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Ensuring the Reliability and Relevance of High-Throughput Assays
A Case Study Testing for Potential Endocrine Activity of Chemicals

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Background

Tens of thousands of chemicals are currently in commerce, and hundreds more are introduced every year. To date, only a small fraction of chemicals have been fully assessed for potential risk. For example, EPA's Endocrine Disruption Screening Program (EDSP) has thousands of chemicals that need to be screened for endocrine disruption and could use a faster method to prioritize these chemicals for additional testing.

There are two major issues with current toxicity testing methods that limit the ability of scientists to assess these chemicals efficiently and effectively:

1. Current *in vivo* (animal) testing is expensive, time consuming and often uses large numbers of animals which has led to a backlog of untested chemicals. Results from animal testing are then extrapolated to evaluate dose, species and life stage differences which lead to uncertainties when assessing hazards and risks.
2. Current whole animal testing cannot provide clear mechanistic insight into human toxicity pathways. However, high-throughput *in vitro* testing methods can provide data on activities at the human cellular level.

The U.S. EPA and its partners have developed methods of toxicity testing that will help usher chemical toxicity testing into the 21st century. These testing methods (referred to as "Tox21" and Toxicity Forecaster

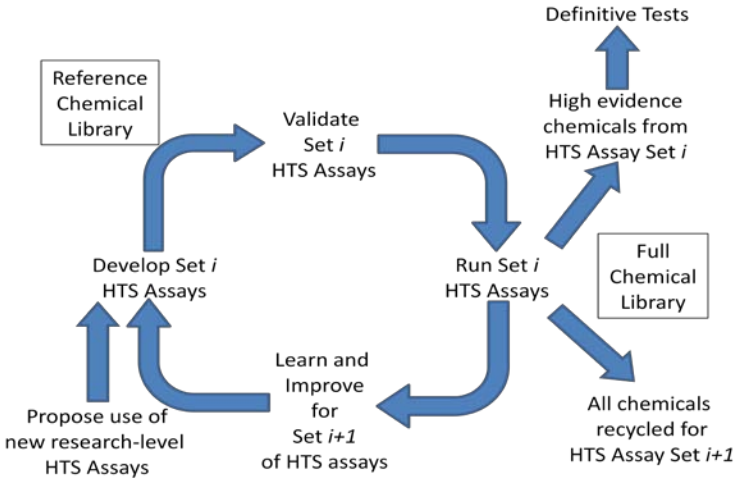

Fig. 1. Conceptual model of a continuously improving battery of HTS assays to be used for prioritization

"ToxCast") include high-throughput screening (HTS) assays and robotic technologies that significantly reduce the time, expense and resource demands of current chemical toxicity testing. Tox21 is currently screening approximately 10,000 chemicals using HTS assays.

Overview

A key obstacle to adoption of these HTS methods by regulators is the need for validation – that is, demonstration that the new approaches are relevant, reliable and fit for the intended purpose. Formal validation is needed to make sure that the data can stand up to legal challenges within regulatory processes. Although they are slow and inefficient, current toxicity testing methods are accepted by the regulated community. In order for all stakeholders to accept the usage of Tox21 and ToxCast assays to help make chemical regulatory decisions, these new methods must be validated to ensure they are of high quality.

Perspectives on Validation of High-Throughput Pathway-Based Assays Supporting the 21st Century Toxicity Testing Vision by Judson et al. describes proposed alterations to standard validation practices that could be used for HTS assays. Alterations are suggested to the validation process developed by Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM), the European Center for the Validation of Alternative Methods (ECVAM) and the Organization for Economic Co-operation and Development (OECD). The validation process includes defining the testing, conducting intra- and inter-laboratory testing and defining the relevance of the work.

Although it is important to ensure the validity of HTS assays, this formal validation process is lengthly and has can be a barrier to innovation.

It is desirable to develop a streamlined validation process to evaluate both the relevance and reliability of HTS assays. "Relevant" means that an assay must test some aspect of biology that will help assess the safety of a chemical. "Reliable" means that the assay must produce similar results over time, across reagent batches, etc. HTS assay data generally provides quantitative read-outs with a focused and mechanistically simple interpretation. These attributes should make evaluation of the relevance and reliability of the HTS assays, peer review and decisions on regulatory acceptance relatively straightforward.

Judson et al. presents two ideas that would lower the cost and time requirements of HTS validation and suggests criteria for assay evaluation and performance without compromising reliability:

1. Eliminating the requirement for round-robin cross-laboratory testing.
2. Developing a streamlined, online peer review process.

The argument for eliminating the need for cross-laboratory validation is based on three premises. Most importantly, with HTS assays one can run many positive and negative reference chemicals both during the validation phase and during routine production which provides ongoing quality assurance. Secondly, many HTS assays are run in laboratories with expensive customized, one-of-a kind equipment that could not be replicated elsewhere, making true replication of assay protocols difficult. Thirdly, for many HTS assays, the originating lab has the capacity to run all of the world's chemicals to be tested for a particular assay, thereby eliminating the need to have other labs be validated.

Developing an online peer review process would simplify review of any number of HTS assays because all the data would be in a common format in a centralized web-accessible database. Any group wishing to propose a new assay for use in a regulatory application would then have immediate access to all existing validation information on similar assays, and could submit their validation package into the central system to be queued up for subsequent peer review. This rapid and continuous preparation of validation documentation would facilitate the continuous improvement of assays to be used in regulatory prioritization [*see Fig. 1*].

One real world application of using HTS data discussed in Judson's paper is using ToxCast and Tox21 to prioritize chemicals for EPA's Endocrine Disruption Screening Program (EDSP). The prioritization would determine which of the thousands of potential endocrine disrupting chemicals are in most need of further testing.

*Using In Vitro High-Throughput Screening Assays to Identify Potential Endocrine Disrupting Chemicals (*Rotroff et al) investigated using EPA's ToxCast assays for assessing chemicals for estrogen, androgen, steroidogenic and thyroid disruption. The study used assay data from the 309 chemicals in ToxCast Phase I and data on an additional 23 reference chemicals and compared it to results from animal toxicity tests. The study demonstrates that current ToxCast HTS assays can accurately identify chemicals with the potential to interact with the estrogenic and androgenic pathways, with a balanced accuracy of over 90%. These results suggest that current ToxCast assays can accurately identify chemicals with potential to interact with the estrogenic and androgenic pathways, and could help prioritize chemicals for EDSP T1S assays.

Conclusions

These two potential alterations to standard assay validation practice proposed by Judson et al. could significantly streamline the acceptance criteria for new HTS technologies used for prioritization applications. The elimination of the requirement to do cross-laboratory testing already has precedent, while the development of an online peer review process offers not only greater efficiency, but also additional transparency relative to current practice.

Although both of these recommendations might be considered controversial due to their departure from current validation practice, both suggestions merit serious consideration given the significant advantages offered by HTS assays.

General Information

U.S. EPA and its partners developed Tox21and ToxCast to create new methods for efficiently screening chemicals and prioritizing limited testing resources to chemicals that represent the greatest potential hazard to human health and the environment.

Tox21 research is performed by the U.S. EPA National Center for Computational Toxicology in collaboration with the National Institutes of Environmental Health Sciences/National Toxicology Program, the National Institutes of Health (NIH)/National Human Genome Research Institute, the NIH Center for Translational Therapeutics (NCTT) and the Food and Drug Administration (FDA).

Originating Organization for Fact Sheet

EPA

Reference

Judson, et al. "Perspectives on Validation of High-Throughput Pathway-Based Assays Supporting the 21st Century Toxicity Testing Vision" (2012).

Rotroff et al. "Using *In Vitro* High-throughput Screening Assays to Identify Potential Endocrine Disrupting Chemicals" (2012).

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