Case Study 1
Predictive Modeling of Endocrine Disruption Pathways

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NAS Workshop on Evidence Integration in Chemical Assessments
4th June, 2019
National Toxicology Program Interagency Center for the Evaluation of Alternative Toxicological Methods (NICEATM), organized as an office under the NTP Division, part of NIEHS
• Interagency Coordinating Committee for the Validation of Alternative Methods


• To establish, wherever feasible, guidelines, recommendations, and regulations that promote the regulatory acceptance of new and revised toxicological tests that protect human and animal health and the environment while reducing, refining, or replacing animal tests and ensuring human safety and product effectiveness.

7 Regulatory Agencies

Consumer Product Safety Commission
Department of Agriculture
Department of the Interior
Department of Transportation
Environmental Protection Agency
Food and Drug Administration
Occupational Safety and Health Administration

9 Research Agencies

Agency for Toxic Substances and Disease Registry
National Institute for Occupational Safety and Health
National Cancer Institute
National Institute of Environmental Health Sciences
National Library of Medicine
National Institutes of Health
Department of Defense
Department of Energy
National Institute of Standards and Technology

• Other participants include: NCATS, Tox21 Representatives
Tox21: From Assays to Pathways

- Identify targets or pathways linked to toxicity/adverse outcomes
- Run corresponding high-throughput screening (HTS) or *in vitro* assays on thousands of chemicals
- Develop predictive systems models: *
  * in silico/in vitro → in vivo
- Use predictive models (qualitative):
  - Prioritize chemicals for targeted testing
  - Suggest / distinguish possible AOPs
- Use predictive models (quantitative):
  - Screen chemicals for hazard
  - Green chemistry design

NICEATM provides **computational toxicology** and **validation** support to Tox21

http://www.ncats.nih.gov/
Endocrine Project Workflow

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
Endocrine Disruptor Screening Program

- Concern over environmental chemical disruption of endocrine hormone signaling (e.g. reproductive and developmental consequences, contribution to chronic disease, metabolic syndrome)

- Congressionally mandated, multiple EDSP testing tiers

- EDSP Tier 1 Testing: for the purposes of prioritization and screening, identify chemicals with the potential to disrupt estrogen, androgen, or thyroid hormone receptor signaling.

- There is a mismatch between resources needed for EDSP Tier 1 testing and the number of chemicals to be tested
  - 10-30,000 chemicals in EDSP Universe
  - ~$1M per chemical for Tier 1
    - 11 low-throughput & animal based tests
  - 50-100 year backlog
Evolution of the EDSP

New Approach: EDSP + Tox21 = EDSP21
- Pathway-based predictive models
- Multiple high-throughput in vitro assays
- Validate to replace selected Tier 1 screening assays
New Approach: EDSP + Tox21 = EDSP21

• Pathway-based predictive models
• Multiple high-throughput *in vitro* assays
• Validate to replace selected Tier 1 screening assays
OECD GD 34, Validation and International Acceptance of New or Updated Test Methods

Validation is a process by which the reliability and relevance of a test method are established for a specific purpose.

EDSP Tier 1

For the purposes of prioritization and screening, identify chemicals with the potential to disrupt estrogen, androgen, or thyroid receptor signaling.
OECD GD 34, Validation and International Acceptance of New or Updated Test Methods

Relevance and reliability should be characterized against data generated with a list of reference chemicals tested in the original method accepted by regulatory agencies.

Reference chemicals: Chemicals selected for use in the validation process, for which responses in the \textit{in vitro} or \textit{in vivo} reference test system or the species of interest are already known.
Performance Based Validation Approach

Estrogen Receptor Pathway Model: Fit for Purpose

Judson et al. 2015, Tox Sci: “Integrated Model of Chemical Perturbations of a Biological Pathway Using 18 In Vitro High Throughput Screening Assays for the Estrogen Receptor"

Kleinstreuer et al. 2015, EHP: “A Curated Database of Rodent Uterotrophic Bioactivity"

Browne et al. 2015, ES&T: “Screening Chemicals for Estrogen Receptor Bioactivity Using a Computational Model"
Estrogen Receptor Signaling Assays

1. ER Binding
2. ER Dimerization
3. DNA Binding
4. RNA Transcription
5. Protein Production
6. Proliferation
Estrogen Receptor Signaling Assays

ER Binding
- hER α
- mER α
- bER α

ER Dimerization
- α/α
- α/β
- β/β
- 8 hr, 24 hr

DNA Binding
- GFP expression
- 2 hr, 8 hr

RNA Transcription
- hER α Trans
- ERE Cis

Protein Production
- hER α/β
- hER α LBD

Proliferation
- hER α Proliferation
Estrogen Receptor Signaling Assays

ER Binding
- hER α
- mER α
- bER α
- Cell Free

ER Dimerization
- α/α
- α/β
- β/β
- HEK293T (Kidney)
- 8 hr, 24 hr

DNA Binding
- GFP expression
- HeLa (Cervix)
- 2 hr, 8 hr

RNA Transcription
- hER α Trans
- ERE Cis
- HepG2 (Liver)

Protein Production
- hER α/β
- vMCF7 (Breast)

Proliferation
- hER α LBD
- HEK293T (Kidney)

- hER α Proliferation
- T47D (Breast)
Estrogen Receptor Signaling Assays

ER Binding
- hER α
- mER α
- bER α
  - Cell Free
  - NovaScreen

ER Dimerization
- α/α
- α/β
- β/β
  - HEK293T (Kidney)
  - Odyssey Thera
  - 8 hr, 24 hr

DNA Binding
- GFP expression
  - HeLa (Cervix)
  - Odyssey Thera
  - 2 hr, 8 hr

RNA Transcription
- hER α Trans
- ERE Cis
  - HepG2 (Liver)
  - Attagene

Protein Production
- hER α/β
- vMCF7 (Breast)
- hER α LBD
  - HEK293T (Kidney)

Proliferation
- hER α Proliferation
- T47D (Breast)
- ACEA
Tox21/ToxCast ER Pathway Model

Combine results from multiple ToxCast in vitro assays

- Orthogonal assays on pathway
  - Different technologies
  - Different points in pathway

- No assay is perfect
  - Assay Interference
  - Noise

- Use mathematical model to integrate assays

For each chemical, the model summarizes results from all assays with a composite dose-response curve, which is used to calculate an AUC relative to 17β-estradiol. 

Judson et al. 2015 Tox Sci
### Mathematical Model

\[ A_i = \sum_j F_{ij} R_j \]

- \( A_i \) is the efficacy of the assay at a given concentration
- \( R_j \) is the “true” efficacy which is unobservable
- \( F \) links receptors to assays

\[ \varepsilon^2 = \sum_i (A_{i\text{pred}} - A_{i\text{meas}})^2 + \text{penalty}(\bar{R}) \]

- Solve a constrained least-squares problem to minimize difference between the measured and predicted assay values

\[ A_{i\text{pred}} \in [1,0] \]

- Penalty enforces physical assumption that chemical will not hit many targets simultaneously

\[ \text{penalty}(\bar{R}) = \alpha \frac{SR^2}{SR^2 + SR_0^2} \]

\[ AUC_j = \frac{1}{N_{\text{conc}}} \sum_{i=1}^{N_{\text{conc}}} \text{sign}(\text{slope}) \times R_j(\text{conc}_i) \]

- AUC Summarizes results normalized to positive control

\[ \sum_{j} \]
Example curves

True Agonist

80-05-7: Bisphenol A
Agonist: 0.65  Antagonist: 0

80-05-7: Bisphenol A
Agonist: 0  Antagonist: 0

True Antagonist

82640-04-8: Raloxifene hydrochloride
Agonist: 0  Antagonist: 0.87

Negative-Narrow Assay Interference

10016-20-3: alpha-Cyclodextrin
Agonist: 0  Antagonist: 0.00022
Performance-based Validation

- **In Vitro Reference Chemicals**
  - Identified by ICCVAM and OECD using multiple validated low throughput *in vitro* ER assays
  - Forty chemicals total (28 agonists and 12 inactive)

- **In Vivo Reference Chemicals**
  - Identified by NICEATM from scientific literature search for rodent uterotrophic data on 1800 ToxCast chemicals
  - Data extracted and data quality reviewed based on minimum guideline-like study criteria
  - Forty-three chemicals total (30 active, 13 inactive)
In Vitro Reference Chemicals

ER Agonist Model Performance

- True Positive: 25
- True Negative: 12
- False Positive: 0
- False Negative: 3

Accuracy: 0.95
Sensitivity: 0.89
Specificity: 1.00

Judson et al. 2015 Tox Sci
Rodent Uterotrophic Bioassay

**Purpose**
- Short term *in vivo* screen to evaluate the ability of a chemical to elicit a biological response similar to that of natural estrogens

**Principle**
- Uterus is under the control of estrogens to stimulate growth
- Production of endogenous estrogens is prevented
  - Ovariectomized (OVX)
  - Immature (Imm)
- Uterus becomes sensitive to external estrogenic substances

Billon-Galés A et al. PNAS 2011
Identifying *In Vivo* Reference Chemicals

**Animal Model**
- OVX Adult Rat: OVX 6-8 weeks, 14 day post-surgery recovery
- OVX Adult Mouse: OVX 6-8 weeks, 7 day post-surgery recovery
- Immature Rat: Begin dosing postnatal day 18-21, complete dosing by postnatal day 25

**Group Size**
- Control groups: minimum three animals
- Treatment groups: minimum five animals

**Route of Administration**
- Oral gavage
- Subcutaneous injection
- Intraperitoneal injection

**Number of Dose Groups**
- Minimum of two dose groups, must have positive and negative control groups

**Dosing Interval**
- Dosing for minimum of three consecutive days; must be completed by PND 25 in immature animals

**Necropsy Timing**
- Between 18-36 hours after last dose

Leverage existing *in vivo* uterotrophic data
- Systematic literature search of publically available data (e.g. PubMed, Scopus)
- Identify chemical activities measured in “guideline-like” uterotrophic studies
- Identify a subset of *in vivo* reference chemicals
  - Active chemicals verified in ≥2 independent studies
  - Inactive chemicals verified in ≥2 independent studies (with no positive results in any study)

*Kleinstreuer et al. 2015 EHP*
Identifying In Vivo Reference Chemicals

- Literature Searches: 1800 Chemicals
- Data Review: 700 Papers, 42 Descriptors, x2
- Uterotrophic Database: 98 Chemicals, 442 GL uterotrophic bioassays
- High-Level Filter
- 6 Minimum Criteria
- "Guideline-Like" (GL)
- Selection Criteria
- In Vivo ER Reference Chemicals: 30 Active, 13 Inactive

Browne et al. “Screening Chemicals for Estrogen Receptor Bioactivity Using a Computational Model” (ES&T 2015)
Kleinstreuer et al: “A Curated Database of Rodent Uterotrophic Bioactivity” (EHP 2016)

M. Gwinn, US EPA
Same Study Design (Immature Rat): BPA

Uterotrophic Reproducibility

LEL or MDT (mg/kg/day)

Injection  Oral

Inactive  Active

Uterotrophic

- Active
- Inactive
ER Agonist Model Performance

In Vivo Reference Chemicals

**ER AUC Rank Order**

- **True Positive**: 29
- **True Negative**: 46
- **False Positive**: 1
- **False Negative**: 1

**Accuracy**: 0.97

**Sensitivity**: 0.97

**Specificity**: 0.97

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Browne et al. 2015 ES&T
ER Agonist Model Performance

In Vivo Reference Chemicals

<p>| | | |</p>
<table>
<thead>
<tr>
<th></th>
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</tr>
</thead>
<tbody>
<tr>
<td>True Positive</td>
<td>29</td>
<td></td>
</tr>
<tr>
<td>True Negative</td>
<td>46</td>
<td></td>
</tr>
<tr>
<td>False Positive</td>
<td></td>
<td>1</td>
</tr>
<tr>
<td>False Negative</td>
<td></td>
<td>1</td>
</tr>
<tr>
<td>Accuracy</td>
<td>0.97</td>
<td></td>
</tr>
<tr>
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<td>0.97</td>
<td></td>
</tr>
<tr>
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<td>0.97</td>
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Browne et al. 2015 ES&T
### Adopting Alternative EDSP Assays

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<tr>
<th>EDSP Tier 1 Battery of Assays</th>
<th>Model Alternative Development</th>
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<tr>
<td>Estrogen Receptor (ER) Binding ★</td>
<td>ER Model FY 2015</td>
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<tr>
<td>Estrogen Receptor Transactivation (ERTA) ★</td>
<td>ER Model FY 2015</td>
</tr>
<tr>
<td>Rodent Uterotrophic ★</td>
<td>ER Model FY 2015</td>
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<tr>
<td>Androgen Receptor (AR) Binding</td>
<td>AR Model FY 2017</td>
</tr>
<tr>
<td>Rodent Hershberger</td>
<td>AR Model FY 2017</td>
</tr>
<tr>
<td>Aromatase</td>
<td>STR Model FY 2017</td>
</tr>
<tr>
<td>Steroidogenesis (STR)</td>
<td>STR Model FY 2017</td>
</tr>
<tr>
<td>Female Rat Pubertal</td>
<td>ER, STR &amp; THY Models FY 2018</td>
</tr>
<tr>
<td>Male Rat Pubertal</td>
<td>AR, STR &amp; THY Models FY 2018</td>
</tr>
<tr>
<td>Fish Short Term Reproduction</td>
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<td>Amphibian Metamorphosis</td>
<td>THY Model FY 2018</td>
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June 19, 2015

FRL-9928-69

"Use of High Throughput Assays and Computational Tools; Endocrine Disruptor Screening Program; Notice of Availability and Opportunity for Comment"
Adopting Alternative EDSP Assays

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July 2018
May 10th - New release (3.0.8) bug fixes

May 10th, 2019 at 5:53:38 AM

An improved version of the CompTox Chemicals Dashboard (version 3.0.8) has been released (May 10th, 2019) to address a number of known bugs, including: (1) the cytotoxicity threshold for the ToxCast summary data tab; and (2) the number of active assay counts between the ToxCast: Summary table and the ToxCast/Tox21 plotting tabs. In the previously released version (March 2019), the Dashboard was showing the median cytotoxicity prediction in the ToxCast: Summary graph, rather than the lower bound on the cytotoxicity prediction, as had been illustrated in previous versions of the Dashboard. The Dashboard should also now accurately reflect the number of active ToxCast hits in the ToxCast/Tox21 plotting tab. Thank you to the many stakeholders who have contacted us to inform us regarding how they use the Dashboard data and of issues they have encountered.
Integrated Chemical Environment: ICE

**Data integrator:**
- Structured format designed for ease of use
- Allows access to data for multiple regulatory endpoints
- Query by CASRN or established reference chemical lists
- Flexible, exportable results

**Workflows:**
- Property predictions, Chemical space characterization, IVIVE, Mechanistic models, AOP mapping

https://ice.ntp.niehs.nih.gov/

Bell et al. 2017 EHP
## Integrated Chemical Environment: ICE

### Endocrine Pathway Models

![Assay Selection Interface]

<table>
<thead>
<tr>
<th>Select Assay Target</th>
<th>Activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acute Oral Toxicity</td>
<td>Any</td>
</tr>
<tr>
<td>Skin Sensitization</td>
<td>Any</td>
</tr>
<tr>
<td>Skin Irritation</td>
<td>Any</td>
</tr>
<tr>
<td>Eye Irritation</td>
<td>Any</td>
</tr>
<tr>
<td><strong>Endocrine</strong></td>
<td>Any</td>
</tr>
<tr>
<td><strong>Androgen</strong></td>
<td>Any</td>
</tr>
<tr>
<td>in vitro</td>
<td>Any</td>
</tr>
<tr>
<td>in silico</td>
<td>Any</td>
</tr>
<tr>
<td><strong>Estrogen</strong></td>
<td>Any</td>
</tr>
<tr>
<td>in vivo</td>
<td>Any</td>
</tr>
<tr>
<td>in vitro</td>
<td>Any</td>
</tr>
<tr>
<td>in silico</td>
<td>Any</td>
</tr>
<tr>
<td>in vitro (all)</td>
<td>Any</td>
</tr>
<tr>
<td><strong>in silico</strong></td>
<td>Any</td>
</tr>
</tbody>
</table>
Integrated Chemical Environment: ICE

Endocrine Pathway Models

Endocrine Call Breakdown

<table>
<thead>
<tr>
<th>Substance Name</th>
<th>CASRN</th>
<th>DSSTOXID</th>
<th>ER Pathway Model, Agonist</th>
<th>ER Pathway Model, Antagonist</th>
</tr>
</thead>
<tbody>
<tr>
<td>Propofol</td>
<td>2078-54-8</td>
<td>DTXSID6023523</td>
<td>1,812 (160)</td>
<td></td>
</tr>
<tr>
<td>Diisopropyl phthalate</td>
<td>605-45-8</td>
<td>DTXSID2040731</td>
<td>1,812 (160)</td>
<td></td>
</tr>
<tr>
<td>Triamcinolone</td>
<td>124-84-7</td>
<td>DTXSID1940742</td>
<td>1,855 (160)</td>
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<tr>
<td>Pyridoxine</td>
<td>65-23-6</td>
<td>DTXSID4023541</td>
<td>1,855 (160)</td>
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<tr>
<td>17-alpha-Hydroxypregesterone</td>
<td>68-96-2</td>
<td>DTXSID6040747</td>
<td>1,855 (160)</td>
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<tr>
<td>Fandosantan potolicoseolate</td>
<td>221246-12-4</td>
<td>DTXSID5047249</td>
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<tr>
<td>Retinol</td>
<td>62-20-8</td>
<td>DTXSID3023332</td>
<td>1,855 (160)</td>
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</tr>
</tbody>
</table>
In Vitro to In Vivo Extrapolation (IVIVE)

Applying physiologically based pharmacokinetic models to use in vitro experimental data to predict biological effects in vivo

Reverse Toxicokinetics: PK/PBPK Models

In Vivo
- Capillary Blood
- Tissue

In Vitro
- Culture Medium
- Cells

Reverse Dosimetry
Bioactivity in Rat / Mouse uterus

In vitro ER activity
Bioactivity in Rat / Mouse uterus

**In vitro ER activity**

For Quantitative Comparison

\[ \text{IVIVE} \]
Use a combination of *in vitro* high-throughput screening assays (as few as 4 assays) and computational model of estrogen receptor (ER) activity to serve as an alternative to low- and medium-throughput *in vitro* and *in vivo* tests.

M. Gwinn, US EPA
CERAPP: QSAR Modeling

Far too many chemicals to test with standard animal-based methods or even *in vitro* HTS
- ~10,000 chemicals to be tested for EDSP, >50,000 for TSCA
- Fill the data gaps and bridge the lack of knowledge
- QSAR models trained on ToxCast ER pathway data

Alternative

Mansouri et al. EHP (2017)

[GitHub Link](https://github.com/NIEHS/OPERA)
Automating Reference Data Identification

- Project with Oak Ridge National Labs (ORNL) and FDA CFSAN to apply text-mining (NLP) approaches & ML to identify high-quality data

- Semi-automated retrieval and evaluation of published literature (trained on uterotrophic database)

- Apply to developmental toxicity studies (with ICCVAM DARTWG)
  - Define literature search keywords, identify corpus
  - Extract/characterize study protocol details from regulatory guidelines: minimum criteria
  - Apply ML algorithms to identify high-quality studies, expert check
Acknowledgments

- ILS/NICEATM
- EPA/OCSSPP
- EPA/ORD
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- Kamel Mansouri
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- Richard Judson
- Patience Browne