EDSP21: High-Throughput Screening (HTS) and Prioritization Supporting the EPA Endocrine Disruptor Screening Program

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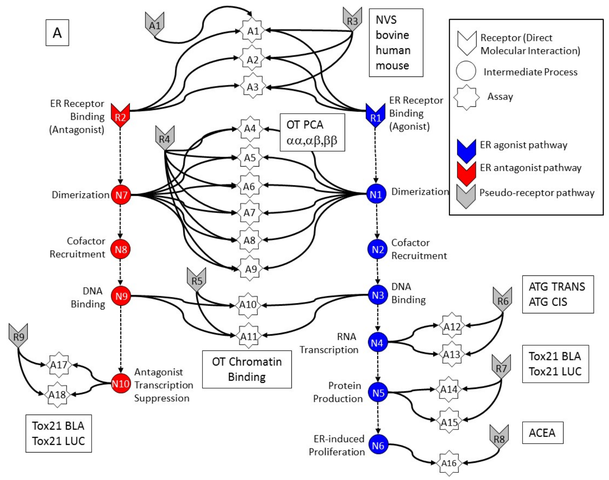
Science Challenge

(1) **EDSP program needs to evaluate many chemicals:** The EPA EDSP program is required to evaluate ~10,000 chemicals for their potential of to be endocrine disruptors.

(2) **EDSP Tier 1 assays are not suited to such large-scale testing:** The current Tier 1 battery of 11 *in vitro* and *in vivo* assays would take many decades to assess these chemicals, driving the need for a new approach

(3) **Assess a new approach:** We have assessed the use of HTS *in vitro* assays and a combining model to prioritize EDSP universe chemicals and to replace certain of the low-throughput Tier 1 assays

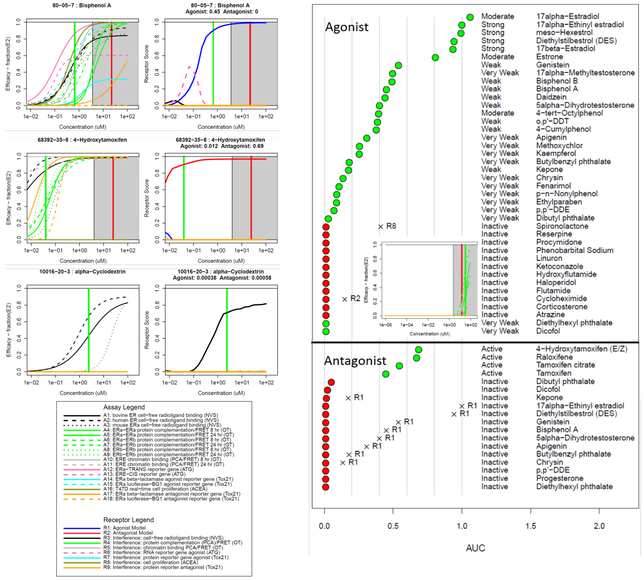
Approach



1) Test 1800 chemicals in 18 Estrogen Receptor (ER) assays
2) Develop model to summarize the results and account for false positive activity
3) Evaluate against known list of in *vitro* reference chemicals
4) Compile database of guideline-like uterotrophic studies
5) Compare uterotrophic data with ER *in vitro* model results to evaluate predictivity
6) Make recommendations for prioritizing chemicals for Tier 1 screening
7) Make recommendations for replacing Tier 1 ER binding and TA assays, and the uterotrophic assay

Case Study Description

Step 1: Assessment of performance of ER Model vs. Reference Chemicals: 45 Positive and negative reference chemicals were evaluated, including agonists and antagonists over a range of potencies. With the exception of 2 very weak agonists, all reference chemicals were correctly classified and largely placed in the correct potency order.



Step 2: A database of guideline uterotrophic assay data: Study protocol descriptors (species, system, dosing, etc.) were extracted from 670 articles testing 235 unique chemicals in 2615 uterotrophic bioassays.

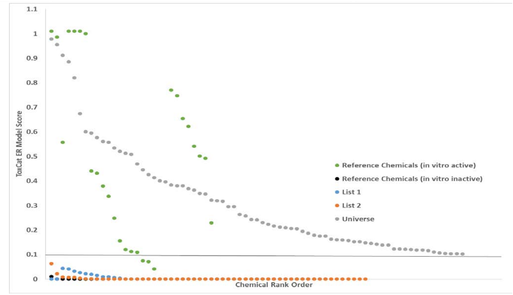
All studies were assessed for adherence to six criteria based on OECD/EPA regulatory test guidelines, and those meeting all criteria (~450 bioassays on 102 ToxCast chemicals) were considered guideline-like (GL) and subsequently analyzed.

Results

Step 3: Performance-based validation of ToxCast ER Model agonist bioactivity versus reference chemicals and methods currently in practice.

Performance	In vitro reference chemicals	In vivo reference chemicals	GL uterotrophic studies	Tier 1 studies
# True Pos	25	28	37	0
# True Neg	11	8	33	41
# False Pos	0	1	4	0
# False Neg	2	1	5	41
Accuracy	0.95	0.95	0.89	1.0
Sensitivity	0.93	0.97	0.88	0
Specificity	1.0	0.89	0.89	1.0

Step 4: Use ToxCast ER model results to determine agonist bioactivity and identify needs for further screening and testing.



ToxCast ER model agonist bioactivity scores for EDSP List 1, List 2, Universe, and reference chemicals. Scores ≥0.1 (indicated by the horizontal line) were considered positive. All List 1 or List 2 chemicals are negative for ER agonist bioactivity, as are about 78% of the remaining chemicals tested. However, about 5% (92) have ER ToxCast model scores that indicate potential agonist bioactivity (scores ≥ 0.1) and would be priority candidates for further screening and testing. Based on the demonstrated performance, EPA would accept ToxCast ER model data *in lieu* of Tier 1 ER binding, ERTA, and Uterotrophic assays.

Conclusions

- These results are being used to develop a new prioritization scheme for chemicals going into EDSP Tier 1.
- EPA will use of the ER model results in lieu of the Tier 1 ER binding, ERTA and uterotrophic assay data (https://federalregister.gov/a/2015-15182)
- A similar approach is being developed for androgen receptor testing and prioritization
- Thyroid-pathway signaling tests and models are currently being developed

Browne et al. "Screening Chemicals for Estrogen Receptor Bioactivity Using a Computational Model", ES&T (2015)

Judson et al. "Integrated Model of Chemical Perturbations of a Biological Pathway Using 18 In Vitro High-Throughput Screening Assays for the Estrogen Receptor" ToxSci (2015)

Klenistreuer et al. "A Curated Database of Rodent Uterotrophic Bioactivity" Environ. Health Persp. (2015)