

Developments in the EPA Computational Toxicology Program to Identify Environmental Endocrine Disruptors



Environmental Endocrine Disruptors Gordon Conference June 22, 2016

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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



First, Let's Celebrate...



Today, President Obama signed a bill to reform the Toxic Substances Control Act (TSCA) that requires EPA to review existing chemicals on a specific, enforceable schedule, as well as to review new chemicals for health risks before they're allowed on the market.



Legislative Mandate

1996 Federal Food, Drug and Cosmetic Act, section 408(p)

Requires the U. S. EPA to develop a screening program using appropriate validated test systems and other scientifically relevant methods to determine whether certain substances may have an effect in humans that is similar to an effect produced by a naturally occurring estrogen, or other such endocrine effect as the Administrator may designate.

1996 Safe Drinking Water Act Amendments, section 1457

Testing of chemical substances that may be found in sources of drinking water, if substantial human populations may be exposed.



Formation of the Initial Screening and Testing Strategy

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Endocrine Disruptor Screening and Testing
 Advisory Committee (EDSTAC) Key
 Recommendations (1998):

- Expand protection to include human health and wildlife
- Include estrogen, androgen and thyroid pathways
- Develop a two-tiered screening and testing program

• EDSTAC Conceptual Framework:

- Tier 1 screening for *potential* to interact with estrogen, androgen or thyroid hormone systems
- Tier 2 testing to verify interaction with the endocrine system and quantify the dose-response relationship



EDSP Screening Batteries

>				Tie	r 1 Sc	reenin	g Batt	ery				Tier	2 Test	ing As	says
Endocrine Pathway	ER Binding	ERα Transcriptional Activation*	AR Binding	Aromatase Inhibition	Steroidogenesis*	Uterotrophic*	Hershberger*	Pubertal Male	Pubertal Female	Amphibian Metamorphosis*	Fish Short Term Reproduction*	Rat 2-gen/ Extended One-Gen*	Medaka Extended One- Gen Repro Test*	Amphibian Growth and Dev Assay*	Japanese Quail Two Gen Toxicity Test
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*OECD harmonized guidelines; In vitro assays indicated by italic font



The Challenge

- 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
- In 2015, EPA issued EDSP ordered additional testing on positive List 1
- The cost of running the Tier 1 battery is ~\$750,000 - \$1 million
- 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



EDSP Chemical Universe List	Number
Conventional Active Ingredients	838
Antimicrobial Active Ingredients	324
Biological Pesticide Active Ingredients	287
Non Food Use Inert Ingredients	2,211
Food Use Inert Ingredients	1,536
Fragrances used as Inert Ingredients	1,529
Safe Drinking Water Act Chemicals	3,616
TOTAL	10,341



High-Throughput Assays Used to Screen Chemicals for Bioactivity





- Understanding of what cellular processes/pathways may be perturbed by a chemical
- Understanding of what amount of a chemical causes these perturbations



Current Application to Estrogen Signaling in EDSP





Validation With *In Vitro* Reference Chemicals





In Vitro Reference Chemicals*

Accuracy	0.93 (0.95)
Sensitivity	0.93 (0.93)
Specificity	0.92 (1.0)



Validation With *In Vivo* Reference Chemicals



Predictive Performance for In Vivo Uterotrophic Studies

Accuracy	0.86 (0.95)
Sensitivity	0.97 (0.97)
Specificity	0.67 (0.89)

*Values in parentheses exclude inconclusive chemicals

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Incorporating a High-Throughput Toxicokinetic Approach





Adding on High-Throughput Exposure





Integrating All Three for Risk-Based Prioritization and Decision Making





Peer Review of New Approaches and Adoption by Regulatory Partners

Prioritization of the Endocrine Disruptor Scr Program Universe of Chemicals for an Estro Receptor Adverse Outcome Pathway Using Computational Toxicology Tools U.S. Environmental Protection Agency Endocine Disruptor Screening Program		Integrated Bioactivity and Exposure Ranking: A Computational Approach for the Prioritization and Screening of Chemicals in the Endocrine Disruptor Screening Program	Table 1 State	Her/Vol. 40, No. 110/Friday, Jane 10 and approach presents to 1/07 status (PA) 400 immediate particular transferred to the present of 1/07. USAND 100 immediate particular of the opportunity to status and additional distance of the present of the pre- operation by the status and additional distance of the pre- sent of the present of the pre- sent of the pre- text of the pre- sent of the pre- sent of the pre- text of the pre- sent of the pre- sent of the pre- sent of the pre- sent of the pre- text of the pre- text of the pre- text of the pre- text of the pre-	 2015-Vitatione relinear exemuting using adversarias net methods is some analysis for Partie I barrary to partie barrare barrari barrary to partie barrare barrari barrary
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FLAGS: Only one conc above baseline, active Borderline active

Efforts to Ensure Data Quality and Increase Transparency

- Public release of Tox21 and ToxCast data on PubChem and EPA web site (raw and processed data)
- Transparent ToxCast data analysis pipeline
 - Data quality flags to indicate concerns with chemical purity and identity, noisy data, and systematic assay errors
 - Publicly available as an R package
- Tox21 and ToxCast chemical libraries have undergone analytical QC and results publicly available
- Public posting of ToxCast procedures
 - Chemical Procurement and QC
 - Data Analysis
 - Assay Characteristics and Performance
- External audit on ToxCast data and data analysis pipeline
- Multiple webinars and workshops to educate stakeholders on high-throughput screening data analysis and interpretation



Enabling Stakeholders to Use ToxCast/Tox21 Data

iCSS Dashboard

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EDSP21 Dashboard



http://actor.epa.gov/edsp21/



But, Version 1.0 is Never Perfect...







Microsoft Windows Version 1.01

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What is Version 2.0 Looking Like?





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Expanding Screening and Modeling to the Androgen Pathway



Kleinstreuer et al. Unpublished

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Agonism

True Positives	8
True Negatives	20
False Positives	1
False Negatives	0
Accuracy	0.97
Sensitivity	1.00
Specificity	0.95

Antagonism

True Positives	18
True Negatives	8
False Positives	0
False Negatives	2*
Accuracy	0.93*
Sensitivity	0.90*
Specificity	1.00

*Two false negatives were correctly identified when Tox21 AR antagonist screen was evaluated at both agonist concentrations



Adding Screening for Disruption of **Steroidogenesis**



- 2060 chemicals screened at single concentration
- 403 chemicals selected for concentration response (altered > 4 hormones)
 - 120 additional chemicals selected for concentration response based • on other needs



Thyroid Toxicity AOP Network



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THR Assays





TSHR Assay

Completed Agonist (Nov '15) Completed Antagonist (April 16)

TRHR Assay

Undergoing Online Validation (Tox21)



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- Gene expression measured
- Cytotoxicity measured in parallel



Bayesian model for NR regulation

SLCO1B1: thyroid hormone transporter in the liver **SULT2A1:** not main SULT that metabolizes TH, but demonstrated to have some activity **UGT1A1/1A6:** mediate T(4) glucuronidation THRSP: thyroid hormone-inducible hepatic protein HIF1a: downstream to TRB1 activation via T3 or T4 signaling



Single-concentration Screen

Thyroid Toxicity AOP Network Molecular-Initiating Adverse Events **Key Events** Outcomes Hypothalamic-pituitary Feedback receptor (hypothalamus) hyrotropin-releasi hormone Recepto Thyroi Thyroid follicular 1 TSH roid hyperplasia tumors (rat) Thyroperoxidase (TPO) inhibition Altered Decreased fitness etamorphosis (amphibian) (amphibian) 4 serum 4 TH synthesis T4/T3 Na*I' symporter (NIS) inhibition 4 tissue [TH] Altered Neurological concentrations dysfunction urodevelop ↓ Transport rotein binding Phase II catabo Xepphintic nuclear 1 receptor activation Cochlean Hearing loss Free Bound-TH Hepatic transpo damage (mammals) Serum binding protein interference **TPO Assay Details** Delodinase L Tissue T4-9 T3 Inhibition conversion • **Tissue Specific** Fluorescent peroxidase old receptor (T) . Red) TH Transporters inhibition •

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- Rat thyroid microsomes
- substrate (Amplex Ultra
- Validated against existing kinetic guaiacol assay
- Luciferase, cytotoxicity ٠ counterscreens



Concentration Response Screen



Stratify by Selectivity



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NIS Assay (300 ToxCast cmpds)



100 uM Chemical

T. Stoker (NHEERL)

Deiodinase (DIO-1) Assay (300 ToxCast cmpds)

NIS and DIO-1 Assay Details

- HEK293T cells expressing NIS
- HEK293 cells expressing DIO-1
- 96-well radioactive iodide uptake
- Screen at single high concentration screen
- Hit follow-up at >20% inhibition for ٠ NIS and >50% inhibition for DIO-1



M. Hornung (NHEERL)



Adding Metabolic Competence





Assessing Cross-Species Conservation of Response

Multispecies Attagene Trans Reporter Assay



Receptor Family	Receptor Name	Species
Estrogen Receptor	ERa	Human
Estrogen Receptor	ERb	Human
Estrogen Receptor	ER1	Zebrafish
Estrogen Receptor	ER2a	Zebrafish
Estrogen Receptor	ER2b	Zebrafish
Estrogen Receptor	ERa	Chicken
Estrogen Receptor	ER1	Frog
Estrogen Receptor	ER2	Frog
Estrogen Receptor	ERa	Turtle
Estrogen Receptor	AR	Human
Estrogen Receptor	AR	Chicken
Estrogen Receptor	AR	Turtle
Estrogen Receptor	AR	Frog
Estrogen Receptor	AR	Zebrafish
Peroxisome Proliferator Activated Receptor γ	PPARg	Mouse
Peroxisome Proliferator Activated Receptor	PPARg	Zebrafish
Peroxisome Proliferator Activated Receptor γ	PPARg	Human
Pregnane X Receptor	PXR	Mouse
Thyroid Receptor	TRa	Turtle
Thyroid Receptor	TRb	Zebrafish
Thyroid Receptor	TRb	Zebrafish
Thyroid Receptor	TRa	Frog
Thyroid Receptor	TRa	Human
Thyroid Receptor	TRb	Human
Controls	M-06	NA
Controls	GAL4	NA
Controls	M-19	NA
Controls	m-32	NA
Controls	m-61	NA .

- Host cell: human HepG2
- Stimulation with EC20 of 6afluorotestosterone for detection of androgen receptor antagonists
- 100 chemicals with ER, AR, PPAR activity tested in concentration-response
- Data calculated as fold-change over control (6a-fluorotestosterone/DMSO)



Cross-Species Conservation of Efficacy



Human/turtle/frog similar; chicken more diverse; zebrafish very different

zfER2a_Emax	zfER1_Emax	frER1_Emax	hERb_Emax	trERa_Emax	chERa_Emax	hERa_Emax	frER2_Emax	zfER2b_Emax	trAR_Emax	hAR_Emax	frAR_Emax	chAR_Emax	zfAR_Emax	zTRb_Emax	hTRb_Emax	hTRa_Emax	trTRa_Emax	frTRa_Emax	zTRa_Emax	chAR_EMax_Inhibition	frAR_EMax_Inhibition	trAR_EMax_Inhibition	hAR_EMax_Inhibition	mPXR_Emax	ZPPAR9_Emax	zfAR_EMax_Inhibitior	hPPARg_Emax	mPPARg_Emax
-4.84					-									0.00	-	-		100										4.8



Improving Exposure Estimates and Characterization





What Does Version 2.0 Look Like?









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Acknowledgements and Questions

Tox21 Colleagues: NTP Crew FDA Collaborators NCATS Collaborators

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NICEATM Colleagues

OSCP Colleagues



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