EPA-Specific Drivers: EDSP

- The US Environmental Protection Agency’s (EPA) Endocrine Disruptor Screening Program (EDSP) was established in response to Congressional mandates in the Federal Food Quality Protection and Safe Drinking Water Acts.
- It evaluates potential risk of endocrine disruption in humans and wildlife from exposure to pesticide chemicals and drinking water contaminants.
- Recommendations from an expert advisory committee established a two-tiered system:
  - Tier 1 screening for potential to interact with the estrogen, androgen or thyroid hormone systems.
  - Tier 2 testing to verify interaction and quantify dose-response relationships.
- In 2011, EPA began a multiyear transition to prioritize and screen thousands of EDSP chemicals using high-throughput in vitro assays and computational modeling approaches.

In 2009, EPA published list of 67 pesticide chemicals (List 1) for Tier 1 screening (15 subsequently withdrawn).

In 2013, EPA published a revised second list (List 2) of 109 chemicals for proposed Tier 1 screening.

The cost of running the Tier 1 battery is ~$1 million per chemical.

The number of animals potentially used for EDSP tier 1 battery is approximately 600 animals for one chemical (~200 Rats, 80 fish and 320 frogs).

At current rate, it would take decades and cost billions of dollars to screen all 10,000 chemicals of interest to EPA for potential endocrine activity.
The Approach

- Developed multiple high-throughput screening assays
  - Use multiple assays per pathway
    - Different technologies
    - Different points in pathway
  - No assay is perfect
    - Assay Interference
    - Noise

- Use a systems biology model to integrate assays
  - Model creates a composite dose-response curve for each chemical to summarize results from all assays

Estrogen Receptor Computational Model
Judson et al., Envi Health Pers (2015)

Androgen Receptor Computational Model
Kleinstreuer et al., Chem Res Toxicol (2017)
Evaluating the Approach

- Comparison to existing literature studies
- Comparison to curated reference chemicals
- Peer-reviewed publications
- FIFRA Scientific Advisory Panel (SAP)
- Organization of Economic Cooperation and Development (OECD) review

Lessons Learned

• **Impact of Cytotoxicity:** Analysis and filtering of cytotoxic ‘burst’

• **Subset Model:** Developed smaller subset pathway models and criteria for assay selection in the subset to allow use of existing/preferred assays.

• **Metabolic Competence:** Lack metabolic competence in in vitro HTS Assays may lead to over- or underestimation of chemical hazard.

• **Uncertainty:** In the analysis of the HTS assays, there is a need to establish uncertainty bounds around potency and efficacy values.
• Most chemicals display a “burst” of potentially non-selective bioactivity near the cytotoxicity concentration.

• This is often “false positive” activity
  • E.g. Activity in an ER assay in the “burst” region is likely due to cell stress and not true ER binding activity

• Statistical method can be used to filter out this false positive activity before drawing conclusions about ER, AR (or other specific target) activity

ER and AR Subset models

- Original ER and AR models used many redundant assays to help understand the types of noise and assay interference occurring in in vitro assays.
- “Subset models” were developed: Rebuild the original models using all subsets of assays (2, 3, 4, … assays) and evaluated against the full model using balanced accuracy as the performance metric.
- Results show that subsets with fewer 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 pathway
  - use diverse assay reporting technologies
  - use diverse cell types
- ER Agonist: 4 or more assays
- AR Antagonist: 5 or more assays

Retrofitting Metabolism: AIME method suitable for biochemical- and cell-based HTS assays

Screening Throughput: Adaptable to 96- and 384-well screening platforms

Regulatory Relevance: Integration of phase I liver metabolism for hazard identification of parent and metabolite endocrine activity

Results: Evaluation of a 63 chemical test set supports metabolic screening for -
  - Refinement of prioritization for ER-active substances based on metabolite effects
  - In some cases, supports more accurate prediction of in vivo effects for biotransformed substances

Alginate Immobilization of Metabolic Enzymes (AIME) Method: S9 fraction immobilization in alginate microspheres on 96- or 384-well peg lids

Parallel evaluation of parent compound and metabolites identifies false positive and false negative effects

Collaboration with Unilever  C. Deisenroth, Unpublished
Uncertainty Analysis

Major sources of uncertainty:
1. Qualitative: is an assay “hit” really due to ER/AR activity, or assay interference?
2. Quantitative: uncertainty around the true potency value (AC50)

Both are now incorporated into the ER and AR model results through the development of statistical methods have been developed to establish uncertainty bounds around potency and efficacy values. These statistical methods involve resampling the data and refitting the concentration response curves thousands of times to quantitatively estimate the uncertainty.

Bootstrap Uncertainty in In Vitro Potency Values

Computational Modeling

ER Pathway Model

Propagation of Uncertainty in Modeling Output

18 ER In Vitro Assays

Watt and Judson, PLOS One 2018 doi.org/10.1371/journal.pone.0196963
CERAPP and CoMPARA

- Large scale QSAR modeling projects to predict ER and AR activity
- CERAPP - Collaborative Estrogen Receptor Activity Prediction Project
- CoMPARA : Collaborative Modeling Project for Androgen Receptor Activity
- Use ER and AR Pathway model results to train QSAR models
- Use data from the open literature to evaluate
- Many expert groups from US, Europe, Japan and China submitted models, from which consensus models were derived
- Modes: Binding, Agonist, Antagonist
- Model types:
  - Qualitative (active, inactive),
  - Semi-quantitative (inactive, very weak, weak, moderate, strong)
- Results available through the CompTox Chemicals Dashboard

Mansouri et al., Environmental Health Perspectives (2016) doi: 10.1289/ehp.1510267
Mansouri et al., Environmental Health Perspectives (in press 2019).
HT-H295R model for Steroidogenesis

- Developed a high-throughput H295R (HT-H295R) assay that includes measurement of 11 hormones, including progestogens, corticosteroids, androgens, and estrogens.

- To date, 2012 chemicals have been screened at 1 concentration; of these, 656 chemicals have been screened in concentration-response. The objectives of this work were to:
  - (1) develop an integrated analysis of chemical-mediated effects on steroidogenesis in the HT-H295R assay and
  - (2) evaluate whether the HT-H295R assay predicts estrogen and androgen production specifically via comparison with the OECD-validated H295R assay.

- Evaluated the robustness, reproducibility, and power of the HT-H295R statistical model per feedback received at Scientific Advisory Panel review.

- Demonstrated the use of the HT-H295R statistical model in a selectivity-based prioritization exercise.
• Considering the thyroid-related AOP network as an outline for HTS screening
  • Ongoing research on the development of screening assays for molecular initiating events and key events
  • Includes development of confirmatory approaches that could be used in a future model
Ongoing and Next Steps

- Expanding acceptance and implementation of this work through OECD
  - ER model Integrated Approach to Testing and Assessment (IATA; published 2019)
  - AR model IATA (initiated 2019)
  - ER Defined Approach (initiated 2019)
- Continue to apply this approach to address other EDSP needs
  - Steroidogenesis
  - Thyroid
- Translation to possible tissue- and organ-level effects
  - Organotypic model development
- Including exposure components to give the risk context
  - In vitro-to-in vivo extrapolation (IVIVE)
Take Home Messages

• EPA has addressed the need to screen and prioritize thousands of chemicals quickly and without the use of animals through:
  • Development of high-throughput screening assays
  • Integrated computational models
  • Development of in silico consensus models

• EPA has made great advances on including uncertainty and metabolic competence in analysis of high-throughput assays and computational approaches.

• Current approaches can be applied more broadly beyond what is described here, and can be used across testing laboratories and decision contexts.

• An important component of scientific confidence in these approaches is performance-based evaluation as compared to curated reference chemicals.
Questions?

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US Environmental Protection Agency
Developing Alternative EDSP Assays

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</table>

ER = estrogen receptor; AR = androgen receptor; STR = steroidogenesis; THY = thyroid
Developing Organotypic Culture Models to Identify Tissue/Organ Effects

Blue, Hoechst 33342 /DNA
Green, Phalloidin/Actin

C. Deisenroth, In Review
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/](https://cran.r-project.org/web/packages/httk/)

Population-Based IVIVE Model

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

- Administered dose required to achieve steady state plasma concentrations equivalent to In Vitro Bioactivity

Reverse Dosimetry

Wetmore *et al.*, *Tox Sci.*, 2012
Wetmore *et al.*, *Tox Sci.*, 2015
Rotroff *et al.*, *Tox Sci.*, 2010

In Vitro Potency Value

Plasma Concentration

Exposure Route

Human Liver Metabolism

Human Plasma Protein Binding

EPA ToxCast Phase I and II Chemicals