Memorandum of Understanding

Between

U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES (HHS)
NATIONAL INSTITUTES OF HEALTH (NIH)
National Institute of Environmental Health Sciences (NIEHS)
Division of the National Toxicology Program (DNTP)

And

U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES (HHS)
NATIONAL INSTITUTES OF HEALTH (NIH)
National Center for Advancing Translational Sciences (NCATS)
Division of Preclinical Innovation (DPI)

And

U.S. ENVIRONMENTAL PROTECTION AGENCY (EPA)
Office of Research and Development (ORD)

And

U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES (HHS)
U.S. Food and Drug Administration (FDA)

Concerning

Research to Advance Toxicity Testing

I. PURPOSE/OBJECTIVES/GOALS

This four-Partner Memorandum of Understanding (MOU) sets in place the mechanism to renew and strengthen the existing collaborations between NIEHS/DNTP, NCATS/DPI, EPA/ORD, and the FDA (hereinafter jointly referred to as "Partners" and individually as "Partner") in the research to advance the science of toxicology, toxicity testing, and its application to regulatory decision making. A central component of previous MOUs was to develop and apply high throughput screening (HTS) and high content (HC) assays using cell culture systems, phylogenetically lower animal species (e.g., zebrafish), and three-dimensional (3D) cell culture systems and tissue models, together with high throughput whole genome analytical methods, for hazard identification and to evaluate mechanisms of toxicity relevant to human health concerns. While continuing to support these areas of research, we are expanding the focus areas to address key challenges in advancing toxicology in the 21st century, that if successful, will have

substantial benefit to each organization regardless of differences in their unique missions. The goals of this MOU are to (1) develop an expanded portfolio of alternative test systems, (2) address technical limitations of *in vitro* test systems, (3) curate legacy *in vivo* toxicity testing data, (4) establish scientific confidence in the *in vitro* test systems with relevance to human health, and (5) refine alternative methods for characterizing pharmacokinetics and *in vitro* disposition. Success in achieving these goals is expected to result in more scientifically and economically efficient test methods for toxicity testing, and more biologically based models for risk assessment. Consequently, the reduction, replacement, and refinement (i.e. the more strategic use of animals in regulatory testing), also known as "the 3Rs," is anticipated to increase the ability of the Partners to more rapidly evaluate the safety profiles of chemicals of interest.

BACKGROUND

The convergence of science, technology, regulatory need, and public opinion has produced an historic opportunity to transform toxicology and risk assessment into more accurate, rapid, and cost-effective sciences. In recognition of the need for a long-term, multiple Federal agency commitment, this MOU is being renewed to expand the focus of the collaboration to address key challenges in advancing toxicology in the 21st century. The three previous MOUs focused on identifying and evaluating hazard and mechanism-based alternative approaches. This MOU shifts that focus toward both advancing the science in toxicology as well as overcoming impediments to implementing these type of approaches, such as understanding the limitations of our current and alternative approaches as well as ways to more efficiently translate the results into regulatory decisions. In addition, this MOU builds on a number of separate and joint efforts among our four organizations. Building on the strengths of the individual organizations is intended to facilitate the advancements necessary to move toxicology to a more predictive science based on the most human-relevant and meaningful tools of modern molecular biology and chemistry and to leverage the resources of these federal agencies to move more effectively and efficiently toward these common goals.

II. AUTHORITIES

EPA/ORD enters into this MOU pursuant to Section 103 of the Clean Air Act [42 U.S.C. §7403 (a) and (b)]; Section 104 of the Clean Water Act; [33 U.S.C. § 1254 (a) and (b)]; Section 300 j-l of the Safe Drinking Water Act (42 U.S.C. §1442); Section 10 of the Toxic Substances Control Act [15 U.S.C. § 2609 (a)]; and Section 20 of the Federal Insecticide, Fungicide, and Rodenticide Act [7 U.S.C. § 136r (a)].

NIEHS/DNTP enters into this MOU pursuant to Sections 301, 401, and 463 of the Public Health Service Act [42 U.S.C. § 241, 281, and 285*l*].

NCATS/DPI enters into this MOU pursuant to Section 301 and 479 of the Public Health Service Act [42 U.S.C. § 241 and 287].

FDA enters this MOU pursuant to Section 301 of the Public Health Service Act [42 U.S.C. § 241] and Section 1003 of the Federal Food, Drug, and Cosmetic Act [21 U.S.C. § 393].

III. ROLES AND RESPONSIBILITIES

Each Partner intends to implement the following provisions of this MOU, under the responsibility of the Assistant Administrator for ORD, the NIEHS/DNTP Scientific Director, the NCATS Director, and the Commissioner of Food and Drugs.

A. Alternative Test Systems: A shared focus of all Partners is to identify and/or develop new approach methodologies (NAMs), or alternative approaches to current *in vivo* hazard models, specifically focusing on *in vitro* assays that comprehensively capture the potential toxicological effects of chemicals, allow translation of molecular and pathway perturbations to effects at the tissue-, organ-, and organism-level, and capture potential population variability in toxicodynamic responses. The Partners acknowledge that new approaches in this area are needed, such as the use of targeted and/or global gene expression methods that allow for the analyses of thousands of genes directly from cell lysates; upgraded imaging modalities to reveal fast transient cellular changes; and complex 3D cellular organoid structures that capture potential effects in a tissue or organ. The Partners also acknowledge that complex model systems such as alternative species (e.g., zebrafish) and organotypic culture models, microscale tissues, and microphysiological systems are promising approaches that incorporate a higher level of complexity to augment the simpler HTS assays previously employed. Furthermore, translating toxicity testing data into risk assessment requires an understanding of population variability and identification of susceptible populations. All Partners intend that this aim will best be accomplished through collaborative experimental investigations, transparent and free public access, regular joint meetings, engagement with external stakeholders, and advice from acknowledged experts in a variety of disciplines in the international scientific community. The Partners intend to identify data gaps where research and development are needed and/or to develop new experimental methods designed to allow a more comprehensive evaluation of how compounds interact with key steps in critical pathways.

B. Toxicokinetics and In Vitro Disposition: The ability to accurately translate *in vitro* potency estimates to the equivalent administered dose requires understanding the toxicokinetics of a substance. Further, it is known that active and passive disposition of chemicals in *in vitro* assays (e.g., binding to plastic, transport inside or outside the cell, binding to media proteins) may significantly bias potency estimates for some chemicals. The Partners intend to collaborate on developing and evaluating new methods and computational modeling approaches that better predict the relationship between target tissue concentrations and external doses of chemicals. In addition, the Partners intend to collect the experimental and computational data necessary to incorporate *in vitro* disposition into estimates of effective potency and efficacy.

C. Artificial Intelligence: Great strides have been made using artificial intelligence approaches (e.g., machine learning, natural language processing) to improve our knowledge of adverse events. All Partners have used such approaches in the past and will in the future to assess potential toxicity of compound classes, predict the types of toxicity (e.g., hepatotoxicity), etc. All Partners agree that this area would be fruitful to improve current approaches and potentially address the 3Rs and will work together.

D. Assay Technical Limitations: Despite considerable progress in developing and advancing *in vitro* approaches, there remain technical limitations in their broad application for toxicity

testing. Specific challenges include a lack of physiologically relevant metabolic competence for many of the assays, testing with a limited range of solvent vehicles (e.g., DMSO), limited coverage of important cellular and intracellular processes, limited duration exposures, and the limited representation of complex cell and tissue interactions. The Partners intend to work together to adapt existing methods or develop new methods to systematically address the main technical challenges that face *in vitro* test systems.

E. In Vivo Study Curation: *In vivo* data provides a rich resource with which to help interpret the *in vitro* test systems. Understanding the qualitative and quantitative variability associated with these studies will be critical for understanding how they may differ from the new *in vitro* testing approaches. The Partners intend to work together to identify and curate these legacy, non-proprietary toxicity studies, enter the data into a computable form, and harmonize ontologies used to characterize the toxicity studies.

F. Establishing Scientific Confidence: Traditional approaches to validating *in vitro* and *in silico* approaches to toxicity testing are largely unsustainable; taking many years to complete, requiring significant resources, and typically focusing on a one-for-one replacement of a specific regulatory endpoint of interest. However, regulatory agencies must have confidence that the new approaches provide data that are reliable, reproducible, and relevant to the intended context of use. The Partners intend to work together to perform research that informs the development of an evaluation framework for the definition of performance standards which can be used to establish confidence in the new approaches.

***G. Chemical Libraries*:** The Partners have previously identified large numbers of compounds with existing toxicological data for testing in the identified assays and alternative animal/tissue models. These compounds were used to create the "Tox21 10K Library." Large scale testing of this compiled "Tox21 10K Library" has been completed for some 70 assays; additional screening for providing new insights into cellular targets and signaling pathways not yet explored will continue. In addition, the Partners intend to create smaller "reference libraries" of compounds relevant to specific toxicities, drawn from the existing Tox21 library based on the known toxicity information held by the Partners. The Partners' member organizations intend to share toxicity and exposure information on compounds selected for testing that is not confidential or trade secret. The Partners also intend to jointly determine appropriate quality assurance/quality control procedures for the compounds chosen for testing, and to make the analytical chemistry information on these compounds publicly available.

H. Analysis and Bioinformatics: Analysis of individual *in vitro* assay results (i.e., identifying active and inactive compounds for a particular assay) and bioinformatics (i.e., evaluating sets of data from multiple *in vitro* and *in vivo* assays while taking into account chemico-physical properties for significant relationships) are critical to the success of the joint initiative. As a result, the Partners intend to (1) collaborate on the development of the most appropriate tools for the analysis of *in vitro* data; (2) share non-confidential data (both *in vitro* as well as that generated using traditional test methods); (3) employ computational approaches to evaluate the information from *in vitro* studies, and (4) work to make all the data publicly accessible through publication and deposition into PubChem. The NIEHS/DNTP, NCATS/DPI, EPA/ORD, and FDA intend to undertake targeted *in vivo* follow-up studies when appropriate to further characterize specific compound activities. The Partners also intend to consider the use of

extramural mechanisms to support these activities. Proof-of-concept studies will be important to demonstrate the feasibility of new approaches and their undertaking will require a critical level of effort across institutions. It is envisioned that these efforts will evolve towards a systems-biology approach as a foundation for constructing and using biologically based dose-response models in risk assessment. Regulatory acceptance of these new approaches will take considerable thought and effort. An important consideration will be the translation of the results of this joint research program into testing strategies that provide data useful to risk assessors.

I. Outreach and Communication: Effective and open communication about this research program, its findings and their use are important to its acceptance by the broader toxicology community and the ultimate success of the program. The Partners intend to conduct joint outreach activities related to the Tox21 research. Such activities might include:

- Sponsoring relevant workshops (e.g., to identify the key pathways for various organ systems, development of new test systems, or to develop best practices for analysis of the new data streams).
- Organizing public meetings and/or symposia that focus on advances in the area of new approaches for toxicity testing and systems-biology models for integration and interpretation of the data.
- Co-organizing a seminar series that addresses key advancements in the application of new approaches for toxicity testing or translation of these data into phenotypic outcomes that would form the basis for more mechanism- based risk assessment practices.
- Contributing via presentations and posters to national and international meetings.
- Co-authoring articles to keep the scientific community informed of progress and advances in this research program.
- Continuing to interact via joint meetings of the EPA's Computational Toxicology Communities of Practice, the NCATS, the DNTP, and the FDA.
- Promoting the regulatory acceptance of alternative approaches as appropriate.

J. Governance: The Partners intend for the activities identified in this MOU to be managed by a Governance Board composed of the Scientific Director, DPI, NCATS; Director, EPA/ORD Center for Computational Toxicology & Exposure, the Chief of the DNTP Predictive Toxicology Branch, and the Senior Advisor for Toxicology in the Office of the Center Director of the FDA Center for Food Safety and Applied Nutrition. The members of the Governance Board, with advice from their management, are to be responsible for developing and implementing a cross-organizational research strategy, promoting cross-organization interactions, identifying and recommending actions to overcome barriers to success, ensuring minimal redundancy of activities, serving as spokespersons for the quadripartite effort within and outside their respective organizations, and reporting on the overall progress of the program to their respective organizations at periodic intervals. The Governance Board is expected to meet by teleconference or in person at least once every two months.

K. Scientific Review: The Partners intend for the activities carried out in support of this MOU to be reviewed at regular intervals by their respective institutional review groups. For NCATS/DPI, NIEHS/DNTP, and EPA/ORD, the reviews will be conducted by their respective Board of Scientific Counselors, and for FDA, by the FDA Agency Toxicology Working Group, and the Science Board to the Food and Drug Administration.

IV. LIMITATIONS

All commitments made in this MOU are subject to the availability of appropriated funds and each Partner's research priorities. Nothing in this MOU, in and of itself, obligates any Partner to expend appropriations or to enter into any contract, assistance agreement, interagency agreement, or other financial obligation.

This MOU is neither a fiscal nor a funds obligation document. Any endeavor involving reimbursement or contribution of funds between the Partners to this MOU will be handled in accordance with applicable laws, regulations, and procedures, and subject to separate subsidiary agreements that will be affected in writing by representatives of the Partners.

Except as provided in this Section (Section IV, LIMITATION) and Section VI, INTELLECTUAL PROPERTY, this MOU is not legally binding and does not create any right or benefit, substantive or procedural, enforceable by law or equity against the NIH/NIEHS/DNTP, the NIH/NCATS/DPI, the EPA/ORD, or the FDA.

V. PROPRIETARY INFORMATION

Not applicable as all Partners are Federal agencies. Note, however, that no sharing by FDA with other Partners to this MOU of information that is confidential or a trade secret is contemplated by this MOU.

VI. INTELLECTUAL PROPERTY

The Partners agree that inventorship of any patentable matter, created by any of the Partners pursuant to the terms of this MOU, will be determined in accordance with U.S. patent laws. Ownership will follow inventorship and vest in the inventors or their employers as determined by contract or law.

The Partners agree to notify each other when jointly-authoring a journal article that includes a non-government employee as a co-author. In such cases, the Partners should ensure that all necessary rights under copyright are acquired to the satisfaction of all Partners.

VII. CONFIDENTIAL INFORMATION

FDA is the custodian of information, including toxicological information that is owned by entities that FDA regulates. FDA will not, as part of the activities covered by this MOU, share with other Partners to the MOU any information that is confidential or trade secret.

VIII. POINTS OF CONTACT

The following individuals are designated points of contact for the MOU:

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IX. MODIFICATION/DURATION/TERMINATION

This MOU is to take effect upon signature of all Partners and remain in effect for a period of five (5) years unless the Partners decide otherwise in writing. This MOU may be amended at any time by the mutual written consent of the Partners. A Partner may terminate its participation in this MOU by providing written notice to the other Partners at least thirty (30) days in advance of the desired termination date.

The Partners anticipate that other Partners may seek to join this effort in the future. If that occurs, a new MOU may be prepared and will, when signed by each of the Partners to this MOU, supersede this MOU.