Current Status of New Approach Methodologies

EMAP 514: Introduction to Environmental Health Risk Assessment and Management
Environmental Metrology and Policy Program
GEORGETOWN UNIVERSITY

April 29, 2019

Dr. Maureen R. Gwinn
National Center for Computational Toxicology
Office of Research and Development
US Environmental Protection Agency

The views expressed in this presentation are those of the author and do not necessarily reflect the views or policies of the U.S. EPA
New Approach Methodologies (NAMs)

- Commonly defined to include *in silico* approaches, *in chemico* and *in vitro* assays, as well as the inclusion of information from the exposure of chemicals in the context of hazard assessment.

- Recently defined in the EPA’s TSCA Alternative Toxicity Strategy as:
  - a broadly descriptive reference to any technology, methodology, approach, or combination thereof that can be used to provide information on chemical hazard and risk assessment that avoids the use of intact animals.


Toxicology Moving to Embrace 21st Century Methods

Use of NAMs in Filling Gaps in Hazard and Exposure Information

- Use of comprehensive screening to inform hazard characterization
  - High- and Medium-Throughput Screening Assays
  - High-Throughput Metabolism
  - High-Throughput Transcriptomics & Phenotypic Profiling

- Higher Tier Adversity
  - Organotypic Cellular Models
  - Virtual Tissue Models

- High-throughput toxicokinetics
  - In-vitro studies
  - In-silico models and tools

- Consensus multi-pathway modeling approaches (e.g., ExpoCast SEEM)

- Use of structure-based machine-learning QSAR models to predict exposure information
  - Functional use
  - Exposure pathways
High-Throughput Assays Used to Screen Chemicals for Potential Toxicity

- Understanding of what cellular processes/pathways may be perturbed by a chemical
- Understanding of what amount of a chemical causes these perturbations
Innovations in Incorporating Xenobiotic Metabolism

“Extracellular” Approach
Chemical metabolism in the media or buffer of cell-based and cell-free assays
More closely models effects of hepatic metabolism and generation of circulating metabolites

“Intracellular” Approach
Chemical metabolism inside the cell in cell-based assays
More closely models effects of target tissue metabolism

Integrated strategy to model *in vivo* metabolic bioactivation and detoxification
High-Throughput Transcriptomics and Phenotypic Profiling

Requirements:

- Low cost
- Whole genome
- Automatable

Thousands of chemicals × Multiple Cell Types

- 384 well
- ~1300 endpoints

Illustrations from Perkin Elmer

(tcpp: “components”)
Application of High-Throughput Assays to Identify Potential Endocrine Disrupting Chemicals

- Use multiple assays per pathway
  - Different technologies
  - Different points in pathway
- Use model to integrate assays
- Model creates a composite dose-response curve for each chemical to summarize results from all assays

18 In Vitro Assays Measure ER-Related Activity

Judson et al., Tox Sci. 2015
Browne et al., ES&T. 2015
Kleinstreuer et al., EHP 2016
Innovating in Organotypic Culture Models to Predict Tissue Effects

How do we generate quantitative linkages from MIE effects to immediate key events in the thyroid DNT AOP framework for hundreds of chemical “hits”?
Consensus Exposure Predictions with SEEM Framework

• Different exposure models incorporate knowledge, assumptions, and data (Macleod, et al., 2010)
• We incorporate multiple models into consensus predictions for 1000s of chemicals within the Systematic Empirical Evaluation of Models (SEEM) framework (Wambaugh et al., 2013, 2014; Ring et al., 2018).
High-Throughput Toxicokinetic Component

- Currently evaluated ~700 ToxCast Phase I and II chemicals
- Models available through “httk” R package (https://cran.r-project.org/web/packages/httk/)

**EPA ToxCast Phase I and II Chemicals**

- Human Liver Metabolism
- Human Plasma Protein Binding

**Population-Based IVIVE Model**

- Upper 95th Percentile Css Among 100 Healthy Individuals of Both Sexes from 20 to 50 Yrs Old

**In Vitro Potency Value**

- Administered Dose Required to Achieve Steady State Plasma Concentrations Equivalent to *In Vitro* Bioactivity

- Rotroff *et al.*, *Tox Sci.*, 2010
- Wetmore *et al.*, *Tox Sci.*, 2012
- Wetmore *et al.*, *Tox Sci.*, 2015
Results from High Throughput Assays Provide a Conservative Estimate of Adverse Effects

POD<sub>ToxCast</sub> trad < POD<sub>ToxCast</sub> < POD<sub>ExpoCast</sub>

Total = 448 chemicals

For ~92% of the chemicals, POD<sub>ToxCast</sub> was conservative. (~100-fold with human HTTK, ~50-fold with rat HTTK)

International case study with EPA, ASTAR, ECHA, Health Canada, and EFSA
Broad Success Derived from High-Throughput Screening Approaches

Group Chemicals by Similar Bioactivity and Predictive Modeling

Provide Mechanistic Support for Hazard ID

Prioritization of Chemicals for Further Testing

Carcinogenicity of perfluorooctanoic acid, tetrafluoroethylene, dichloromethane, 1,2-dichloropropane, and 1,3-propane sulfone

In June 2014, 18 experts from 11 countries met at the International Agency for Research on Cancer (IARC) to assess the carcinogenicity of perfluorooctanoic acid (PFOS). The working group considered the evidence of carcinogenicity in experiments in mammals and in humans. The group concluded that there is no evidence of carcinogenicity in humans, but there is some evidence of carcinogenicity in animals. The IARC monograph on carcinogenicity of perfluorooctanoic acid (PFOS) is available online at http://monographs.iarc.fr.

Carcinogenicity of tetrachlorvinphos, parathion, malathion, diazinon, and glyphosate

In March 2013, 17 experts from 11 countries met at the International Agency for Research on Cancer (IARC) to assess the carcinogenicity of tetrachlorvinphos (TCV, Parathion, Intermittent Process, Intermediate Product, Assay, Non-Linear, ELISA antigen, ELISA antibody, Interference pathway). The working group considered the evidence of carcinogenicity in experiments in mammals and in humans. The group concluded that there is no evidence of carcinogenicity in humans, but there is some evidence of carcinogenicity in animals. The IARC monograph on carcinogenicity of tetrachlorvinphos (TCV, Parathion) is available online at http://monographs.iarc.fr.

Carcinogenicity of indane, DDT, and 2,4-dichlorophenoxyacetic acid

In June 2013, 30 experts from 11 countries met at the International Agency for Research on Cancer (IARC) to assess the carcinogenicity of indane and DDT (DDT) in the workplace. The working group considered the evidence of carcinogenicity in experiments in mammals and in humans. The group concluded that there is no evidence of carcinogenicity in humans, but there is some evidence of carcinogenicity in animals. The IARC monograph on carcinogenicity of indane and DDT (DDT) is available online at http://monographs.iarc.fr.

Prioritization of Chemicals for Further Testing

Assays/Pathways

IARC Monographs 110, 112, 113
Conclusions

• Incorporating new technologies and innovations in toxicology can more rapidly and inexpensively screen chemicals for potential adverse biological effects

• Incorporating dosimetry and exposure provides an important dose and exposure context

• Uncertainty analysis of NAMs is an ongoing part of research and development of these new technologies

• Data management systems and decision support tools will be increasingly important for interpreting and integrating the expanding and diverse landscape of chemical safety information for use in weigh-of-evidence decisions
Thank You for Your Attention!

Tox21 Colleagues:
  NTP Crew
  FDA Collaborators
  NCATS Collaborators

EPA Colleagues:
  NERL
  NHEERL
  NCEA

EPA’s National Center for Computational Toxicology
Additional slides
Case Study on the Use of an Integrated Approach to Testing and Assessment for Identifying Estrogen Receptor Active Chemicals

Dr. Maureen R. Gwinn (gwinn.maureen@epa.gov)
U.S. Environmental Protection Agency
Office of Research and Development
Washington, DC

The views expressed in this presentation are that of the presenter and do not represent the views and/or policies of the US Environmental Protection Agency.
The intended application of this IATA is for

- screening of environmental chemicals based on their ER agonist activity
- determining whether further evaluation of endocrine-related activity in higher tier in vivo tests (e.g., female pubertal assay, two generation reproductive toxicity study) is needed
Purpose

- To use a combination of 16 in vitro high throughput screening (HTS) assays and a computational model for estrogen receptor (ER) agonist activity, as an alternative to low and medium throughput in vitro and in vivo tests for ER activity.
Overall Approach

Internal

ER High-Throughput Screening Data

ER Pathway Computational Model

External

*In vitro* reference chemicals

*In vivo* reference chemicals

Model Performance Evaluation

Validated Model for Chemical Screening
Results of this analysis demonstrate that one could use one of multiple subset models to accurately predict estrogenic activity of a chemical.

Subsets of as few as 4 of the original 16 agonist assays have acceptable performance against the full model, and the in vitro and in vivo reference chemicals.

The acceptable subsets all have assays that:
- probe diverse points in the ER pathway
- use diverse assay reporting technologies
- use diverse cell types
Summary of Proposed Case Study

- Outlines the curation of lists of reference chemicals for *in vitro* and *in vivo* ER activity
- Integrates results from multiple *in vitro* assays using pathway-based ER computational model as an IATA
- Evaluates performance of the IATA using the curated lists of reference chemicals
- Demonstrates equivalent performance for subsets of *in vitro* assays
- Characterizes the uncertainty associated with the *in vitro* assays and computational model
- Discusses potential application to regulatory decisions