cyclosporine ophthalmic ointments. *Int J Pharm*. 2018;544(1):254-264. doi:10.1016/j.ijpharm.2018.04.042.

The studies may identify translational biomarkers that can be used to distinguish patients with different risk levels for anticancer drug-induced heart damage and, ultimately, guide clinical cardio-oncology practice in patient stratification and therapeutic regimen selection.

The comprehensive analysis of the functional parameters and cellular/molecular endpoints generated with patient-specific iPSC-CMs can also aid in establishing regulatory standards in evaluating the quality of human iPSC-CMs.

Figure 8: Human ALI Airway Epithelial Tissue Model



Human ALI Airway Epithelial Tissue Model

Rodent and nonrodent studies are considered valuable for evaluating the health risks posed by respiratory toxicants. However, there are challenges with these studies, such as differences in physiology and anatomy between animals and humans, the complexity of conducting standardized in vivo inhalation studies, and the significant resources required to conduct such studies.

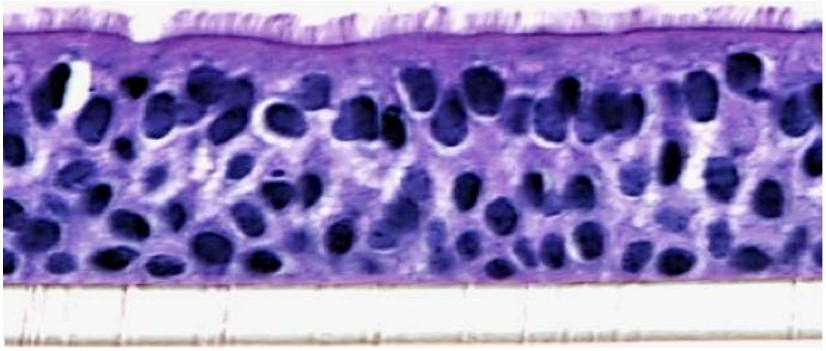
Alternative in vitro approaches, therefore, are under development to supplement or possibly replace animal studies. A key consideration of selecting an appropriate in vitro respiratory test system is its relevance to human lung physiology and ability to respond to inhalation exposures similarly to humans. A possible alternative approach is the human in vitro air-liquid-interface (ALI) airway epithelial tissue model which closely mimics the structure and function of in vivo airway epithelium. In addition, it is possible to measure a battery of tissue responses in these cultures that are highly related to the etiology of human respiratory diseases.

Research to integrate genotoxicity assays into the ALI culture-based test system for assessing the mutagenicity and carcinogenicity of carcinogens is also underway to extend the applicability of this system. A feature of note is the presence of the air interface in the ALI cultures that

allows mimicking human inhalation exposures in an in vitro system with regard to the physical form and exposure doses of test articles. These characteristics recommend the ALI airway system as a potential alternative respiratory model for toxicity assessment.

By using a test platform consisting of the ALI cultures and disease-relevant toxicity endpoints, NCTR scientists have evaluated the respiratory toxicity of a panel of inhaled substances, including tobacco smoke constituents, tobacco smoke, active ingredients in consumer products, and environmental pollutants.

Figure 9: H&E staining of the ALI airway tissue models



The luminal surface facing the air is up and basolateral side adhering to the microporous membrane faces down. CC: ciliated cell; GC: goblet cell; BC: basal cell.

Read More:

Cao, X.; Wang, Y.; Xiong, R.; Muskhelishvili, L.; Davis, K; Richter, P.; Heflich, R.H. (2018) Cigarette Whole Smoke Solutions Disturb Mucin Homeostasis in a Human *in vitro* Airway Tissue Model. *Toxicology*, 409:119-128.

Xiong, R.; Wu, Q.; Muskhelishvili, L.; Davis, K.; Shemansky, J.M.; Bryant, M.; Healy, S.; Rosenfeldt, H.; Cao, X. (2018) Evaluating Mode of Action of Acrolein Toxicity in an in vitro Human Airway Tissue Model. Toxicological Sciences, 166:451-464.

Wang, Y.; Wu, Q.; Muskhelishvili, L.; Davis, K.; Bryant, M.; Cao, X. (2019) Acute Toxicity of Aerosolized Dihydroxyacetone in an in vitro Human Airway Epithelial Tissue Model. Toxicology In Vitro, 59:78-86.

Development of In Vitro Testicular Models For Evaluating The Reproductive Toxicity Of Regulated Products

Somatic testicular cells and male germ cells are susceptible to chemical and physical agents that may adversely affect fertility or the health of offspring. However, the conventional tests used for identifying testicular toxicity and male germline mutagenicity are labor-intensive and

require large numbers of animals, while suitable in vitro models for investigating toxic events in testicular cells are lacking.

Development of in vitro testicular models for evaluating the potential reproductive toxicity of regulated products would advance safety assessments while reducing the number of animals needed for toxicity testing. To this end, in vitro 3D testicular models using testicular cells from laboratory animals are currently under development and characterization. To date, the 3D testicular models appear to organize with the same cell types and similar tissue architecture as found in native testicular tissues. We also have succeeded in measuring cytotoxicity and changes in metabolic function and gene expression following exposure of 3D testicular models to toxic substances.

Using this in vitro testicular model approach to evaluate product safety is expected to complement animal studies by providing information for study design, agent prioritization, and dose selection before committing to animal studies, thus reducing the number of animals used. These models also may provide information for more accurate interpretation of testicular toxicity data derived from animal studies.

Agency-wide Working Groups

Maintaining a well-trained, well-connected cadre of scientists with state-of-the-art expertise is critical to FDA's ability to successfully integrate emerging sciences and technologies into the Agency's research and review processes. To that end, FDA's Office of the Chief Scientist established cross-agency working groups to focus on the Agency's highest-priority scientific areas. Drawing on the know-how of FDA scientists at the forefront of their fields, the working groups serve FDA senior leadership as a scientific information resource. Among other activities, the working groups publish reports, conduct public workshops, share their knowledge with the scientific community in educational webcasts, and offer specialized training for FDA scientists.

The Alternative Methods Working Group

For years, FDA has promoted the development and use of new technologies to better predict human and animal responses to substances relevant to its regulatory mission. Recognizing that a platform was needed for informing the public of FDA's progress in fostering the development and implementation of alternative test methods, in 2019 the Agency formed its Alternative Methods Working Group (Working Group).

The Working Group seeks opportunities to advance innovative technologies and tools as well as new and potential applications of alternative systems (in vitro, in vivo, in silico, and systems toxicology modeling)[14] that offer alternative methods to traditional toxicity and efficacy testing across FDA's product areas.

[14] *In vitro* refers to the technique of performing a given procedure in a controlled environment outside of a living organism; *In vivo* refers to experimentation using a whole, living organism as opposed to a partial or dead organism; *In silico* is an expression used to mean "performed on computer or via computer simulation;" systems toxicology is the integration of classical toxicology with quantitative analysis of large networks of molecular and functional changes occurring across multiple levels of biological organization.

Additionally, the Working Group explores opportunities and viable ways by which emerging methods and new technologies can be advanced to support scientific and regulatory review of FDA-regulated therapeutic products. The Working Group recently launched a webinar series that will give developers the opportunity to present their new methods and methodologies exclusively to FDA scientists. Continuing education in new predictive in vitro, in vivo, and in silico methods is vital to ensuring that FDA regulators and researchers have a broad skill set and remain current with the latest trends in science and technology.

The activities of the Working Group are informational and do not serve as official regulatory guidance.

FDA Stem Cell and In Vitro Microphysiological Systems (MPS) User Group

FDA has a stem cell-MPS user group to discuss internal research, familiarize the Agency with platforms created by external individuals and companies, and obtain seminars from outside researchers. This *horizon-scanning* will position FDA to make certain it has appropriate training and research internally to enable it to use novel data submitted by a sponsor.

Modeling and Simulation Working Group

Computational (in silico) modeling and simulation are powerful tools that complement traditional methods—such as in vitro and in vivo testing—for gathering evidence about FDA-regulated products or developing FDA policy.[15] FDA scientists routinely review modeling and simulation results submitted by industry and use modeling and simulation for scientific research and regulatory decision-making.

In 2016, to reduce the Agency's silos of knowledge and experience in this field, disseminate information across different product centers, and collaborate on projects, FDA launched the Modeling and Simulation Working Group, bringing together the expertise of nearly 200 FDA scientists from across the Agency.

FDA's efforts towards advancing the use of modeling and simulation to transform regulatory pathways were presented by the Working Group's Chair in FDA's monthly Grand Rounds webcast to the scientific community. Her highly informative discussion detailed the FDA Working Group's national and international outreach activities with government and industry to raise awareness about how FDA is harnessing modeling and simulation. Methodologies, success stories with simulation from across FDA product centers, and discussion on the potential for in silico clinical trials for advancing medical products were also presented. The full webcast is available on the FDA.gov website.[16]

The Working Group, managed out of FDA's Office of the Chief Scientist, is organized across six interest groups, with a leadership circle comprising two representatives from each FDA product

[15] See FDA Grand Rounds presentation: https://collaboration.fda.gov/p4r7q3qweuv/?proto=true on slide Simulating Potential Policy Effects on U.S. Public (0:14:57)

[16] Dr. Tina Morrison's informative talk can be viewed here: https://collaboration.fda.gov/p4r7q3qweuv/?proto=true

center and FDA's National Center for Toxicological Research (NCTR), along with one representative from the Office of Regulatory Affairs (ORA).

Affiliated with the Working Group are six modeling interest groups: statistical modeling, risk assessment modeling, big data-based modeling, as well as chemical-based, mechanistic-based, and physics-based modeling interest groups. The Modeling and Simulation Working Group also welcomes the participation of FDA employees and contractors.

Key objectives of the Working Group include:

- Raising awareness about modeling and simulation to advance regulatory science for public health
- Fostering enhanced communication about modeling and simulation efforts among stakeholders
- Serving as a scientific resource on modeling and simulation and emerging technologies for FDA
- Collaborating with national and international organizations pursuing similar activities and
- Promoting consistent review and decision-making with modeling and simulation across FDA

Figure 10: Modeling and Simulation Working Group Membership

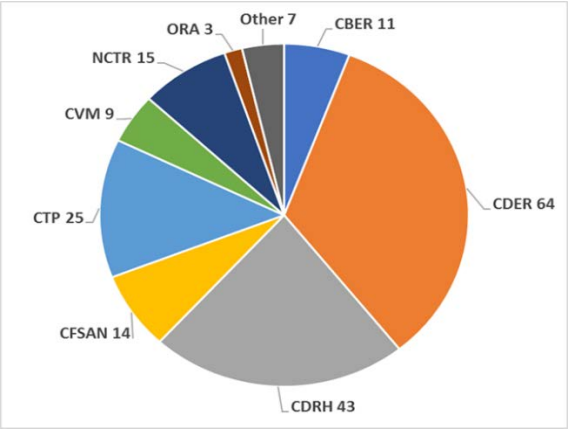
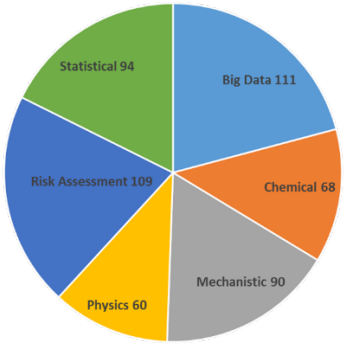


Figure 11: Modeling and Simulation Working Group Interest Groups:



FDA Partnerships

Botanical Safety Consortium

In February 2019, FDA announced policies to modernize the oversight and regulation of dietary supplements. The steps FDA is taking to implement these policies include collaborating with industry to support the development of new products, ingredients, and delivery systems while protecting public health and safety. To support this collaboration, FDA, the National Institute of Environmental Health Sciences (NIEHS) , and the non-profit Health and Environmental Sciences Institute (HESI) signed a memorandum of understanding establishing the Botanical Safety Consortium (BSC). The consortium includes more than 20 participants from industry, academia, and government to promote scientific advances in evaluating the safety of botanical ingredients and mixtures in dietary supplements. This group will look at original ways to use novel toxicology tools, including alternatives to animal testing, to promote the goals of safety and effectiveness.

Health and Environmental Sciences Institute

The Health and Environmental Sciences Institute (HESI) and FDA have partnered to improve public health by reducing unanticipated cardiovascular-related adverse effects from drugs. This partnership is largely through the HESI Cardiac Safety Committee, a multi-sector, international group of experts who identify and address cardiovascular safety issues.

HESI, FDA's Center for Drug Evaluation and Research, and the National Toxicology Program entered into a memorandum of understanding in 2018 to address the need for newer, mechanistic-based technologies to assess cardiovascular risk and drug safety. Through this partnership, HESI is convening numerous stakeholders to gather new evidence to support real-world applications and gaining confidence in new technologies that will support accurate and efficient decision-making for drug development.

These new technologies include the use of 2D and 3D cell-based assays using iPSC-CMs and platforms such as microelectrode arrays, high content imaging/optical analyses, microphysiological systems, and computer-based models.

The Developmental and Reproductive Toxicology Technical Committee has several projects focused on non-animal/alternative approaches, each of which has active FDA participation. These include a program focused on developing quantitative adverse outcome pathways (AOP) and knowledge of development and reproductive toxicity (DART) effects ("DARTable genome") and another using quantitative structure-activity relationship (QSAR) models to predict placental transfer of xenobiotics.

A new working group focused specifically on New Approach Methodologies (NAMs) was also recently initiated. In collaboration with the BSC DART group, the NAMs group aims to create a toolbox that can help define and clarify the context of use for alternative assays that comply with various regulatory guidelines.

The Genetic Toxicology Technical Committee recently formed an in vitro working group that has several FDA participants. This working group is focused on critically evaluating NAMs for in vitro genotoxicity testing, envisioning how NAMs could expand current in vitro genotoxicity testing strategies, and making recommendations for creating an "in vitro only" approach for genotoxicity testing that would meet the needs of various regulatory decision-makers. This working group is also communicating with the BSC Genotox group on possible synergies and collaborations.

Interagency Coordinating Committee on the Validation of Alternative Methods

The Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM) is an interagency committee of the U.S. government that coordinates technical reviews of alternative test methods and cross-agency activities relating to validation, acceptance, and harmonization of test methods. ICCVAM facilitates the development, validation, and regulatory acceptance of test methods that replace, reduce, or refine the use of animals in testing.

FDA representatives from all centers participate in the ICCVAM monthly meetings, public forums, and Scientific Advisory Committee on Alternative Toxicological Methods meetings and work groups. FDA chairs the ICCVAM Metrics Work Group and the Developmental and Reproductive Testing Special Interest Group.

Tox21

Tox 21 is a federal consortium consisting of the EPA, NCATS, NTP, and FDA. During the last decade, the Tox21 consortium has developed and evaluated in vitro high throughput screening (HTS) methods for hazard identification and mechanistic insight.

Tox21 has generated data on thousands of pharmaceuticals and data-poor chemicals; characterized the limits and applications of in vitro methods; and enabled incorporation of HTS data into regulatory decisions. To more broadly address challenges in the field of toxicology, Tox21 released a new strategic and operational plan[17] that expands the focus of its research activities. This plan proposed that Tox21 expand its portfolio of alternative test systems;

[17] See The U.S. Federal Tox21 Program: A strategic and operational plan for continued leadership at: https://www.altex.org/index.php/altex/article/view/711

address technical limitations of in vitro test systems; curate legacy in vivo toxicity testing data, establish scientific confidence in in vitro test systems; and refine alternative methods for characterizing pharmacokinetics and in vitro assay disposition. The new Tox21 strategic and operational plan addresses key challenges to advance toxicology testing and will benefit both the organizations involved and the toxicology community.

Global Partnerships

International Cooperation on Cosmetic Regulation

FDA is one of the founding Steering Committee members of the International Cooperation on Cosmetics Regulation (ICCR). Established in 2006, ICCR is an international group of regulatory authorities from Brazil, Canada, the European Union, Japan, and the United States. ICCR members work together to promote regulatory alignment to maximize consumer protection while minimizing barriers to trade.

Since its inception, ICCR has recognized the importance of reducing, refining, and replacing (the 3Rs) animal testing. In addition, ICCR has encouraged regulators, industry, and validation centers in developing and validating scientific alternatives to animal testing.

It has worked with ICCVAM, the European Union Reference Laboratory for Alternatives to Animal Testing (ECVAM), the Japanese Center for the Validation of Alternative Methods (JaCVAM), and appropriate representatives from other governments associated with ICCR to foster these efforts. This includes establishing the International Cooperation on Alternative Test Methods (ICATM) to address validation studies; independent peer review of scientific validity of test methods; and the development of formal test method recommendations.

In conjunction with ICATM, ICCR has provided and updates the list of such validated tests on its website and has also continued to engage in discussions with animal rights groups during annual stakeholder meetings.

Organisation for Economic Cooperation and Development Test Guidelines Programme

The website of the Organisation for Economic Cooperation and Development (OECD) Test Guidelines Programme defines the OECD Guidelines for the testing of chemicals as "a collection of the most relevant internationally agreed testing methods used by governments, industry and independent laboratories to assess the safety of chemicals. They are primarily used in regulatory safety testing and subsequent chemical notification and registration. The set of Test Guidelines is updated on a regular basis to keep pace with progress in science and countries' regulatory needs. OECD-wide networks of national coordinators and national experts provide input from scientists in government, academia, and industry."

FDA experts have participated in many OECD Working Expert Groups such as Developmental Neurotoxicity, Miniaturised AMES Test, Detailed Review Paper (DRP) Retinoid Pathways, Transgenic Gene Mutation Assays, Phototoxicity, Non-Genotoxic Carcinogenicity, Skin

Sensitisation, Skin Irritation/Corrosion, Ocular Corrosives and Irritations, Toxicokinetic, and Defined Approaches to Skin Sensitization.

FDA subject matter experts have reviewed and provided comments on various requests from the OECD respecting draft test guidelines, standard project submission forms, and related discussions. Many of these efforts have as a goal the eventual development of an OECD Test Guideline or Guidance that incorporates New Approach Methodologies.

ICH and 3Rs

International harmonization of the nonclinical recommendations for pharmaceutical development through the International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use (ICH) continues to reduce repetition of studies and reduces and refines animal use in overall drug development.

Harmonization of recommendations for nonclinical studies provides a common understanding of a standard development program, which means that pharmaceutical companies are less likely to conduct different or additional studies to meet different requirements for the participating ICH regulatory authorities.

In addition, ICH guidances describe a stepwise approach to the conduct of nonclinical studies, which avoids conducting unnecessary studies if a drug does not progress to later stages of development. All ICH guidances on nonclinical topics encourage and allow the use of alternative methods, when available and scientifically relevant. Newly finalized guidances will continue to contribute to the 3Rs as will future guidances.

Revisions to the ICH guidance on the evaluation of toxicity to reproduction (ICH S5(R3)) have been finalized. The revisions expand circumstances under which the outcome of "preliminary EFD studies" (per ICH M3(R3)) can support clinical trials, which could lead to fewer animal studies being conducted. In addition, the revised guidance provides basic principles that will assist in the development and potential regulatory use of alternative assays for evaluating adverse effects on embryofetal development.

EU-ToxRisk

The EU-ToxRisk is a European collaborative project funded by the EU Framework Program for Research and Innovation Horizon 2020 to advance mechanism-based toxicity testing and risk assessment of chemicals. EU-ToxRisk is focused on developing alternatives in four specific areas: Organs on a Chip, Developmental and Reproductive Toxicity, Thresholds of Toxicological Concern, and Repeat Dose Toxicity Testing, concentrating on case studies in these four areas. FDA scientists, members of the EU-ToxRisk Regulatory Advisory Board, provide critical regulatory information and offer critiques on the progress of the case studies and in the general discussion of how the science is progressing.[18] FDA works with members of EU-ToxRisk through out the year, meeting annually with EU-ToxRisk representatives at the Society of Toxicology Meeting.

[18] https://www.eu-toxrisk.eu/page/en/partners/regulatory-advisory-board.php

Berlin 2019 Stakeholder Meeting on Adopting Microphysiological Systems

Stakeholders Working Together to Advance Alternatives

FDA representatives were among the 46 experts from global regulatory agencies, MPS suppliers, industry, and academia who gathered at a three-day workshop in Berlin in June 2019 to identify the challenges and solutions for adopting microphysiological systems (MPS).[19] Together, the experts analyzed the hurdles to overcome for MPS systems to be adopted by the pharmaceutical industry and ultimately acceptance in regulatory approval processes.

Discussion spanned the state-of-the-art of the technology, research highlights, industrial application in drug development, barriers for acceptance, community-building initiatives, as well as regulatory and ethical dilemmas and the future perspectives. Experts perceived qualification and testing of MPS-based assays as major challenges. Workshop stakeholders promoted independent testing centers as a means of building confidence in the usefulness of MPS-based models. A notable outcome of the workshop was the recommendation to close the communication gap between all stakeholders involved.

A report titled, *Biology-inspired Microphysiological Systems to Advance Patient Benefit and Animal Welfare in Drug Development*[20] summarizing workshop discussions was issued in February 2020. The report also provides information on the different MPS systems and assays currently used in industry for internal portfolio decision-making. All stakeholders were moving in the same direction of global regulatory acceptance.

Global Summit on Regulatory Science

The Global Summit on Regulatory Science (GSRS) was established in 2011 to foster the exchange of views among international researchers, regulators, and policy-makers on how best to develop and implement innovative methodologies--including alternative methods in regulatory assessments. The annual GSRS is organized by the Global Coalition for Regulatory Science Research (GCRSR), comprising regulatory science leaders from around the globe. The FDA NCTR Director serves as co-chair of the GCRSR's executive committee and works with

[19] The workshop was sponsored by Center for Alternatives to Animal Testing (CAAT) Europe.
[20] See: https://www.altex.org/index.php/altex/article/view/1526

coalition members to promote global research interactions. The 9th Annual GSRS was hosted by the GCRSR and the Joint Research Centre/European Commission (JRC/EC). A report on the summit proceedings is available on the JRC/EC website.[21]

The 2019 Global Summit on Regulatory Science, hosted by the JRC at Lago Maggiore, Italy, attracted some 200 participants from around the world

Due to the COVID-19 pandemic, the 2020 Global Summit on Regulatory Science was held virtually on September 29-30. More than 1,100 people worldwide registered to hear international experts, including FDA senior scientific leadership, speak on *Emerging Technologies and Their Application to Regulatory Science*. Sessions included a global perspective on artificial intelligence, microphysiological systems, stem cells, bioimaging, OMICs (genomics, proteomics, metabolomics, glycomics), biomarkers, precision medicine, and the human microbiome. For more information on the 2020 GSRS and recorded speaker presentations, visit the GSRS webpage.[22]

[21] See: https://ec.europa.eu/jrc/sites/jrcsh/files/gsrs19-agenda-and-abstract-online-after.pdf
[22] https://www.fda.gov/media/142526/download

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