

TRANSFORM TOX TESTING CHALLENGE

INNOVATING FOR METABOLISM

CHALLENGE OVERVIEW





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ABOUT THE CHALLENGE

Out of thousands of chemicals in use today, very few have been fully evaluated for potential health risks.

The U.S. Environmental Protection Agency (EPA), the National Institutes of Health's (NIH) National Center for Advancing Translational Sciences (NCATS), and the National Toxicology Program (NTP), headquartered at the National Institute of Environmental Health Sciences (NIEHS), are pleased to announce the launch of the Transform Tox Testing Challenge.

Scientists from EPA, NTP and NCATS have used high-throughput screening (HTS) assays to evaluate the potential health effects of thousands of chemicals. The Transform Tox Testing Challenge: Innovating for Metabolism is calling on innovative thinkers to find new ways to incorporate physiological levels of chemical metabolism into HTS assays. Since current HTS assays do not fully incorporate chemical metabolism, they may miss chemicals that are metabolized to a more toxic form. Adding metabolic competence to HTS assays will help researchers more accurately assess chemical effects and better protect human health.

We are looking for innovative technological solutions to retrofit these assays to allow both chemicals and their metabolite products to be evaluated. With your help, we can transform chemical testing methods to make them more relevant to real life and more effective at predicting health effects.



IMPACT

Only a small number of chemicals in use today have enough toxicity data to fully evaluate their potential health risks. What's more, hundreds more chemicals are introduced into commerce every year.

Being able to use HTS assays with metabolic competence could revolutionize the current approach used to evaluate the safety of chemicals and better protect human health.



COMPETITION OVERVIEW

The *Transform Tox Testing Challenge* has multiple stages, with prizes (amounts are subject to change) awarded as applicants advance to each of the stages.

In the first stage of the Challenge, solvers submitted practical designs for how HTS assays could be retrofitted to include xenobiotic metabolic competence. Ten impressive submissions were selected as semi-finalists who were awarded a prize of \$10,000 each and participated in the semi-finalist workshop discussions.

Federal agency sponsors are now asking semi-finalists to put their amazing ideas into practice. Up to five applicants may be selected as finalists, awarded a prize of up to \$100,000 each, and invited to participate in the final stage of the competition.

TEAMS WILL COMPETE IN THREE STAGES FOR A TOTAL PRIZE OF \$1 MILLION

1



Stage 1 - Up to ten submissions will be selected as semi-finalists, awarded a prize of \$10,000 each, and invited to participate in Stage 2.

2



Stage 2 - Up to five applicants may be selected as finalists, awarded a prize of up to \$100,000 each, and invited to participate in the final stage of the competition.

3



Stage 3 – Based on the testing and overall feasibility, one winner may be awarded up to a \$400,000 prize for delivery of a commercially viable method or device that will ultimately result in technologies that can provide metabolic competence to commonly used HTS assays. ultimately result in technologies that can provide metabolic competence to commonly-used HTS assays.

JUDGES

Stephen Ferguson, Ph.D.



Dr. Ferguson is a scientist in the Biomolecular Screening Branch of the Division of the National Toxicology Program (NTP). Dr. Ferguson's research focuses on the development and application of *in vitro* toxicology models and assay approaches that support physiologically-relevant cellular functionality, response to xenobiotic exposure, and xenobiotic metabolism competence. Prior to joining the NTP, Dr. Ferguson led the ADME Tox R and D team of Life Technologies developing *in vitro* liver model systems and assay approaches (primarily for pharmaceutical research) to predict human drug metabolism, drug clearance (i.e. metabolism and transport) and drug-drug interactions. Dr. Ferguson earned B.S. and Ph.D. degrees from North Carolina State University followed by a postdoctoral fellowship at the National Institute of Environmental Health Sciences, studying mechanisms of liver enzyme (i.e. cytochromes P450) induction/transcriptional regulation and the impact of human genetic variants on drug metabolism. Dr. Ferguson also maintains an adjunct faculty appointment in the Toxicology Curriculum at the University of North Carolina at Chapel Hill.

Keith Houck, Ph.D.



Dr. Houck is a research toxicologist in the National Center for Computational Toxicology, Office of Research and Development, US Environmental Protection Agency. He leads a research team developing methods for chemical prioritization and predictive toxicology based on *in vitro* approaches with the Chemical Safety for Sustainability National Research Program. He also leads efforts within the Tox21 multiagency collaboration in acquisition of assays for quantitative high-throughput screening. Dr. Houck sits on the Validation Management Group for Non-Animal Testing of the OECD which advises on endocrine disruptors testing and assessment.

Dr. Houck obtained his undergraduate degree in biology from Guilford College, his MS degree in chemistry from the University of North Carolina at Chapel Hill and his doctoral degree in pathology and toxicology from Duke University. He worked in the field of drug discovery at Genentech, Sphinx Pharmaceuticals and Eli Lilly and Co. prior to joining EPA in 2006.

Steven Simmons, Ph.D.



Dr. Simmons is a research toxicologist with the U.S. EPA's National Center for Computational Toxicology. He leads a research team that develops and implements *in vitro* methods to identify potential environmental toxicants, focusing on xenobiotic metabolism and endocrine signaling pathways. Dr. Simmons first joined EPA in 2006 as a postdoctoral fellow where he developed high-throughput screening assays to measure cellular stress response endpoints. He later joined EPA's National Health and Environmental Effects Research Laboratory (NHEERL) in 2008 as a research biologist where he continued to develop *in vitro* screening assays to address critical knowledge gaps in areas such as DNA damage responses oxidative stress. He earned his B.S. (Biology) from Lamar University (Beaumont, Texas) in 1999. He earned his Ph.D. in Molecular and Cellular Toxicology from North Carolina State University in 2006. His doctorate work characterized transcriptional dysregulation leading to prostate tumorigenesis.

Russell Thomas, Ph.D.



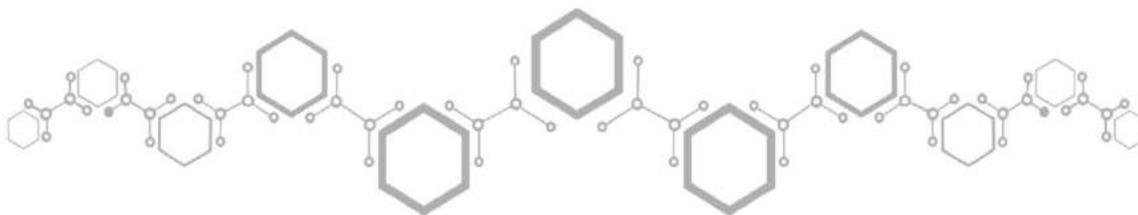
Dr. Thomas is the director of the National Center for Computational Toxicology at the U.S. Environmental Protection Agency. The Center is researching new, more efficient, ways to evaluate the safety of chemicals, particularly in assessing chemicals for human health effects. Prior to coming to the U.S. EPA, Dr. Thomas was the director of the Institute for Chemical Safety Sciences at The Hamner Institutes for Health Sciences and worked in the biotech and biopharmaceutical industry. Dr. Thomas' academic training includes a B.A. in chemistry from Tabor College, an M.S. in radiation ecology and health physics from Colorado State University, and a Ph.D. in toxicology also at Colorado State. Following his doctoral studies, Dr. Thomas performed postdoctoral research in molecular biology and genomics at the McArdle Cancer Research Laboratory at the University of Wisconsin. Dr. Thomas also maintains an adjunct faculty appointment in the Division of Pharmacogenomics and Individualized Therapy at the University of North Carolina at Chapel Hill.

Menghang Xia, Ph.D.



Dr. Xia is a group leader for Laboratory Systems Toxicology in the Division of Preclinical Innovation, National Center for Advancing Translational Sciences (NCATS), NIH. Serving as a co-chair in the Assays/Pathways working group of the Tox21 program, Dr. Xia led the major effort to develop and validate a battery of *in vitro* toxicological assays in a quantitative high-throughput screening (qHTS) platform. She is interested in studying the mechanism of action of drugs/chemicals in multiple cellular signaling pathways. She has also developed and implemented numerous assays into a HTS format using advanced technologies. She is the author and inventor of more than 100 peer-reviewed research articles, book chapters and patents.

Dr. Xia received her bachelor of medicine from the Shanghai Medical University in China and her Ph.D in pharmacology and toxicology from the State University of New York at Buffalo. She did her postdoctoral training at the University of California at San Francisco. She joined the NIH in 2005 from Merck Research Lab, where she identified and validated several targets for drug development.



TRANSFORM TOX TESTING CHALLENGE

INNOVATING FOR METABOLISM

STAGE 2 COMPETITION BRIEF





STAGE 2 SCHEDULE



CALL FOR STAGE 2 SUBMISSIONS

September 27, 2016



SUBMISSION DEADLINE

January 12, 2017

(due at 11:59 PM EST)



FINALISTS ANNOUNCED

March, 2017

Five applicants chosen to receive \$100,000 each and advance to Stage 3





The U.S. Environmental Protection Agency (EPA), the National Institutes of Health's (NIH) National Center for Advancing Translational Sciences (NCATS), and the National Toxicology Program (NTP), headquartered at the National Institute of Environmental Health Sciences (NIEHS) invite semi-finalists to compete in the second stage of the Transform Tox Testing Challenge.

To compete in Stage 2, participants must have entered a Stage 1 submission, been named as a semi-finalist and awarded a Stage 1 prize. Tox Test Challenge semi-finalists are now charged with:

1. Meeting a general level of performance
2. Technically characterizing the metabolic 'competence' of their system
3. Functionally characterizing their system in pilot screen(s)

Up to five applicants may be selected as finalists, awarded a prize of up to \$100,000 each, and invited to participate in the final stage of the competition.

Written responses to prompts and analytical data should be emailed to Kevin Kuhn (Kuhn.Kevin@epa.gov) and Jennee Kuang (Kuang.Jennee@epa.gov). Samples for analytical testing, if applicable, should be shipped per instructions to be included with reference chemicals.

Complete entries, including samples, must be received by the deadline of Stage 2 of the Transform Tox Testing Challenge (11:59pm EST on January 12, 2017). To enter, submit the required information below:

GENERAL INFORMATION

Please provide the following information in writing:

1. Name and full address of organization(s), or individual(s) applying in Stage 2.
2. Point of Contact for application (name, position, title, and contact information).
3. Team members (including affiliations) and partner organizations.
4. A general description of your solution for use with a general audience and for publicity purposes.
5. A quote about the Tox Test Challenge: a statement expressing your team's views on the value of the challenge that can be used for general audience and for publicity purposes.

GENERAL PERFORMANCE

Based on your progress in Stage 2, please respond to the following prompts in writing.

General Applicability: As in Stage 1, describe how your approach will supply currently used HTS assays with metabolic competence, including: the working principle, the process flow, and the functional operation of the solution. Add a diagram to help illustrate your description and, if appropriate, the approximate footprint of the HTS design.

Methods: Thoroughly describe the experimental and analysis methods used to carry out the pilot study. (Attachment requested: Provide methodology to validate the solution)

Ease of Use: Clearly outline the time required to implement your solution in the pilot study.

Economic Viability: Provide per sample costs associated with implementing the solution when applied to screen 1,000 – 10,000 chemicals in concentration response format.

Commercial Viability: Describe a pathway and timeline to commercial viability and/or broad use of the proposed solution.

CHARACTERIZATION

Successful incorporation of metabolism into ToxCast/Tox21 assays will be capable of retrofitting all existing cell-based and cell-free assay platforms with multiple human xenobiotic metabolizing enzymes (i.e., CYP3A4/5, CYP2E1, UGTs, carboxyesterases, etc.). Ideally, the solution would incorporate physiologically-relevant metabolic ‘competence’ over a broad range of xenobiotic enzymes (minimum of 5 simultaneously). Assay formats include 96- and 384-well plates with potential scalability to 1536-well format. Useful systems will support screening with and without metabolic competence.

If requested, Federal Agency sponsors will supply reference chemicals as a set of 500x stocks in a DMSO vehicle and complete a subset of analytical measurements to support semi-finalist participation in the challenge. Reference chemicals will be shipped in October 2016. Please see Appendix B for instructions.

Stage 2 participants are charged with technically characterizing the metabolic ‘competence’ of their designed prototypes.

To assess the metabolic competence of designed/constructed prototypes in Stage 2 of the Challenge, two modes of xenobiotic metabolism assays are required:

1. Specific activity measurements of human drug metabolizing enzymes that must include CYP3A4/5 assessments along with 4 additional enzymes as relevant designed prototypes,
2. Extent of parent chemical depletion experiments that must minimally include the provided CYP3A4 clinical substrate terfenadine.

Specific activity measurements: Participants may choose to run their own evaluations as appropriate to their model systems using a variety of platforms such as LC-MS/MS, luminescence, fluorescence, or HPLC-based methods in their own laboratories (or in collaborations with other labs) with appropriate quantitation methods (i.e., standard curves).

Participants are also encouraged to characterize their designed prototypes for specific activity measurements using Federal Agency sponsor-provided probe substrates according to the methodology described in the “In Situ Metabolism Methodology for the Transform ToxTest Challenge” (Appendix A) with appropriate customization for each designed prototype system.

Briefly, probe substrates should be incubated by Stage 2 Participants at 2 time points (30 min & 18 h) with designed prototypes. At the conclusion of each time point, a portion of in situ metabolism incubations is removed from prototypes, transferred into 2 aliquots and immediately frozen at -80°C. One aliquot is for shipment to Federal Agency sponsors for analytical measurements. The other may be utilized or stored for participant analysis as needed. Table 1 provides a summary of the 5 Federal Agency sponsor-provided probe substrates and corresponding marker metabolites. Assay measurements of marker metabolite formation rates (e.g., pmol of marker metabolite per minute per million cells) will be calculated to assess xenobiotic metabolism competence.

Table 1: Summary of Federal Agency Sponsor-Provided Substrates & Marker Metabolites for Xenobiotic Metabolism Specific Activity Assays

Substrate	Substrate (1X) Final Concentration	Incubation Times	Marker Metabolite	Enzyme(s)
terfenadine	100 μ M (50 mM DMSO stock)	30 min & 18h	hydroxyterfenadine	CYP3A4
phenacetin	100 μ M (400 mM DMSO stock)	30 min & 18h	acetaminophen	CYP1A2
bupropion	500 μ M (250 mM DMSO stock)	30 min & 18h	1-hydroxybupropion	CYP2B6
chlorzoxazone	200 μ M (100 mM EtOH stock)	30 min & 18h	6-hydroxychlorzoxazone	CYP2E1
7-hydroxycoumarin	500 μ M (250 mM EtOH stock)	30 min & 18h	7-hydroxycoumarin glucuronide	UGTs

Parent chemical depletion experiments: Participants may choose to run their own evaluations of relevant substrates as appropriate to their designed prototypes and analytical capabilities. However, incubations with the CYP3A4 substrate terfenadine (100 μ M, 30 min & 18h) are minimally required to evaluate the total metabolic capacity of the designed prototypes.

Participants are encouraged, if interested, to evaluate 5 Federal Agency sponsor-provided probe substrates shown in Table 1 to be shipped to Federal Agency sponsors for analytical measurements. A generalized method for this is provided in the “In Situ Metabolism Methodology for the Transform Tox Testing Challenge” that multiplexes these incubations with the specific activity assessments described above. Each designed prototype system may require customization of this protocol, and the 5 Federal Agency sponsor-provided substrates may not be relevant to all designed prototypes. The provided methods would allow up to 5 parent chemical depletion evaluations to be assessed.

Briefly, probe substrates are incubated by Stage 2 Participants at 2 time points (30min & 18h) in designed prototypes. At the conclusion of each time point, reactions are quenched with 2:1 volumes of chilled (on ice) acetonitrile, aliquoted into 2 separate polypropylene round-bottom plates, and immediately frozen at -80°C for subsequent shipment to Federal Agency sponsors and analytical measurements. Table 1 provides a summary of the 5 Federal Agency sponsor-provided probe substrates that may be evaluated. Assay measurements of parent chemical depletion rates will be calculated (e.g., % depletion).

Stage 2 participants are charged with functionally characterizing their system in a series of pilot screens.

Cytotoxicity screening: Participants are encouraged to provide data from the application of their solution in a cytotoxicity screen of 10 reference chemicals. The reference chemicals to be supplied by Federal Agency sponsors are benzo[a]pyrene (CAS #: 50-32-8), aflatoxin B1 (CAS #: 1162-65-8), cyclophosphamide monohydrate (CAS #: 6055-19-2), 2-naphthylamine (CAS #: 91-59-8), acrylamide (CAS #: 79-06-1), 1,8-dinitropyrene (CAS #: 42397-65-9), doxorubicin hydrochloride (CAS #: 25316-40-9), 6-aminochrysene (CAS #: 2642-98-0), 8-methoxypsoralen (CAS #: 298-81-7), and 4-nitrophenol (CAS #: 100-02-7).

- The screen should be run in 96- or 384-well format in triplicate. The target cell type for measuring cytotoxicity should be HEK293 cells (ATCC #:CRL-1573) in a medium formulation of DMEM with 10% FBS and antibiotics.
- Viability should be assessed based on cellular ATP levels.
- The reference chemicals should be tested in concentration response format with and without metabolic competence for an assay duration of 24 hours. A total of 8 concentrations should be run starting with a 1:200 dilution and progressing at 2-fold dilutions (1:200, 1:400, 1:800, 1:1600, 1:3200, 1:6400, 1:12800, and 1:25600). Multiple vehicle controls should be included on each plate (DMSO at 0.5%).
- Solutions adding metabolically competent cells (e.g., primary hepatocytes in trans-well format) or subcellular fractions (e.g., S9) to the existing assay should have a denatured or metabolically incompetent control to adjust for non-specific chemical protein binding.

Stretch Goal: An optional addition to the dataset that will increase participant's scores include application of their solution in an estrogen receptor (ER) transactivation screen of 4 reference chemicals (reference chemicals and cells will not be provided).

- The screen should be run in 96- or 384-well format in triplicate.
- The reference chemicals should include methoxychlor (CAS #: 72-43-5), 4-benzylphenol (CAS #: 101-53-1), benzophenone (CAS #: 119-61-9), and azobenzene (CAS #: 103-33-3).
- The target cell type for measuring ER transactivation should be T47D-KBluc (ATCC# CRL-2865) with culture conditions outlined in Conley et al. (PMID: 26936661).
- The reference chemicals should be measured in concentration response. Cytotoxicity should be measured in parallel.



STAGE 2 SCORING CRITERIA

Submissions will be judged based on the criteria outlined below. As described in the Entry Section, Tox Test Challenge participants are charged with:

1. Meeting a general level of performance (25%)
2. Technically characterizing the metabolic 'competence' of their system (35%)
3. Functionally characterizing their system in pilot screen(s) (40%)

Scoring will be based on the weighted (% of total score) criteria provided in the table below. Each performance criteria will be scored on a scale of 0-5 with 0 being the lowest and 5 being the highest.

General Performance Criteria	Points	Weighting
APPLICABILITY <ul style="list-style-type: none"> Demonstrated capability of retrofitting onto all existing cell-based and cell-free ToxCast and Tox21 assays 	1 to 5	10%
QUALITY OF THE METHODS AND ANALYSIS <ul style="list-style-type: none"> Thoroughly described the experimental and analysis methods used to carry out the pilot study Provided methodology to validate the solution 	1 to 5	5%
EASE OF USE <ul style="list-style-type: none"> Clearly outlined the time required to implement their solution in the pilot study Did not require constant mixing or multiple wash steps or solution exchanges during the HTS assay 	1 to 5	5%
ECONOMIC VIABILITY <ul style="list-style-type: none"> Tracked and provided the per sample costs associated with implementing the solution The costs associated with implementing the solution are reasonable when applied to screen 1,000 – 10,000 chemicals in concentration response format 	1 to 5	5%

Technical Characterization	Points	Weighting
ENZYME RANGE <ul style="list-style-type: none"> Incorporated CYP3A4/5 Incorporated a minimum of 4 additional human xenobiotic metabolizing enzymes. Relevant enzymes include, but are not limited to: CYP1A2, CYP2A6, CYP2B6, CYP2C8, CYP2C9, CYP2C19, CYP2D6, CYP2E1, various UDP- glucuronosyl transferases, various sulfotransferases, flavin-containing monooxygenase, monoamine oxidases, and/or carboxyesterases 	1 to 5	10%
RELATIVE ENZYME ACTIVITIES <ul style="list-style-type: none"> The quantitative or relative measurements of specific enzyme activities were within the range of human specific activities from liver tissue, subcellular fractions, and suspensions of primary human hepatocytes (i.e., pooled donors) The specific enzyme activities showed physiological differences in the proportions of enzymes 	1 to 5	15%
TOTAL ENZYMATIC ACTIVITY <ul style="list-style-type: none"> Demonstrated sufficient metabolic activity to metabolize terfenadine 	1 to 5	10%

Functional Characterization	Points	Weighting
SCALABILITY <ul style="list-style-type: none"> Demonstrated scalability to 96- and/or 384-well platforms Has the technical capability to be scalable to hundreds of multi-well plates in a single HTS experiment 	1 to 5	10%
REFERENCE CHEMICAL PERFORMANCE <ul style="list-style-type: none"> Showed the expected performance across the full range of reference chemicals Allowed screening with and without metabolic competence 	1 to 5	30%



STAGE 2 RULES, TERMS AND CONDITIONS

By participating in the Challenge, each Applicant agrees to comply with and abide by these Official Rules, Terms & Conditions and the decisions of the U.S. Environmental Protection Agency (EPA), the National Institutes of Health's (NIH) National Center for Advancing Translational Sciences (NCATS), and the National Toxicology Program (NTP), headquartered at the National Institute of Environmental Health Sciences (NIEHS) and/or the individual judges, which shall be final and binding in all respects.

The following Rules, Terms & Conditions must be carefully followed and agreed to by all Applicants to Stage 2 of this competition.

The Transform Tox Testing Challenge is a three-stage event, with prizes awarded as Applicants advance to the next stage of the Challenge; prize amounts are subject to change. The government reserves the right to cancel the later stages or award smaller prizes for partial solutions. In addition, the Transform Tox Testing Challenge may reopen, at the sole discretion of NIH and EPA.

ELIGIBILITY RULES

To be eligible to win Stage 2 of this Challenge, an individual or entity:

- Must have entered a Stage 1 submission to the Transform Tox Testing Challenge, been named as a semi-finalist and awarded a Stage 1 prize.
- Must have submitted a complete response to Stage 2 of the Transform Tox Testing Challenge under the rules promulgated by Federal Agency sponsors.
- Must be an individual or team comprised of members each of whom are 18 years of age or over.
- Must not be on the Excluded Parties List System located at <https://www.epls.gov/>.
- May not be a Federal entity or Federal employee acting within the scope of their employment.
- The applicant shall not be deemed ineligible because the applicant used Federal facilities or consulted with Federal employees during a competition if the facilities and employees are made equitably available to all applicants participating in the competition.

- Federal grantees may not use Federal funds to develop challenge applications unless consistent with the purpose of their grant award. Federal contractors may not use Federal funds from a contract to develop challenge applications or to fund efforts in support of a challenge submission.
- Employees of EPA and NIH, and/or any other individual or entity associated with the development, evaluation, or administration of the Challenge as well as members of such persons' immediate families (spouses, children, siblings, parents), and persons living in the same household as such persons, whether or not related, are not eligible to participate in the Challenge.
- Applicants must agree to assume any and all risks and waive claims against the Federal Government and its related entities, except in the case of willful misconduct, for any injury, death, damage, or loss of property, revenue, or profits, whether direct, indirect, or consequential, arising from their participation in a competition, whether the injury, death, damage, or loss arises through negligence or otherwise.
- Applicants must also agree to indemnify the Federal Government against third party claims for damages arising from or related to competition activities.
- Applicants are not required to obtain liability insurance or demonstrate financial responsibility in order to participate in the Challenge.

SUBMISSION AND PARTICIPANT RULES

- The written portion of Stage 2 entries must be submitted in English.
- Samples supplied for testing must be submitted according to the protocol developed for Stage 2 entries by the Federal Agency sponsors.
- Applicants will be required to sign Material Transfer Agreements (MTAs) in order to send samples for testing. Please see Appendix B for an example MTA.
- Complete entries, including samples, must be received by the deadline of Stage 2 of the Transform Tox Testing Challenge (11:59PM EST on January 12, 2017).
- No additions or modifications to the applications will be accepted after the submission deadline.
- Federal Sponsors bear no responsibility for submission errors.

REPRESENTATION, WARRANTIES, AND INDEMNIFICATION

By entering in the Challenge, each Applicant represents, warrants, and covenants as follows:

- Applicant is the sole author, creator, and owner of the Submission;
- The Submission is not the subject of any actual or threatened litigation or claim;
- The Submission does not and will not violate or infringe upon the intellectual property rights, privacy rights, publicity rights, or other legal rights of any third party;
- The Submission does not and will not contain any harmful computer code (sometimes referred to as “malware,” “viruses” or “worms”); and
- The Submission, and Applicants’ use of the Submission, does not and will not violate any applicable laws or regulations, including, without limitation, applicable export control laws and regulations of the U.S. and other jurisdictions.

If the Submission includes any third party works (such as third party content or open source code), Applicant must be able to provide, upon request, documentation of all appropriate licenses and releases for such third party works. If Applicant cannot provide documentation of all required licenses and releases, Federal Agency sponsors reserve the right, at their sole discretion, to disqualify the applicable Submission. Conversely, they may seek to secure the licenses and releases and allow the applicable Submission to remain in the Challenge, while reserving all rights with respect to such licenses and releases.

Applicants must indemnify, defend, and hold harmless the Federal Government from and against all third party claims, actions, or proceedings of any kind and from any and all damages, liabilities, costs, and expenses relating to or arising from Applicant’s Submission or any breach or alleged breach of any of the representations, warranties, and covenants of Applicant hereunder.

The Federal Agency sponsors reserve the right to disqualify any Submission that, in their discretion, deems to violate these Official Rules, Terms & Conditions.

INTELLECTUAL PROPERTY

Applicants are free to discuss their submission and the ideas or technologies that it contains with other parties; encouraged to share ideas/technologies publicly; encouraged to collaborate or combine with other teams to

strengthen their solutions; and are free to contract with any third parties, as long as they do not sign any agreement or undertake any obligation that conflicts with the Challenge rules, terms and conditions.

Upon submission, each Applicant warrants that he or she is the sole author and owner of the work and any pertinent Intellectual Property (IP) rights, that the work is wholly original to the Applicant (or is an improved version of an existing work that the Applicant has sufficient rights to use—including the substantial improvement of existing open-source work), and that it does not infringe any copyright or any other rights of any third party of which Applicant is aware. Each Applicant also warrants that the work is free of malware.

Each Applicant must clearly delineate any Intellectual Property (IP) included in the application that is owned by the Applicant, and which the Applicant wishes to protect as proprietary data.

All materials submitted to NIH and EPA as part of a submission become NIH and EPA records and cannot be returned. Any confidential commercial information contained in a submission must be designated at the time of submission.

FOIA: Submitters will be notified of any Freedom of Information Act requests for their submissions in accordance with 29 CFR 70.26.

Applicants retain ownership of their concepts, including any software, research or other intellectual property (“IP”) that they develop in connection therewith, subject to the license granted to Federal Agency sponsors as set forth herein.

Upon submission, each Applicant grants to the U.S. government and others acting on behalf of the U.S. government, a royalty-free, irrevocable, non-exclusive worldwide license to use, reproduce, publicly perform, publicly display the submission to the extent necessary to administer the challenge, and to publicly perform and publicly display the submission abstract, including, without limitation, for advertising and promotional purposes relating to the challenge.

Before award of a Stage 2 prize, prospective finalists must grant the U.S. government a 5-year royalty-free, irrevocable, non-exclusive, non-transferrable worldwide license to practice or have practiced for or on behalf of the United States any invention throughout the world owned or controlled by the Applicant that covers the submission.

PRIZES

The total prize pool for the Challenge is up to \$1,000,000 across all three stages. Prizes awarded under this competition will be paid by electronic funds transfer and may be subject to Federal income taxes. EPA will comply with Internal Revenue Service withholding and reporting requirements, where applicable.

DATES/DEADLINES

The Federal Government reserves the right to modify any dates or deadlines set forth in these Official Rules, Terms & Conditions or otherwise governing the Challenge.

CHALLENGE TERMINATION

The Federal Government reserves the right to suspend, postpone, cease, terminate or otherwise modify this Challenge, or any Applicant's participation in the Challenge, at any time at its discretion.



CONTACT US

For general information, technical or process questions about the challenge, please contact:

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For media inquiries, please contact:

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Strategic Communications & Outreach, Office of Research and Development, US EPA

Phone: 919-541-1522

Appendix A:

In Situ Metabolism Methodology for the Transform Tox Testing Challenge

Stage 2 Challenge participants are encouraged to evaluate the metabolic competence of their designed prototype solutions for the Transform Tox Testing Challenge. Participants are required to minimally assess CYP3A4/5 metabolic activities and the extent of terfenadine disappearance with their designed/constructed prototypes along with 4 additional human xenobiotic metabolizing enzymes.

Although participants may choose to run their own evaluations of metabolic competence (e.g., specific activities of marker metabolite formation and disappearance of parent chemicals) as appropriate to their model systems using a variety of platforms such as LC-MS, luminescence, fluorescence, and HPLC-based methods in their own laboratories, participants are encouraged to perform metabolism incubations with constructed prototype solutions for external analytical measurements (frozen and shipped to Federal Agency sponsors for analytical measurements). For these externally-analyzed samples, participants should incubate the provided probe substrates as described below.

These data will be used to calculate specific activity measurements of marker metabolite formation rates (e.g., pmol of marker metabolite per minute per million cells) as summarized in Table 1. Parent chemical depletion measurements may also be measured to further characterize the metabolic competence of prototype solutions. Due to the variation across proposed technologies/prototypes, a generalized protocol for these evaluations is provided below. For technologies incorporating alternative transformation pathways that are not covered in Table 1, no externally-provided analytical measurements are available at this time.

Table 1: Metabolism substrates, concentrations, incubation times, marker metabolites, and corresponding enzymes for metabolism competence evaluations

Substrate	Substrate (1X) Final Concentration	Incubation Times	Marker Metabolite	Enzyme(s)
terfenadine	100 μ M (50 mM DMSO stock)	30 min & 18h	hydroxyterfenadine	CYP3A4
phenacetin	100 μ M (400 mM DMSO stock)	30 min & 18h	acetaminophen	CYP1A2
bupropion	500 μ M (250 mM DMSO stock)	30 min & 18h	1-hydroxybupropion	CYP2B6
chlorzoxazone	200 μ M (100 mM EtOH stock)	30 min & 18h	6-hydroxychlorzoxazone	CYP2E1
7-hydroxycoumarin	500 μ M (250 mM EtOH stock)	30 min & 18h	7-hydroxycoumarin glucuronide	UGTs

MATERIALS

- Humidified incubator capable of maintaining constructed prototypes at 37°C for the duration of incubations
- Terfenadine (CAS: # 50679-08-8) stock solution [provided by Federal Agency sponsors upon request]
- Phenacetin (CAS: # 62-44-2) stock solution [provided by Federal Agency sponsors upon request]
- Bupropion (CAS: # 34841-39-9) stock solution [provided by Federal Agency sponsors upon request]
- Chlorzoxazone (CAS: # 95-25-0) stock solution [provided by Federal Agency sponsors upon request]
- 7-hydroxycoumarin (CAS: # 93-35-6) stock solution [provided by Federal Agency sponsors upon request]
- Chilled acetonitrile solution (on ice)
- Prototype (96- or 384-well) system configured for xenobiotic metabolism incorporation of 5 or more enzymes capable of retrofitting existing ToxCast/Tox21 assays systems (should include necessary components such as liver cells, transduced/transfected cells, liver subcellular fractions, recombinant enzymes, etc...)
 - Appropriate cell culture media [e.g., buffered (e.g. HEPES) William's E medium or Dulbecco's minimal essential medium) or shorter-term assay buffers (e.g., buffered Hank's Balanced Salt Solution-HBSS, Krebs-Henseleit buffer (pH 7.4), or potassium phosphate buffer (100 mM, pH 7.4) for 1 hour incubation)] with requisite cofactors such as NADPH to dilute in situ metabolism substrates to 1X final concentrations [Note: an aliquot (e.g., ≥ 1 mL) of matrix medium/buffer should be provided for analytical measurements as matrix control]
- Pipettors capable of accurately transferring volumes from 2 μ L up to 1 mL
- Water bath or incubator to warm media/assay buffer to 37°C
- Appropriate plasticware/glassware to store solutions under ambient conditions or warmed in water bath
- 96-well or 384-well round bottom polypropylene 'deep-well' plates appropriate to transfer in situ metabolism incubation samples for storage (with covering), freezing at -80°C, and shipment on dry ice to Federal Agency sponsors
- PBS or HBSS to wash prototype wells (if required by the constructed prototype)
- Equipment to document photographs and photomicrographs of the prototype(s) at a macro and individual well level

METHODS

1. Thaw the 5 xenobiotic metabolism substrate stock solutions (all in 100% DMSO).
2. Prepare in situ metabolism substrate solutions in appropriate cell culture media or assay buffer as compatible with constructed prototypes (e.g., 1X substrate in buffered cell culture media/assay buffer, 2X substrate solution mixed 1:1 with 2X prototype assay buffer, etc...).
3. Prepare constructed prototypes in 96- or 384-well formats consistent with the scope of the Transforming Tox Testing Challenge objectives. For data normalization, it is recommended to include either the number of millions of metabolizing cells incorporated into an individual well or the measurement of total mg of protein present from non-treated wells of the metabolism-generating system (triplicate wells) as appropriate to the constructed prototypes.
4. Capture representative photographs and photomicrographs of designed prototypes to assist evaluators in understanding technical characteristics of constructed solutions.
5. Add prepared in situ metabolism substrate solutions (from Step 2) to a total of 120 μ L for 96-well formats and 30 μ L for 384-well formats in each well of constructed prototypes to be tested for xenobiotic metabolism competence at 1X final concentrations. If these incubation volumes are not compatible with constructed prototypes, alternate volumes may be applicable and should be documented in submitted sample list information for each sample. Incubations with each probe substrate should include no-cell controls for each time point assessed in assay media/buffer. An example plate map is shown below for 96-well format (Table 2). Use of separate plates for each time point is recommended if compatible with constructed prototype availability.
6. Incubate substrates for 30min at 37°C (non-shaking).
7. At the conclusions of the 30 min incubation period, remove ~60 μ L of incubation media and aliquot half (~30 μ L) into 2 separate round bottom polypropylene plates for freezing, storage, and shipment (1 of the 2 aliquots). Participants are encouraged to retain an aliquot.
8. Add 120 μ L (96-well) of chilled (on ice) acetonitrile to the remaining 60 μ L within assay plates and aliquot half (~90 μ L) into 2 separate round bottom polypropylene plates for freezing, storage, and shipment (1 of the 2 aliquots). Participants are encouraged to retain an aliquot.
9. Seal wells prior to 'immediate' freezing at -80°C and subsequent shipment to Federal Agency sponsors for LC-MS analysis.
10. Repeat steps 6 through 8 for each time point assessed.

Table 2: An example plate map for 96-well format

30 min TERF	30 min TERF	30 min TERF	30 min no cell control TERF	30 min no cell control TERF	30 min no cell control TERF	18 hr TERF	18 hr TERF	18 hr TERF	18 hr no cell control TERF	18 hr no cell control TERF	18 hr no cell control TERF
30 min PHEN	30 min PHEN	30 min PHEN	30 min no cell control PHEN	30 min no cell control PHEN	30 min no cell control PHEN	18 hr PHEN	18 hr PHEN	18 hr PHEN	18 hr no cell control PHEN	18 hr no cell control PHEN	18 hr no cell control PHEN
30 min BUP	30 min BUP	30 min BUP	30 min no cell control BUP	30 min no cell control BUP	30 min no cell control BUP	18 hr BUP	18 hr BUP	18 hr BUP	18 hr no cell control BUP	18 hr no cell control BUP	18 hr no cell control BUP
30 min CHLZ	30 min CHLZ	30 min CHLZ	30 min no cell control CHLZ	30 min no cell control CHLZ	30 min no cell control CHLZ	18 hr CHLZ	18 hr CHLZ	18 hr CHLZ	18 hr no cell control CHLZ	18 hr no cell control CHLZ	18 hr no cell control CHLZ
30 min 7-HC	30 min 7-HC	30 min 7-HC	30 min no cell control 7-HC	30 min no cell control 7-HC	30 min no cell control 7-HC	18 hr 7-HC	18 hr 7-HC	18 hr 7-HC	18 hr no cell control 7-HC	18 hr no cell control 7-HC	18 hr no cell control 7-HC
non-treated well	non-treated well	non-treated well									

Ship samples and matched matrix media/buffer to Federal Agency sponsors (a couple of mL should suffice). Include in shipping information a detail sample list with appropriate labels to identify samples, applied substrate concentrations, applied incubation time for each sample, actual incubation volumes used for each sample, and either the number of millions of metabolizing cells incorporated into an individual well or the measurement of total mg of protein present from non-treated wells of the metabolism-generating system (triplicate wells), as appropriate to the constructed prototypes, to allow calculation of pmol/min-million cells or pmol/min-mg protein from measured marker metabolite formation quantification data as appropriate to the design prototype solutions.

Appendix B:

Requesting Reference Chemicals and Analytical Support

If requested, Federal Agency sponsors will supply reference chemicals as a set of 500x stocks in a DMSO vehicle and complete a subset of analytical measurements to support semi-finalist participation in the challenge. Reference chemicals will be shipped in October 2016.

To receive reference chemicals and to send samples to Federal Agency sponsors, semi-finalists must first sign a Materials Transfer Agreement (MTA). The draft MTA for the Transform Tox Testing Challenge is below.

If you would like to complete the MTA and receive chemicals, please contact Kevin Kuhn (Kuhn.Kevin@epa.gov) and Jennee Kuang (Kuang.Jennee@epa.gov) to begin the process.

MATERIALS TRANSFER AGREEMENT

Provider:

U.S. Environmental Protection Agency (EPA)
Office of Research and Development (ORD)
National Center for Computational Toxicology (NCCT)

Recipient:

Point of Contact for Submission _____

Organization _____

Address _____

1a. Provider agrees to transfer to Recipient's Investigator named below the following Research Material:

Chemicals and Materials

Five xenobiotic metabolism probe substrates (50uL of 500X stock solutions in DMSO). The substrates are:

- Terfenadine (CAS: # 50679-08-8),
- Phenacetin (CAS: # 62-44-2),
- Bupropion (CAS: # 34841-39-9),
- Chlorzoxazone (CAS: # 95-25-0), and
- 7-hydroxycoumarin (CAS: # 93-35-6).

Ten reference chemicals for cytotoxicity screening (50uL of 500X stock solutions in DMSO). The reference chemicals are:

- Benzo[a]pyrene (CAS #: 50-32-8),
- Aflatoxin B1 (CAS #: 1162-65-8),
- Cyclophosphamide monohydrate (CAS #: 6055-19-2),
- 2-naphthylamine (CAS #: 91-59-8),
- Acrylamide (CAS #: 79-06-1),
- 1,8-dinitropyrene (CAS #: 42397-65-9),
- doxorubicin hydrochloride (CAS #: 25316-40-9),
- 6-aminochrysene (CAS #: 2642-98-0),
- 8-methoxypsoralen (CAS #: 298-81-7), and
- 4-nitrophenol (CAS #: 100-02-7).

1b. The Recipient agrees to transfer to the EPA Investigator named below the following Research Material:

- All data or data summaries requested in the Transform Tox Testing Challenge Stage 2 Brief resulting from chemical screening performed on the probe substrates and reference chemicals.
- Samples for analytical testing by Federal Agency Sponsors of the Transform Tox Testing Challenges as described in the Stage 2 Challenge Brief.

2. This Research Material may not be used in human subjects. The Research Material will be used only for research purposes by Recipient's investigator in his/her laboratory, for the research project described below, under suitable containment conditions. This Research Material will not be used for screening, production or sale, for which a commercialization license may be required. Recipient agrees to comply with all Federal rules and regulations applicable to the Research Project and the handling of the Research Material.

3. If the data or material that are being transferred constitute human subjects research, please visit the following intranet site to determine if your project needs review and approval by the HSRRO: <http://intranet.ord.epa.gov/p2/hsr/human-subjects-review>

Does the research involve specimens or data derived or collected from human subjects?

No

Yes – I am seeking review and approval from the HSSRO. Assurance Number _____

4. The Dual Use Research of Concern (DURC) Internal Review Entity (IRE) has determined that:

- This research does not meet the DURC definition and no additional review and oversight are required. The PI must report to the IRE any results or changes in the research such that one or more of the 7 categories of experimental effects may apply, or if the PI feels that the research may be DURC.
- This research meets the DURC definition and requires additional oversight under the *USG Policy for Institutional Oversight of DURC*. Corresponding USG funding agency will be notified and a draft of the mitigation plan will be submitted within 90 days of this determination.
- Mitigation Plan submitted to the funding agency on _____
- Approved mitigation Plan on file

5. This Research Material will be used by Recipient's investigator solely in connection with the Transform Tox Testing Challenge described with specificity as follows. **Please insert description here:**

6. In all oral presentations or written publications concerning the Research Project, Recipient will acknowledge Provider's contribution of this Research Material unless requested otherwise. To the extent permitted by law, Recipient agrees to treat as confidential, any of Provider's written information about this Research Material that is stamped "CONFIDENTIAL" for a period of three (3) years from the date of its disclosure to recipient. The foregoing shall not apply to information that is or becomes publicly available or which is disclosed to Recipient without a confidentiality obligation. Any oral disclosures from Provider to Recipient which Provider wishes to be treated as confidential shall be identified as being Confidential at the time of the disclosure and by written notice delivered to Recipient within thirty (30) days after the date of the oral disclosure. Recipient may publish or otherwise publicly disclose the results of the Research Project, but if Provider has given Confidential information to Recipient, such public disclosure may be made only after Provider has had thirty (30) days to review the proposed disclosure to determine if it includes any Confidential information, to the extent such review period is permitted by law.

7. This Research Material represents a significant investment on the part of Provider and is considered proprietary to Provider. Recipient's investigator therefore agrees to retain control over this Research Material and further agrees not to transfer the Research Material to other people not under his/her direct supervision without advance written approval of Provider. Provider reserves the right to distribute the Research Material to others and to use it for its own purposes. When the Research Project is completed, the Research Material will be returned to the Provider or disposed, if directed by Provider.

8. This Research Material is provided as a service to the research community. It is being supplied to Recipient with no warranties, express or implied, including any warranty of merchantability or fitness for a particular purpose. Provider makes no representations that the use of the Research Material will not infringe any patent or proprietary rights of third parties.

9. Recipient shall retain title to any patent or other intellectual property rights in inventions made by its employees in the course of the Research Project. However, if said inventions contain any portion of the Research Material, are derived from the Research Material, or could not have been produced but for the use of the Research Material, Recipient agrees to contact the Provider to determine what ownership interests, if any, the Provider may have, and, where applicable, to negotiate in good faith the terms of a commercial license. Inventorship for a patent application or a commercialized product based on said inventions shall be determined according to United States patent law.

10. When Provider is the EPA: Recipient agrees not to claim, infer, or imply endorsement by the Government of the United States of America (hereinafter referred to as "Government") of the Research Project, the institution or personnel conducting the Research Project or any resulting product(s). Recipient agrees to hold the Government harmless and to indemnify the Government for all liabilities, demands, damages, expenses and losses arising out of Recipient's use for any purpose of the Research Material.

11. When Recipient is the EPA: Provider will not be liable to EPA for any claims or damages arising from EPA's use of the Research Material.

12. The Provider shall have the right to terminate this Agreement at any time if Recipient breaches any of the terms of this Agreement. Upon termination, Recipient shall return to the Provider all unused portions of the Research Materials.

13. Will EPA develop any products or services from information or materials provided by the Recipient?

Yes – go to item A

No – skip to #13 (next clause)

Item A: The EPA has a long history of applying principles of quality assurance/quality control to all technical work conducted by or for the Agency (CIO 2106: USEPA Quality Policy). Given EPA is receiving metabolomics and screening data and will use the metabolomics and screening data for Agency purposes, the Recipient is required to provide EPA with documentation such as a quality manual, describing their organization's quality system. In lieu of such documentation, Standard Operating Protocols for compound handling and the assays performed are acceptable or documentation showing third party accreditation to a relevant standard and scope is also acceptable for documenting an organization's quality system. EPA requirements for quality management plans can be found at this URL: http://www.epa.gov/quality/qa_docs.html

14. All notices pertaining to or required by this Agreement shall be in writing and shall be signed by an authorized representative and shall be delivered by hand (including private courier mail service) or sent by certified mail, return receipt requested, with postage prepaid, addressed as follows:

Provider's Contact Information:

Russell Thomas
National Center for Computational Toxicology (NCCT)
US EPA
109 TW Alexander (MD-D143-03)
Research Triangle Park, NC 27711
Tel: 919-541-5776
Thomas.russell@epa.gov

With a copy to:

Sandra Roberts
National Center for Computational Toxicology (NCCT)
US EPA
109 TW Alexander (MD-D143-03)
Research Triangle Park, NC 27711
919-541-3850
Roberts.sandra@epa.gov

For commercial courier address use:

4930 Old Page Rd.
Durham, NC 27703

AND

Sarah Bauer

EPA FTTA Program Coordinator

(Overnight courier address)

US EPA MC 8106R

Ronald Reagan building Room 71175

1300 Pennsylvania Ave NW

Washington, DC 20004

202-564-3267

Recipient's Contact Information:

Official's Name & Title

Mailing Address

Phone & Fax Number

Email address

With a copy to:

Official's Name & Title

Mailing Address

Phone & Fax Number

Email address

15. Paragraphs 2, 7, 9 and 10 shall survive termination.

16. This Agreement shall be construed in accordance with law as applied by the Federal courts in the District of Columbia.

17. The undersigned Provider and Recipient expressly certify and affirm that the contents of any statements made herein are truthful and accurate.

18. This agreement shall enter into force as of the date of the last signature of the parties and shall remain in effect for three years from said date.

Any false or misleading statements made, presented, or submitted to the Government, including any material omissions, under this Agreement and during the course of negotiation of this Agreement are subject to all applicable civil and criminal statutes including 31 U.S.C. ' ' 3801-3812 (civil liability), 18 U.S.C. ' 1001 (criminal liability), and 31 U.S.C. ' ' 3729-33 (False Claims Act).

SIGNATURES

FOR THE RECIPIENT:

Principal Investigator

Investigator's Name

Date

Title

Email address: _____

Authorized Representative of Institution

Representative's Name

Date

Title

CERTIFICATION OF NO CONFLICT OF INTEREST (EPA ONLY)

I hereby certify that neither I nor any member of my immediate family will benefit in any material way from the execution or failure to execute the attached FTTA Cooperative Agreement or Licensing Agreement except to the extent of participation in royalty sharing as authorized by section 13 of the Stevenson-Wydler Technology Innovation Act, as amended by the Federal Technology Transfer Act of 1986 (15 U.S.C. 3710a et seq.).

I further certify that I have no knowledge of any such conflict by any other person who has participated in any material way in the initiation, design or development of the attached Agreement or who will participate in carrying it out.

FOR THE PROVIDER:

Principal Investigator

Name

e-mail address

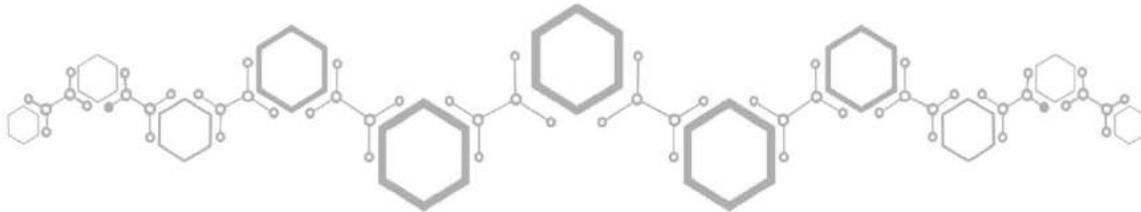
Date

Authorized Representative of Institution

Russell Thomas, Ph.D.

Director, EPA/ORD/NCCT

Date

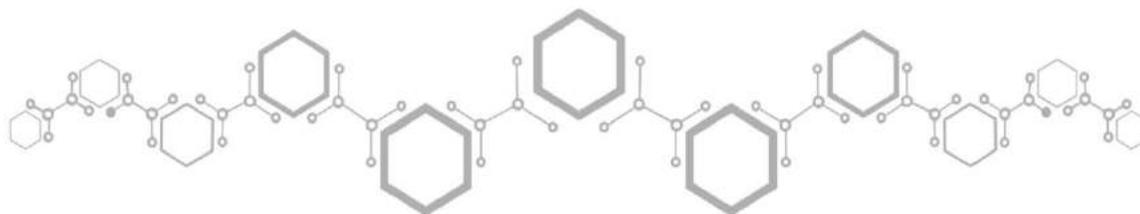


TRANSFORM TOX TESTING CHALLENGE

INNOVATING FOR METABOLISM

APPENDIX C: STAGE 1 ARCHIVE





TRANSFORM TOX TESTING CHALLENGE

INNOVATING FOR METABOLISM

APPENDIX C: STAGE 1 ARCHIVE





DETAILED SCHEDULE

CALL FOR STAGE 1 SUBMISSIONS

January 8, 2016



SUBMISSION DEADLINE

April 8, 2016

The Challenge will stop accepting submissions at 11:59PM EST on Thursday, April 8



SEMI-FINALISTS ANNOUNCED

May 16, 2016

Up to ten applicants will be chosen by a panel of experts to receive \$10,000 each and advance to Stage 2



WORKSHOP DAY

July 8, 2016

Semi-finalists will participate in an in-person workshop in Washington, D.C. to prepare for the launch of Stage 2 of the competition.





TOTAL PRIZE POOL

\$1,000,000

From the \$1 million prize pool, up to ten semi-finalists will be awarded \$10,000 each and invited to participate in Stage 2. Up to five Stage 2 participants may be selected as finalists, awarded up to \$100,000 each and invited to participate in the final stage. After final judging, one winner may be awarded up to \$400,000.

STAGE 1 APPLICANTS' RULES, TERMS, AND CONDITIONS

Thank you for your efforts towards integrating metabolic competence into toxicity testing. The following Rules, Terms & Conditions must be carefully followed and agreed to by all Applicants to Stage 1 of this competition - the submission of a practical design.

The Transform Tox Testing Challenge is a three-stage event, with prizes awarded as Applicants advance to the next stage of the Challenge; prize amounts are subject to change. The government reserves the right to cancel the later stages or award smaller prizes for partial solutions.

RULES FOR ELIGIBILITY

To be eligible to win Stage 1 of this Challenge, an individual or entity:

- (a) Must have entered a submission on transformtoxtesting.com under the rules promulgated by the U.S. Environmental Protection Agency (EPA), the National Institutes of Health's (NIH) National Center for Advancing Translational Sciences (NCATS), and the National Toxicology Program (NTP), headquartered at the National Institute of Environmental Health Sciences (NIEHS).
- (b) Must be an individual or team comprised of members each of whom are 18 years of age or over.
- (c) Must not be on the Excluded Parties List System located at <https://www.epls.gov/>.
- (d) May not be a Federal entity or Federal employee acting within the scope of their employment.
- (e) The applicant shall not be deemed ineligible because the applicant used Federal facilities or consulted with Federal employees during a competition if the facilities and employees are made equitably available to all applicants participating in the competition.
- (f) Federal grantees may not use Federal funds to develop challenge applications unless consistent with the purpose of their grant award. Federal contractors may not use Federal funds from a contract to develop challenge applications or to fund efforts in support of a challenge submission.
- (g) Employees of EPA and NIH, and/or any other individual or entity associated with the development, evaluation, or administration of the Challenge as well as members of such persons' immediate families (spouses, children, siblings, parents), and persons living in the same household as such persons, whether or not related, are not eligible to participate in the Challenge.
- (h) Applicants must agree to assume any and all risks and waive claims against the Federal Government and its related entities, except in the case of willful misconduct, for any injury, death, damage, or loss of property, revenue, or profits, whether direct, indirect, or consequential, arising from their participation in a competition, whether the injury, death, damage, or loss arises through negligence or otherwise.
- (i) Applicants must also agree to indemnify the Federal Government against third party claims for damages arising from or related to competition activities.
- (j) Applicants are not required to obtain liability insurance or demonstrate financial responsibility in order to participate in the Challenge.
- (k) By participating in the Challenge, each Applicant agrees to comply with and abide by these Official Rules, Terms & Conditions and the decisions of the Federal Agency sponsors and/or the individual judges, which shall be final and binding in all respects.

APPLICATION SUBMISSION AND PARTICIPANT RULES FOR STAGE 1

- Applications must be submitted in English.
- Applications submitted via regular mail, facsimile, or email will not be accepted.
- Complete applications must be submitted by the deadline of the Transform Tox Testing Challenge (11:59pm EST on April 8, 2016) using the online platform. No additions or modifications to the applications will be accepted after the submission deadline.
- NIH and EPA bear no responsibility for submission errors resulting from transmission or conversion processes associated with electronic submissions.
- If no qualifying application can be verified at the completion of the Stage 1 Application Submission, the Transform Tox Testing Challenge may reopen, at the sole discretion of NIH, and EPA.

REPRESENTATION, WARRANTIES, AND INDEMNIFICATION

By entering in the Challenge, each Applicant represents, warrants, and covenants as follows:

- (a) Applicant is the sole author, creator, and owner of the Submission;
- (b) The Submission is not the subject of any actual or threatened litigation or claim;
- (c) The Submission does not and will not violate or infringe upon the intellectual property rights, privacy rights, publicity rights, or other legal rights of any third party;
- (d) The Submission does not and will not contain any harmful computer code (sometimes referred to as “malware,” “viruses” or “worms”); and
- (e) The Submission, and Applicants’ use of the Submission, does not and will not violate any applicable laws or regulations, including, without limitation, applicable export control laws and regulations of the U.S. and other jurisdictions.

If the Submission includes any third party works (such as third party content or open source code), Applicant must be able to provide, upon request, documentation of all appropriate licenses and releases for such third party works. If Applicant cannot provide documentation of all required licenses and releases, Federal Agency sponsors reserve the right, at their sole discretion, to disqualify the applicable Submission. Conversely, they may seek to secure the licenses and releases and allow the applicable Submission to remain in the Challenge, while reserving all rights with respect to such licenses and releases.

Applicants must indemnify, defend, and hold harmless the Federal Government from and against all third party claims, actions, or proceedings of any kind and from any and all damages, liabilities, costs, and expenses relating to or arising from Applicant’s Submission or any breach or alleged breach of any of the representations, warranties, and covenants of Applicant hereunder.

The Federal Agency sponsors reserve the right to disqualify any Submission that, in their discretion, deems to violate these Official Rules, Terms & Conditions.

INTELLECTUAL PROPERTY

- Applicants are free to discuss their submission and the ideas or technologies that it contains with other parties; encouraged to share ideas/technologies publicly; encouraged to collaborate or combine with other teams to strengthen their solutions; and are free to contract with any third parties, as long as they do not sign any agreement or undertake any obligation that conflicts with the Challenge rules, terms and conditions.
- Upon submission, each Applicant warrants that he or she is the sole author and owner of the work and any pertinent Intellectual Property (IP) rights, that the work is wholly original of the Applicant (or is an improved version of an existing work that the Applicant has sufficient rights to use—including the substantial improvement of existing open-source work), and that it does not infringe any copyright or any other rights of any third party of which Applicant is aware. Each Applicant also warrants that the work is free of malware.
- Applicants retain ownership of their concepts, including any software, research or other intellectual property (“IP”) that they develop in connection therewith, subject to the license granted to Federal Agency sponsors as set forth herein.
- Upon award of a Stage 2 prize and/or Stage 3 prize, each Applicant must grant the U.S. government a 5-year royalty-free, irrevocable, non-exclusive, non-transferrable worldwide license to practice or have practiced for or on behalf of the United States any invention throughout the world owned or controlled by the Applicant that covers the Entry.
- Each Applicant must clearly delineate any Intellectual Property (IP) included in the application that is owned by the Applicant, and which the Applicant wishes to protect as proprietary data.
- All materials submitted to NIH and EPA as part of a submission become NIH and EPA records and cannot be returned. Any confidential commercial information contained in a submission must be designated at the time of submission.
- FOIA: Submitters will be notified of any Freedom of Information Act requests for their submissions in accordance with 29 CFR 70.26.

PRIZES

The total prize pool for the Challenge is up to \$1,000,000 across all three stages. Prizes awarded under this competition will be paid by electronic funds transfer and may be subject to Federal income taxes. EPA will comply with Internal Revenue Service withholding and reporting requirements, where applicable.

DATES/DEADLINES

The Federal Government reserves the right to modify any dates or deadlines set forth in these Official Rules, Terms & Conditions or otherwise governing the Challenge. Final deadlines and other dates will be posted online.

CHALLENGE TERMINATION

The Federal Government reserves the right to suspend, postpone, cease, terminate or otherwise modify this Challenge, or any Applicant's participation in the Challenge, at any time at its discretion.

STAGE 1 SUBMISSIONS

Using the Submission Platform, submit your concept for how high-throughput screening (HTS) assays could be retrofitted to include xenobiotic metabolic competence. Applicants will be given from January 8, 2016 to April 8, 2016 to present detailed descriptions, specifications, precedents, and requirements necessary to show that their idea can be turned into a product or method able to solve the problem.

Submissions for Stage 1 should include the following:

- Written design proposal using the submission template
- Background information that shows evidence to support the idea
- A description on how you arrived at your idea
- A description of the methods and technologies needed to develop the product
- An evaluation that describes your ability to execute the idea in Stage 2

STAGE 1 SCORING CRITERIA

The first stage of this competition welcomes ideas from all innovators. Entrants are required to describe, in detail, their approach to currently used HTS systems with xenobiotic metabolic competence. Submissions will be judged based on the criteria outlined below. Before being scored against weighted performance criteria, entries must meet the absolute performance criteria provided in the table below:

Absolute Criteria	Score Range
Technological Approach	Yes or No
Applicability	Yes or No
Ease of Use	Yes or No

Technological Approach (Yes or No)

The overall technological approach will be evaluated by the Judging Panel. A clear and concise description of the proposed solution to supply currently used HTS systems with xenobiotic metabolic competence (i.e. human Phase I and Phase II enzymes) will allow the Judging Panel to assess the feasibility of achieving the performance criteria. Entries must include a diagram and text that clearly and thoroughly describe the working principle, process flow, and functional operation of the solution. If appropriate, the diagram must include the approximate footprint of the HTS design.

Ease of Use (Yes or No)

- The proposed solution requires less than 48 hours to implement in advance of an HTS experiment.
- The proposed solution does not require constant mixing or multiple wash steps or solution exchanges during the HTS assay.

Entries meeting the absolute criteria will be scored based on the weighted (% of total score) criteria provided in the table below. Each performance criteria will be scored on a scale of 0-5 with 0 being the lowest and 5 being the highest.

Performance Criteria	Weight
Enzyme Range and Activity*	40%
Scalability	15%
Interference	15%
Differential Enzyme Activity	15%
Opportunity and Potential	15%

Enzyme Range and Activity

- The ideal solution conveys xenobiotic metabolism competence relevant to toxicological effects (e.g. CYP2E1).
- The ideal solution is expandable beyond cytochrome P450s (CYPs).
- The proposed solution will incorporate a minimum of five of the following human xenobiotic metabolizing enzymes:

PHASE I ENZYMES

Enzyme	Substrate	Metabolite
hCYP1A1	EROD	Resorufin
hCYP1A2	Phenacetin	Acetaminophen
hCYP2A6	Coumarin	7-hydroxycoumarin
hCYP2B6	Bupropion	Hydroxybupropion
hCYP2C8	Paclitaxel	6 α -hydroxypaclitaxel
hCYP2C9	Diclofenac	4'-hydroxydiclofenac
hCYP2C19	Mephenytoin	4'-hydroxymephenytoin
hCYP2D6	Dextromethorphan	Dextrorphan
hCYP2E1	Chlorzoxazone	6-hydroxychlorzoxazone
hCYP3A4	Midazolam	1-hydroxymidazolam

PHASE II ENZYMES

Enzyme	Substrate	Metabolite
UGT	7-hydroxycoumarin	7-hydroxycoumarin glucuronide
SULT	7-hydroxycoumarin	7-hydroxycoumarin sulfate

Scalability

- The proposed solution must be scalable to a 96-well platform. The ideal solution will be scalable to 384 and 1,536-well plate formats.
- The ideal solution will be scalable to hundreds of multi-well plates in a single HTS experiment.

Interference

- The ideal solution will not sensitize cell-based assays or cause cytotoxicity.
- The ideal solution will not interfere with (potentiate or attenuate) enzyme or receptor function in cell-free assays.
- The ideal solution will not interfere with established fluorescence, luminescent, or absorbance HTS endpoints.

Differential Enzyme Activity

- The ideal solution has the ability to mimic physiological differences in the proportions of enzymes.

Opportunity and Potential

- Is the proposed technology/method economically viable and scalable/replicable?
- Does the entry clearly define possible barriers to success?
- Does the entry identify the competitive advantage of product/service?
- Does the entry address a pathway or timeline to commercial viability and/or broad use?
- Is there appropriate expertise and capability to bring the idea to the testing/deployment stage?
- Do the team members have the relevant expertise and resources available to carry out proposed work?

TECHNICAL REFERENCES

Proposed solutions must be capable of retrofitting onto existing ToxCast and Tox21 HTS assays, be compatible with cell-based and/or cell-free platforms currently used in ToxCast and Tox21 HTS assays, and have a stable activity for the length of standard assays. Challenge participants should familiarize themselves with the resources below as they prepare their solutions for application.

TOXCAST & TOX21 RESOURCES

EPA's Toxicity Forecaster (ToxCast) generates data and predictive models on thousands of chemicals of interest to the EPA. Toxicology Testing in the 21st Century (Tox21) is a federal collaboration among EPA, NIH, including NCATS and NTP at NIEHS, and the FDA.

- [ToxCast & Tox21 High-Throughput Assay Information](#)
- [ToxCast Users Guide](#)
- [EPA's National Center for Computational Toxicology](#)
- [EPA's Rapid Chemical Exposure and Dose Research \(ExpoCast\)](#)
- [Video overview of ToxCast & Tox21](#)
- [NCATS High-Throughput Screening Assay Guidance](#)

SELECTED ARTICLES

In addition to the selected articles below, please also visit the [Index of ToxCast-Relevant Articles](#) and the [EPA Science Inventory](#) for more information about ToxCast and Tox21.

- Huang R, Sakamuru S, Martin MT, Reif DM, Judson RS, Houck KA, Casey W, Hsieh JH, Shockley KR, Ceger P, Fostel J, Witt KL, Tong W, Rotroff DM, Zhao T, Shinn P, Simeonov A, Dix DJ, Austin CP, Kavlock RJ, Tice RR, Xia M. Profiling of the Tox21 10K compound library for agonists and antagonists of the estrogen receptor alpha signaling pathway. *Sci Rep.* 2014 Jul 11; 4: 5664.
- Huang R, Xia M, Cho MH, Sakamuru S, Shinn P, Houck KA, Dix DJ, Judson RS, Witt KL, Kavlock RJ, Tice RR, Austin CP. Chemical genomics profiling of environmental chemical modulation of human nuclear receptors. *Environ Health Perspect.* 2011 Aug; 119(8): 1142-8.
- Martin MT, Dix DJ, Judson RS, Kavlock RJ, Reif DM, Richard AM, Rotroff DM, Romanov S, Medvedev A, Poltoratskaya N, Gambarian M, Moeser M, Makarov SS, Houck KA. Impact of environmental chemicals on key transcription regulators and correlation to toxicity end points within EPA's ToxCast program. *Chem Res Toxicol.* 2010 Mar 15; 23(3): 578-90.

- Rotroff DM, Dix DJ, Houck KA, Kavlock RJ, Knudsen TB, Martin MT, Reif DM, Richard AM, Sipes NS, Abassi YA, Jin C, Stampfl M, Judson RS. Real-time growth kinetics measuring hormone mimicry for ToxCast chemicals in T-47D human ductal carcinoma cells. *Chem Res Toxicol.* 2013 Jul 15; 26(7): 1097-107.
- Shukla SJ, Huang R, Simmons SO, Tice RR, Witt KL, Vanleer D, Ramabhadran R, Austin CP, Xia M. Profiling environmental chemicals for activity in the antioxidant response element signaling pathway using a high throughput screening approach. *Environ Health Perspect.* 2012 Aug; 120(8): 1150-6.
- Sipes NS, Martin MT, Kothiya P, Reif DM, Judson RS, Richard AM, Houck KA, Dix DJ, Kavlock RJ, Knudsen TB. Profiling 976 ToxCast chemicals across 331 enzymatic and receptor signaling assays. *Chem Res Toxicol.* 2013 Jun 17; 26(6): 878-95.
- Tice, R.R., Austin, C.P., Kavlock, R.J., Bucher, J.R., 2013. Improving the human hazard characterization of chemicals: a Tox21 update. *Environ. Health Perspect.* 121 (7): 756-765.
- Attene-Ramos et al., (2015) Profiling of the Tox21 chemical collection for mitochondrial function to identify compounds that acutely decrease mitochondrial membrane potential. *Environmental Health Perspectives* 123: 49-56.

ENTRY FORM

The Transform Tox Testing Challenge invites you to submit your innovative solution to help retrofit high-throughput screening (HTS) assays with chemical metabolic competence. This is a multi-stage event that will award up to \$1,000,000. Get started by completing this entry form and submitting your idea to Stage 1 of the challenge.

Submit the required information below. You can save and preview your entry as many times as you want until the Stage 1 deadline – April 8, 2016.

Good luck!

1. Describe how your approach will supply currently used HTS assays with metabolic competence, including: the working principle, the process flow, and the functional operation of the solution. Add a diagram to help illustrate your description and, if appropriate, the approximate footprint of the HTS design. (Content limited to 2500 characters)

Failure to respond Yes to all questions does NOT disqualify applicants from advancing in the Challenge. Judges are not expecting a perfect solution in Stage 1.

2. Is the proposed solution capable of retrofitting onto existing ToxCast and Tox21 HTS assays? (Yes or No)

3. Is the proposed solution compatible with cell-based and/or cell-free platforms currently used in ToxCast and Tox21 HTS assays? (Yes or No)

4. Does the proposed solution have stable metabolic activity for the length of ToxCast and Tox21 HTS assays (up to 48 hours)? (Yes or No)

5. Does the proposed solution require fewer than 48 hours to implement in advance of an HTS experiment? (Yes or No)

6. Does the proposed solution require constant mixing or multiple wash steps or solution exchanges during the HTS assay? (Yes or No)

7. Does the proposed solution provide chemical metabolism relevant to toxicological effects? (Yes or No)

8. Is the solution expandable beyond cytochrome P450s (CYPs)? (Yes or No)

9. Will the proposed solution incorporate a minimum of five of the most common enzymes (hCYP1A1, hCYP1A2, hCYP2A6, hCYP2B6, hCYP2C8, hCYP2C9, hCYP2C19, hCYP2D6, hCYP2E1, hCYP3A4, UGT, SULT) that metabolize chemicals? (Yes or No)

PHASE I ENZYMES

Enzyme	Substrate	Metabolite
hCYP1A1	EROD	Resorufin
hCYP1A2	Phenacetin	Acetaminophen
hCYP2A6	Coumarin	7-hydroxycoumarin
hCYP2B6	Bupropion	Hydroxybupropion
hCYP2C8	Paclitaxel	6 α -hydroxypaclitaxel
hCYP2C9	Diclofenac	4'-hydroxydiclofenac
hCYP2C19	Mephenytoin	4'-hydroxymephenytoin
hCYP2D6	Dextromethorphan	Dextrorphan
hCYP2E1	Chlorzoxazone	6-hydroxychlorzoxazone
hCYP3A4	Midazolam	1-hydroxymidazolam

PHASE II ENZYMES

Enzyme	Substrate	Metabolite
UGT	7-hydroxycoumarin	7-hydroxycoumarin glucuronide
SULT	7-hydroxycoumarin	7-hydroxycoumarin sulfate

10. Is the proposed solution scalable to a 96-well platform? (Yes or No)

11. Would the proposed solution be scalable to hundreds of multi-well plates in a single HTS experiment? If yes, please provide more details. (Content limited to 2000 characters)

12. Does the solution sensitize cell-based assays or cause toxicity? If yes, please provide more details. (Content limited to 2000 characters)

13. Does the solution interfere with (potentiate or attenuate) enzyme or receptor function in cell-free assays? If yes, please provide more details. (Content limited to 2000 characters)

14. Does the solution interfere with established fluorescence, luminescent, or absorbance HTS endpoints? If yes, please provide more details.
(Content limited to 2000 characters)

15. Does the solution have the ability to mimic physiological differences in the proportions of enzymes? If yes, please provide more details.
(Content limited to 2000 characters)

16. How much do you estimate your technology/method would cost? Please provide a detailed description of cost which includes estimates for all relevant materials, reagents, personnel time, and any additional drivers. (Content limited to 2000 characters)

17. Is your proposed technology/method scalable/replicable? If yes, please provide more details. (Content limited to 2200 characters)

18. Describe some of the possible barriers that could impede the proposed solution from succeeding. (Content limited to 2200 characters)

19. Describe the competitive advantage of your product/service. (Content limited to 2200 characters)

20. Briefly describe a pathway or timeline to commercial viability and/or broad use of the proposed solution. (Content limited to 2200 characters)

21. Give examples of appropriate expertise and capability you would use to bring the idea to the testing/deployment stage. (Content limited to 2200 characters)

22. Does your team have the relevant experience and resources available to carry out the proposed work? If yes, please provide more details.
(Content limited to 2200 characters)

23. Does your team have the relevant experience and resources available to carry out the proposed work? If yes, please provide more details.
(Content limited to 2200 characters)

APPENDIX D: STAGE 1 SEMI-FINALISTS



Dr. Lawrence Verneti
HanKayTox Consulting

We propose a 96- and 384-well microtiter plate with capabilities to: 1) supply rodent or human hepatocytes in an on-demand format suitable to co-culture with a second cell or cell free assay; 2) supply 96- and 384-well microtiter plates of hepatocytes in an on-demand format suitable to pre-condition test agents for the sequential transfer of test agents and metabolites directly to the assay test plates, and; 3) prepare and store 384- and 1536-well daughter plates for on-demand use of test agents in media pre-conditioned by hepatocytes.



Dr. Christopher Vulpe
University of Florida

Toxicity testing of chemicals in cultured cells can potentially decrease costs, increase throughput, and reduce animal use. However, cultured cells may not metabolize chemicals properly and may not accurately predict potential adverse effects of a chemical on a person. University of Florida and the State University of New York Polytechnic Institute toxicologists propose to take advantage of a powerful new technology, CRISPR mediated gene targeting, to turn metabolism back on in cultured cells. These CRISPR modified cells would more accurately mimic normal metabolism and improve the accuracy of toxicity tests.



Dr. Hongbing Wang
University of Maryland School of Pharmacy

We propose to develop a human primary hepatocyte (HPH)-immortalized cell co-culture model by utilizing a transwell platform that can be scaled up to a HTS format, allowing currently used, cell culture-based screening assays to run in an environment that produces physiologically relevant metabolites.

Properly cultured HPHs are well-recognized as one of the most relevant and practical models that maintain a broad spectrum of drug-metabolizing enzymes, transporter proteins, and hepatocyte-specific transcription factors.

Co-culture in a transwell platform allows the transfer of culture media, compounds, and their metabolites between the HPHs and immortalized cells, enabling our system to detect physiologically relevant activators and deactivators that will improve upon existing HTS assays.



Dr. Remco Westerink
Institute for Risk Assessment Sciences, Utrecht University

Hepa-HTS™ provides metabolic competence to existing HTS assays by allowing co-culture and perfusion of up to 96 3D cultures of hepatocytes in a membrane-free, standard microtiter plate format.

Hepa-HTS™ is based on MIMETAS' OrganoPlate® platform that offers superior optical quality for fluorescence-based determination of metabolic competence and resulting (hepa)toxicity.

Hepa-HTS™ can easily be combined with chemical analysis as well as existing Organoplate® applications such as Neuroscreen-3D to allow for the transition of multiple organ-on-a-chips towards an integrated body-on-a-chip.



Dr. Moo-Yeal Lee and Mr. Rayton Gerald

Cleveland State University and Mr. Rayton Gerald, Solidus Biosciences

Dr. Moo-Yeal Lee, teamed up with Mr. Rayton Gerald of Solidus Biosciences, Inc. proposes to develop a 384-pillar plate that is complementary to a conventional 384-well plate for high-throughput toxicity screening.

The 384-pillar plate, made of polystyrene with functional polymer coating, supports 3D cell cultures and comprises an array of human hepatic cells for gene expression and high-content toxicity screening.

For metabolism-induced toxicity screening, hepatic cell lines in hydrogels can be printed on the 384-pillar plate using a liquid dispenser or a microarray spotter and cultured in the 384-well plate to create miniaturized hepatic cell spheroids, which can be combined with drug metabolizing enzymes (DMEs) or recombinant adenoviruses carrying genes for DMEs in additional 384-well plates. We envision that the 384-pillar plate combined with the 384-well plate can be a robust and flexible system for high-throughput screening of compounds and will enable retrofitting of existing ToxCast assays to have metabolic competence.



Dr. Albert Li

In Vitro ADMET Laboratories LLC

The IVAL Exogenous Metabolism System (patent-pending) consists of a transwell insert containing human or animal hepatocytes. The EXM insert is placed into a cell culture well containing the target cells used for toxicity evaluation, serving as an exogenous hepatic metabolic system.

The chemical to be evaluated is added to the EXM transwell, allowing metabolism by the hepatocytes in the insert. Both the parent chemical and its metabolites would migrate across the semi-permeable membrane of the EXM transwell insert to interact with the target cells in the culture well. The IVAL EXM should be compatible with current ToxCast assays which employ multi-well (e.g. 96-well) plates.



Dr. James Rusling

University of Connecticut

Our project proposes to use magnetic beads coated with metabolic enzyme sources including microsomes and supersomes containing cyt P450s, bioconjugation enzymes and other human enzyme sources.

The enzyme-coated beads react with solutions of test chemicals in 96-well filter plates, and vacuum filtration transfers the metabolite product into a new 96-well plate ready for Tox Test assays.

Our group has already developed this approach for investigations as a front end for LC-MS/MS investigations of metabolite-related genotoxicity pathways, and should be readily converted as a front end for existing toxicity tests.



Dr. David Thompson

MilliporeSigma

Our approach will use a synthetic, polycistronic, self-replicating RNA vector that is capable of expressing multiple drug metabolism enzymes in the cell line of choice.

This system uses a single RNA species capable of self-replicating for a limited number of cell divisions, allowing continued expression of the drug metabolism enzymes during expansion and cryopreservation of the modified cell lines.

This approach was recently used to introduce the four reprogramming factors necessary to generate integration-free induced pluripotent stem (iPS) cells from human fibroblasts.



Dr. Stéphane C. Corgié

ZYMtronix

ZYMtronix commercializes a universal enzyme immobilization platform where magnetic particles self-assemble while encapsulating and stabilizing enzymes or combinations of enzymes. With full control over the immobilized enzymes, ZYMtronix will adapt its current screening product towards the production of metabolically competent bioassays that are compatible with the Tox21 and ToxCast high-throughput screening platforms.



Dr. Brian Johnson

Onexio Biosystems LLC

Our system, dubbed MICRO MT (Metabolism Integrated Cell RepOrter MicroTiter plate), uses the natural metabolic activity of human liver cell lines to generate chemical metabolites and then deliver these metabolites to existing reporter assays in a highly reproducible fashion. The MICRO MT format is technically simple, requires little additional equipment and is amenable to the high volume and high throughput needs of 21st century toxicology.