SETAC Europe Continuing Education Course: TC07-The Endocrine System: Global Perspectives on Testing Methods and Evaluation of Endocrine Activity (https://dublin.setac.org/programme/scientific-programme/training-courses/)

Slides given as part of this training course in a virtual training recorded on May 8, 2020

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The views expressed in this presentation are those of the authors and do not necessarily reflect the views or policies of the U.S. EPA
What does the future hold?
Goals

1. To provide broad coverage of chemicals, chemical mixtures, outcomes, and life stages

2. To reduce the cost and time of testing

3. To use fewer animals and cause minimal suffering in the animals used

4. To develop a more robust scientific basis for assessing health effects of environmental agents
Why can’t we just use traditional approaches?

**Challenge**
- Too many chemicals
- Traditional methods are slow, costly
- Not enough data

**Solution**
- New Approach Methods (NAMs)
  - Interagency Collaboration: Toxicity Testing in the 21st century (Tox21)
    - > 10K chemicals
    - > 100 biological targets
    - Cell painting, transcriptomics (HTTr, S1500+)
- USEPA’s Toxicity Forecaster (ToxCast) program
  - > 3K chemicals screened in
  - > 1K assays
  - ~400 biological targets
New Assessment Methodologies

• Animal testing isn’t always the answer
  • Time consuming, expensive, ethically challenging

• New Assessment Methodologies (NAMs)
  • Categories, read across, (Q)SARs, and other model predictions
    • Need for regulatory acceptance (e.g., confidence in applying data to decisions)
    • Challenges in interpretation (e.g., linking molecular/cellular changes to adversity)
    • Need for flexibility (e.g., NAMs may be context/pathway specific)
ToxCast and Tox21 have generated a lot of publicly available bioactivity data for hazard screening and prediction.

- ToxCast: more assays, fewer chemicals, EPA-driven
- Tox21: fewer assays, all 1536 well plate, driven by consortium
- All Tox21 data are analyzed by multiple partners
- Tox21 data is analyzed in the ToxCast Data Pipeline
Exposure provides context for high-throughput science

Bioactivity

Exposure

mg/kg BW/day

No Priority (Hold)  No Priority (Hold)  Low Priority  Medium Priority  Higher Priority
Endocrine hazard and risk evaluation using public tools: approach outline

- Publicly available data from ToxCast is actively being applied to endocrine hazard labeling in the EU.

- Risk-based approaches that incorporate bioactivity and exposure make the best use of new approach methodologies.

This presentation will demonstrate where to find these information and suggest an approach for utilizing them in endocrine hazard and risk evaluation.
CompTox Chemicals Dashboard

875 Thousand Chemicals

Latest News

August 9th 2019 - New release (3.0.9) in time for ACS Fall Meeting

August 14th, 2019 at 4:39:37 PM

A new version of the Dashboard has been released in time for the ACS Fall meeting. Included in this release are updates to data in the ToxVal database, an update to the in vitro database (version 3.2), and the release also addresses a number of minor bugs and includes a short list of additional functionality as described in the Release Notes here.
The CompTox Chemical Dashboard

- Freely accessible website and integration hub:
  - Chemical substances – the majority with structures
  - Searchable by chemical, product use, and gene
  - Experimental and predicted physicochemical property data
  - Experimental and predicted fate and transport data
  - Information regarding consumer products containing chemicals
  - Bioactivity data for the ToxCast/Tox21 project
  - “Literature” searches for chemicals using public resources
  - Links to other agency websites and public data resources
  - “Batch searching” for thousands of chemicals
  - Chemical lists of interest – pesticides, leachables, PFAS, (but not a list of endocrine disruptors)
  - Downloadable Open Data for reuse and repurposing
A single application integrating...
A data integration hub
LOTS of data!

• >875,000 chemicals curated over 20 years
• >700,000 toxicity data points from >30 sources
• Millions of synonyms and identifiers
• Tens of thousands of experimental data points
• Millions of QSAR prediction reports
• Millions of bioactivity data points for >4000 chemicals and hundreds of assay end points
• Searching of Pubmed’s 30 million abstracts
# Review of Bioassay Data

![Image of bioassay data](image-url)

## TOXCAST DATA

<table>
<thead>
<tr>
<th>Name</th>
<th>Description</th>
<th>Gene Symbol</th>
<th>AOP</th>
<th>Hit Call</th>
<th>Scaled Top</th>
<th>AC50</th>
<th>logAC50</th>
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Endocrine-related subset of assays
Examine physicochemical properties such as logP, vapor pressure, and MW to get a better sense of whether the chemical was suitable for the current *in vitro* assay suite.

**Analytical chemistry:** was the chemical present and in the DOA for current ToxCast?

**ToxCast negatives:** what does a negative mean? Outside of domain of applicability?

Consider some aspects of the Lipinski’s rules: logP -0.4 to 5.6 range; MW 180-480; Vapor Pressure < 1.
Examine QC data (if available) to see if we expect that the chemical was present for screening.

Analytical chemistry: was the chemical present and in the DOA for current ToxCast?
A note on ToxCast versioning

• Data change: curve-fitting, addition of new data
• Models change: improvements, more data, etc.
• The CompTox Chemicals Dashboard release from August 9, 2019 is now using ToxCast invitrodb version 3.2: https://doi.org/10.23645/epacomptox.6062623.v4
• All ToxCast data and endocrine models (CERAPP, COMPARA, ER, AR, steroidogenesis) can currently be accessed from within invitrodb.
• Data downloads for CCTE: https://www.epa.gov/chemical-research/exploring-toxcast-data-downloadable-data
Models >>> single assays. And equivocals happen.

CERAPP = consensus ER QSAR (from 17 groups)
COMPARA = consensus AR QSAR
ToxCast Pathway Model AUC ER = full ER model (18 assays)
ToxCast Pathway Model AUC AR = full AR model (11 assays)

>0.1 = positive; 0.001-0.1 = equivocal
HT-H295R model for steroidogenesis

Endocrine models available?

• Supplemental File 4 has fold-change by hormone
• Supplemental File 9 has mM (model values)
• Invitrodb v3.2 has a hth295r model table with both of these included in it.
• Hope to include this in future release of the Dashboard.
This is the cytotoxicity threshold or “burst” based on the method described in Judson et al. 2016. It is the lower bound on the estimate of a cytotoxicity threshold. (see tcplCytoPt() function in the tcpl R package).
Summary of the assay data is in a table.
“Burst:” thinking and updates

- The latest Comptox Chemicals Dashboard release (version 3.0.9, August 9, 2019) demonstrates a cytotoxicity threshold based on the latest ToxCast database (invitrodb version 3.2, released August 2019). This value can change as more cytotoxicity data become available, curve-fitting approaches for existing data change, or the “burst” calculation approach is updated.

- In invitrodb version 3.2, 88 assays are considered for the cytotoxicity threshold. A positive hit must be observed in 5% of these assays (noting that not all chemicals are screened in all 88 assays) in order to assign a cytotoxicity threshold. The cytotoxicity threshold is a median of AC50 potency values from the N assays with a hit. The cytotoxicity threshold visualized in the Dashboard is a lower bound on this estimate, calculated as the median cytotoxicity potency minus 3 times the global median absolute deviation.

- This is discussed further in a publication (10.1093/toxsci/kfw148) and the ToxCast Pipeline R package (tcpl) function, tcplCytoPt() (available on CRAN: https://cran.r-project.org/web/packages/tcpl/index.html).

- If fewer than 5 cytotoxicity assays demonstrate a positive hit, a default of 1000 micromolar is assigned for the chemical.

- The lower bound estimate of the cytotoxicity threshold or “burst” is useful context for ToxCast results. Bioactivity observed below the cytotoxicity threshold may represent more specific activity that is less likely to be confounded by cytotoxicity.

- It is possible that AC50 values above the cytotoxicity threshold are informative. If an assay has a parallel cytotoxicity assay in the same cell type, that may be more informative for interpreting that assay. Or, if a result is consistent with an AOP relevant to the chemical with assay AC50 values above and below the cytotoxicity threshold, those data may be meaningful.
User application dictates “selectivity”

• AC50 < burst?
• AC50 \(0.5\log_{10}\) distance from burst?
• AC50 < parallel viability assays?
• How else to filter ToxCast data: 3+ caution flags & hit-percent?
• Other related ideas:
  • What other assays appear active in a similar concentration range?
  • Is there consistent support for MOA(s), or is it nonspecific activity?
Steady state in vitro-in vivo extrapolation (IVIVE) assumption: blood::tissue partitioning ≈ cells::medium partitioning

\[ C_{ss} = \frac{\text{oral dose rate}}{(GFR \times F_d) + \left( Q_i \times F_{ib} \times \frac{Cl_{int}}{Q_i + F_{ib} \times Cl_{int}} \right)} \]

Identification of a potency value to use for IVIVE of a threshold dose

Wetmore et al. (2012)

- Swap the axes (this is the “reverse” part of reverse dosimetry)
- Can divide bioactive concentration by \( C_{ss} \) for a 1 mg/kg/day dose to get oral equivalent dose
IVIVE via high-throughput toxicokinetic data and models

- Operationally, the httk R package (v 1.10.0) can be downloaded from CRAN or GitHub for reproducible generation of administered equivalent doses (AEDs)
- For some substances, there is a beta tab in the Dashboard with Css and other values needed (no models). More chemicals have information in the httk package.
- AC50 or LEC (micromolar) * (1 mg/kg/day/Css (micromolar)) = AED prediction
- Httk package optionally implements multiple models that can have increasing complexity based on data available
Bioactivity:exposure ratio requires exposure

- Currently the Dashboard shows SEEM2 (2014) values
Consensus modeling of chemical exposure based on pathways: ExpoCast SEEM3

• “ExpoCast SEEM3” model:
  • uses twelve different exposure predictors including both near- and far-field models;
  • covers four distinct exposure pathways: non-pesticidal dietary, consumer products, far-field pesticide, and far-field industrial.
  • In SEEM3 each exposure predictor is scaled and centered such that chemicals without a value for a predictor relevant to its exposure pathways are assigned the average value.

Comparison to exposure predictions for a bioactivity:exposure ratio
Including risk-based approaches like BER in chemical safety decisions

- Specific vs. nonspecific modes-of-action and the challenge of hazard labeling

Thomas et al. 2013 suggested a framework for hazard assessment that would be largely customized based on MOE (or now, BER).
Including risk-based approaches like BER in chemical safety decisions

- Now, ~6 years later, Thomas et al. (2019) suggest a computational toxicology blueprint that represents evolution of the same concept.
Screening level assessment example: combine NAMs for exposure, *in vitro* bioactivity, and toxicokinetics

- Conducted by Accelerating the Pace of Chemical Risk Assessment (APCRA)
  - “international cooperative collaboration of government agencies convened to address barriers and opportunities for the use of new approach methodologies (NAMs) in chemical risk assessment” (Paul Friedman et al., accepted)
Case study workflow

Apply high-throughput toxicokinetics (httk) to get mg/kg/day

Is BER useful for prioritization?
Are there addressable weaknesses?

Is log10-POD ratio > 0 for most chemicals?
Can we learn from log10-POD ratio < 0?

POD

NAM

POD

trad

ToxCast AC50s (µM)
ASTAR HIPPTox EC10s (µM)

Bioactivity-exposure ratio

POD\textsubscript{trad} : POD\textsubscript{NAM} ratio

95\textsuperscript{th} %

5\textsuperscript{th} %

95\textsuperscript{th} %

5\textsuperscript{th} %

EPA - ExpoCast
Health Canada

EPA - ToxValDB
Health Canada
EFSA
ECHA

• NOEL, LOEL, NOAEL, or LOAEL
• Oral exposures
• Mg/kg/day
Prioritizing chemicals based on BER for all bioactivity or for some target bioactivity

For 448 substances, ~89% of the time, the point-of-departure based on ToxCast (POD-NAM) was less than the NOAEL/LOAEL values available from animals.

Figure 3 from Paul Friedman et al.  
https://doi.org/10.1093/toxsci/kfz201