Identifying endocrine disrupting chemicals using *in vitro* and computational approaches

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State of the Science on Development and Use of New Approach Methods (NAMs) for Chemical Safety Testing

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

EPA-Specific Drivers: EDSP

- **The US Environmental Protection Agency's (EPA) Endocrine Disruptor Screening Program (EDSP)**
  - established in response to Congressional mandates in the Federal Food Quality Protection and Safe Drinking Water Acts
  - evaluating 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 relationship
  - 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

https://www.federalregister.gov/articles/2015/06/19/2015-15182/use-of-high-throughput-assays-and-computational-tools-endocrine-disruptor-screening-program-notice

FEDERAL REGISTER

The Daily Journal of the United States Government

the procedures in TSCA section 14 and 40 CFR part 2.

Burden statement: The annual public reporting and recordkeeping burden for this collection of information is estimated to average 31.5 hours per response. Burden is defined in 5 CFR 1320.3(b).

The ICR, which is available in the docket along with other related materials, provides a detailed explanation of the collection activities and the burden estimate that is only briefly summarized here:

Respondents/Affected Entities: Entities potentially affected by this ICR are companies that manufacture, process or import chemical substances, mixtures or categories.

Estimated total number of potential respondents: 1.

Frequency of response: On occasion.

Estimated total average number of responses for each respondent: 1.

Estimated total annual burden hours: 31.5 hours.

Estimated total annual costs: $2,388. This includes an estimated burden cost of $2,388 and an estimated cost of $0 for capital investment or maintenance and operational costs.

III. Are There Changes in the Estimates from the Last Approval?

There is a decrease of 916 hours in the total estimated respondent burden compared with that identified in the ICR currently approved by OMB. This decrease reflects additional both adjustment changes from a reduction in the assumed number of PAIR reports filed annually, and program changes resulting from mandatory electronic submissions of PAIR reports. In recent years (FY 2011–FY 2014), EPA has received no PAIR submissions and, for the purposes of this analysis, EPA assumes an annual rate of one submission per year. At the time OMB last renewed this ICR, EPA estimated an average of 33 reports from 14.8 submitters based on fiscal year 2006–2010 data. The ICR supporting statement provides a detailed analysis of the change in burden estimate. This change is both an adjustment and a program change.

IV. What is the Next Step in the Process for this ICR?

EPA will consider the comments received and amend the ICR as appropriate. The final ICR package will then be submitted to OMB for review

opportunity to submit additional comments to OMB. If you have any questions about this ICR or the approval process, please contact the technical person listed under **FOR FURTHER INFORMATION CONTACT.**

Authority: 44 U.S.C. 3501 *et seq.*

Dated: June 10, 2015.

James Jones,

Assistant Administrator, Office of Chemical Safety and Pollution Prevention.

[FR Doc. 2015-14946 Filed 6-18-15; 8:45 am]

BILLING CODE 6560-50-P

ENVIRONMENTAL PROTECTION AGENCY

[EPA–HQ–OPPT–2015–0305; FRL–9928–69]

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

AGENCY: Environmental Protection Agency (EPA).

ACTION: Notice.

SUMMARY: This document describes how EPA is planning to incorporate an alternative scientific approach to screen chemicals for their ability to interact with the endocrine system. This will improve the Agency's ability to fulfill its statutory mandate to screen pesticide chemicals and other substances for their ability to cause adverse effects by their interaction with the endocrine system. The approach incorporates validated high throughput assays and a computational model and, based on current research, can serve as an alternative for some of the current assays in the Endocrine Disruptor Screening Program (EDSP) Tier 1 battery. EPA has partial screening results for over 1800 chemicals that have been evaluated using high throughput assays and a computational model for the estrogen receptor pathway. In the future, EPA anticipates that additional alternative methods will be available for EDSP chemical screening based on further advancements of high throughput assays and computational models for other endocrine pathways. Use of these alternative methods will accelerate the pace of screening, decrease costs, and reduce animal testing. In addition, this approach advances the goal of providing sensitive, specific, quantitative, and

DATES: Comments must be received on or before August 18, 2015.

ADDRESSES: Submit your comments, identified by docket identification (ID) number EPA–HQ–OPPT–2015–0305, by one of the following methods:

- *Federal eRulemaking Portal: http://www.regulations.gov.* Follow the online instructions for submitting comments. Do not submit electronically any information you consider to be Confidential Business Information (CBI) or other information whose disclosure is restricted by statute.
- *Mail:* Document Control Office (7407M), Office of Pollution Prevention and Toxics (OPPT), Environmental Protection Agency, 1200 Pennsylvania Ave. NW., Washington, DC 20460–0001.
- *Hand Delivery:* To make special arrangements for hand delivery or delivery of boxed information, please follow the instructions at *http://www.epa.gov/dockets/contacts.html.*

Additional instructions on commenting or visiting the docket, along with more information about dockets generally, is available at *http://www.epa.gov/dockets.*

FOR FURTHER INFORMATION CONTACT: *For technical information contact:* Jane Robbins, Office of Science Coordination and Policy (OSCP), Office of Chemical Safety and Pollution Prevention, Environmental Protection Agency, 1200 Pennsylvania Ave. NW., Washington, DC 20460–0001; telephone number: (202) 564–6625; email address: robbins.jane@epa.gov.

For general information contact: The TSCA-Hotline, ABVI-Goodwill, 422 South Clinton Ave., Rochester, NY 14620; telephone number: (202) 554–1404; email address: *TSCA-Hotline@epa.gov.*

SUPPLEMENTARY INFORMATION:

I. General Information

A. Does this action apply to me?

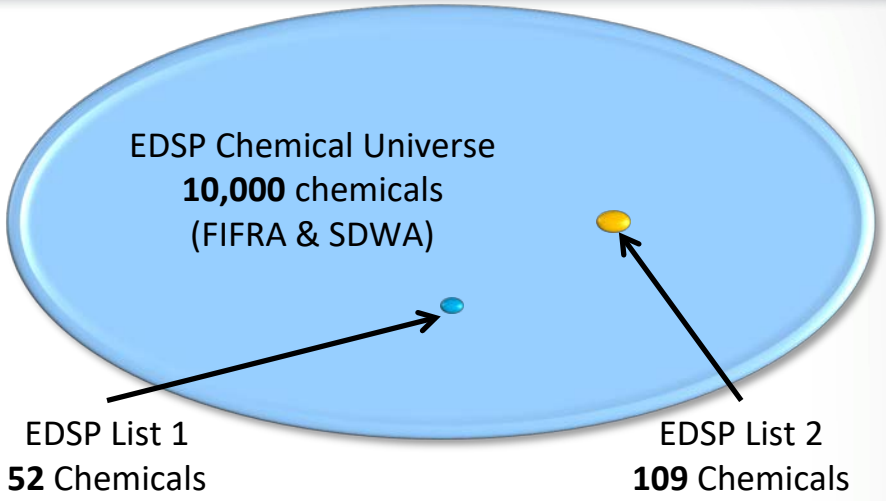
This action is directed to the public in general, and may be of interest to a wide range of stakeholders including those interested in endocrine testing of chemicals (including pesticides), and the EDSP in general. Since others also may be interested, the Agency has not attempted to describe all the specific entities that may be affected by this action.

B. What is the agency authority for taking this action?

The EDSP is established under section 408(p) of the Federal Food, Drug and

EDSP Pivot

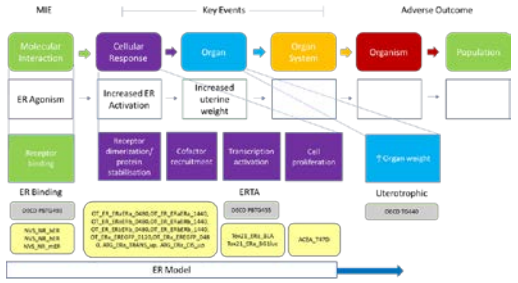
- In 2009, EPA published list of 67 pesticide chemicals (List 1) for Tier 1 screening (15 subsequently withdrawn).
- In 2013, EPA published a revised second list (List 2) of 109 chemicals for proposed Tier 1 screening.
- The cost of running the Tier 1 battery is ~$1 million per chemical.
- The number 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.



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**

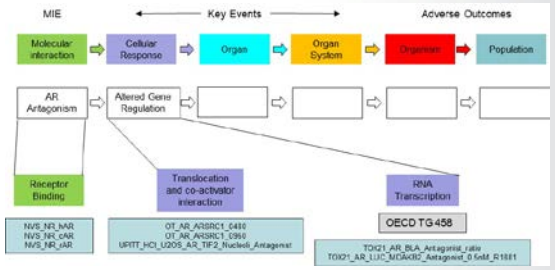
The Approach

- 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



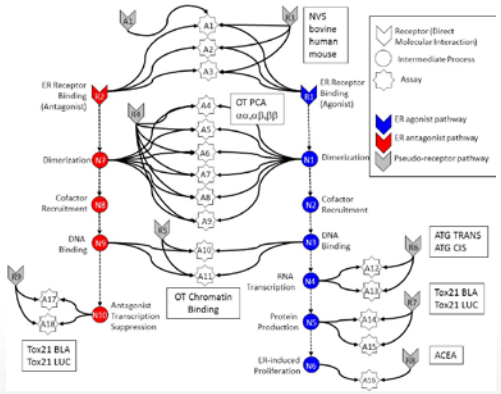
Estrogen Receptor Computational Model

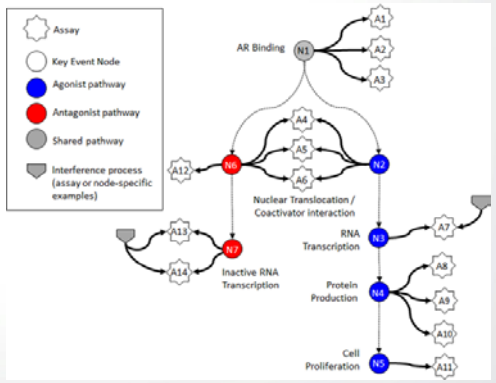
Judson et al., Envi Health Pers (2015)



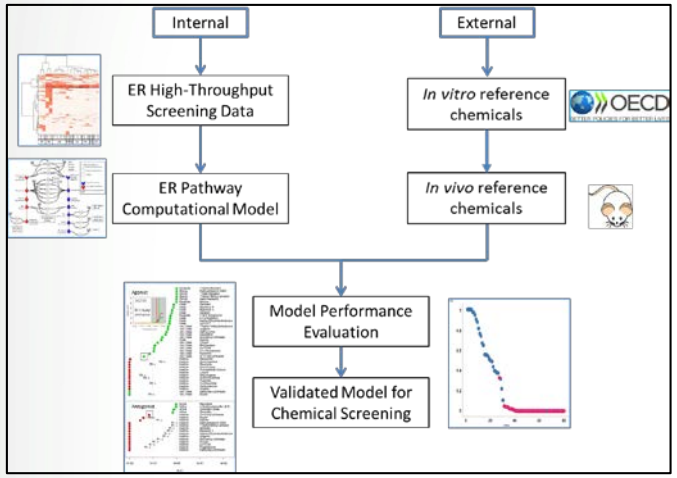
Androgen Receptor Computational Model

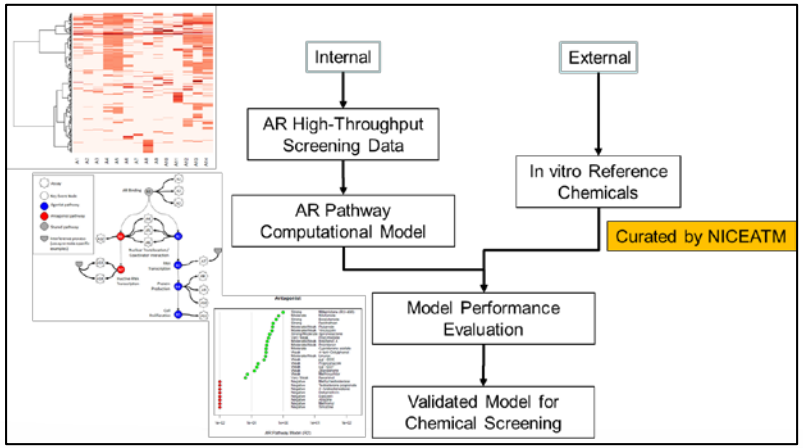
Kleinstreuer et al., Chem Res Toxicol (2017)





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

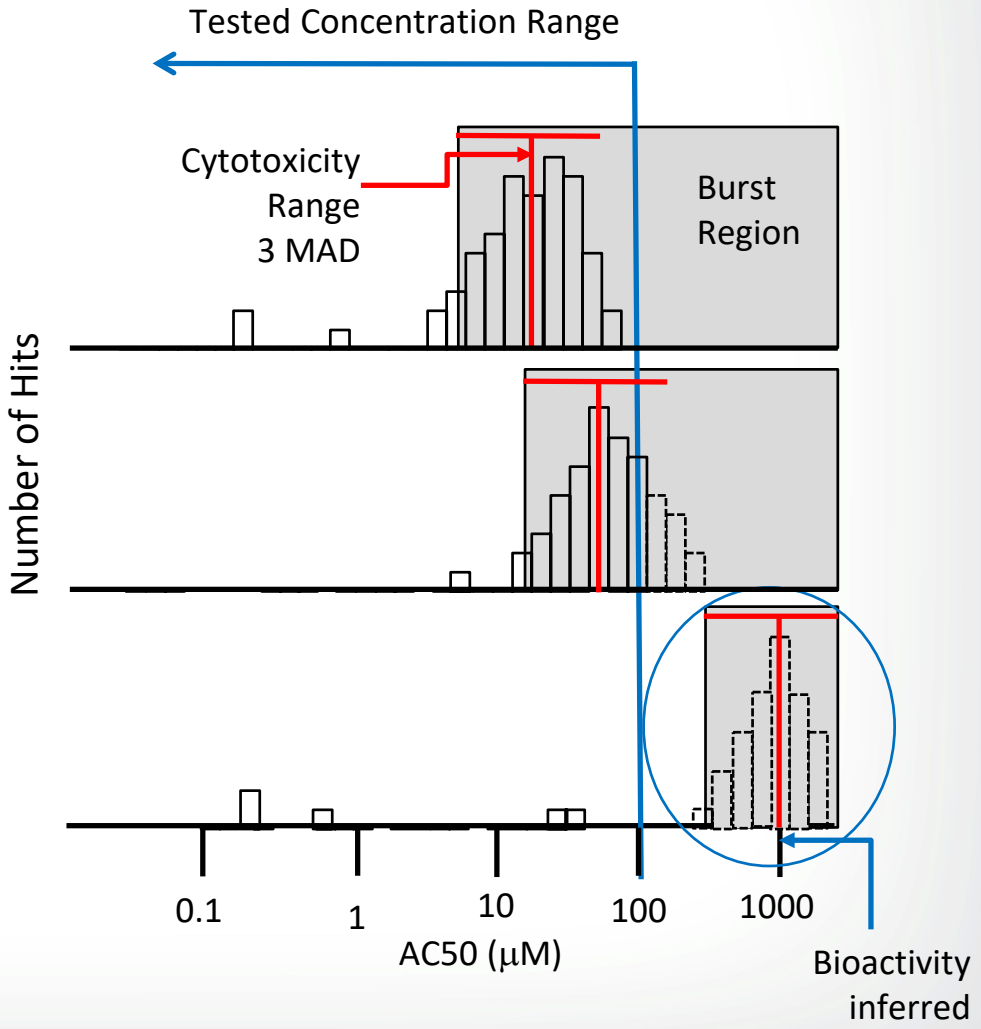
Judson et al. Env Health Pers (2015) doi: 10.1093/toxsci/kfv168; Kleinstreuer et al. Reprod Toxicol 2018 doi: 10.1016/j.reprotox.2018.08.017

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.

Cytotoxic 'burst'

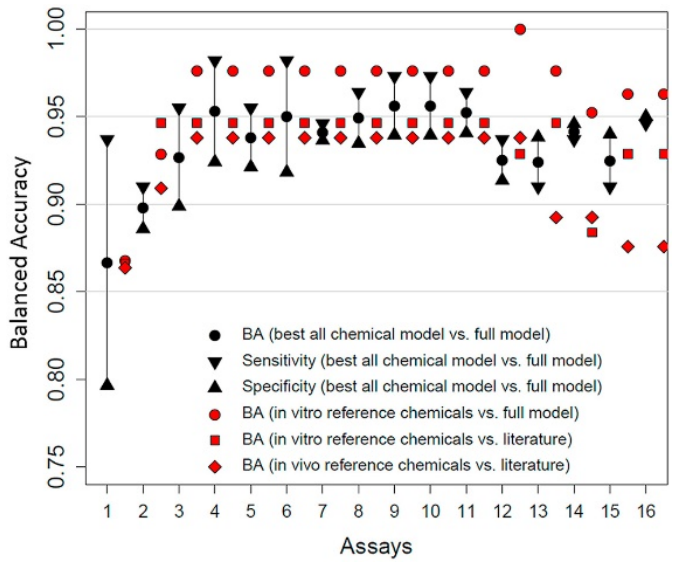
- Most chemicals display a "burst" of potentially non-selective bioactivity near the cytotoxicity concentration.
- This is often "false positive" activity
  - E.g. Activity in an ER assay in the "burst" region is likely due to cell stress and not true ER binding activity
- Statistical method can be used to filter out this false positive activity before drawing conclusions about ER, AR (or other specific target) activity

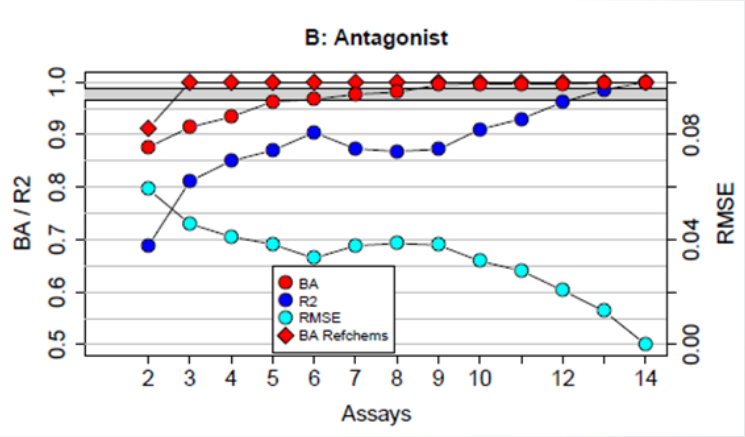


Judson et al. Tox.Sci. (2016) doi.org/10.1093/toxsci/kfw092

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



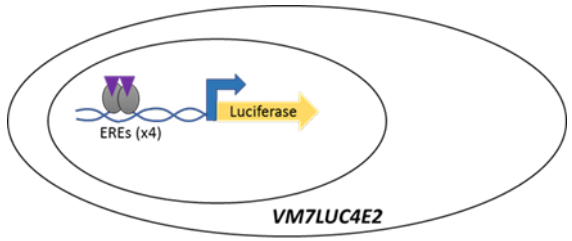


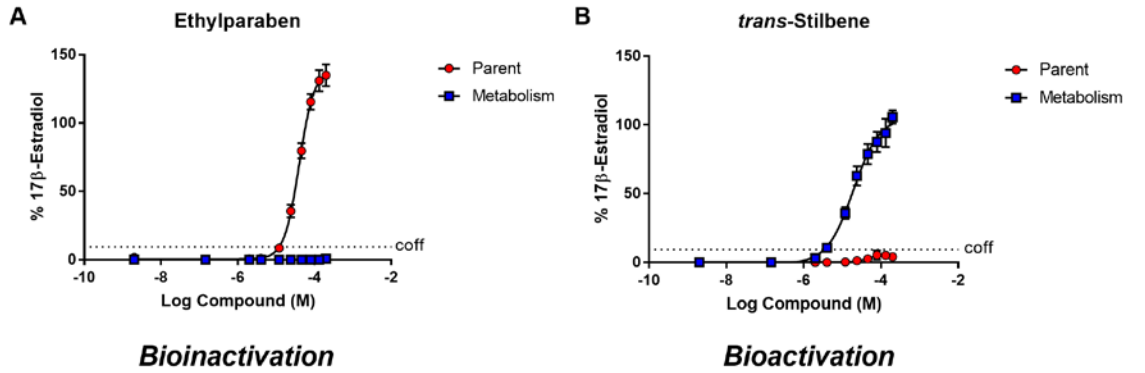
Judson et al., Reg. Tox. Pharm. (2017) doi: 10.1016/j.yrtph.**2017**.09.022 (ER)
Judson, et al. In preparation (2019) (AR)

Metabolic Competence

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

- **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





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

Collaboration with Unilever C. Deisenroth, Unpublished

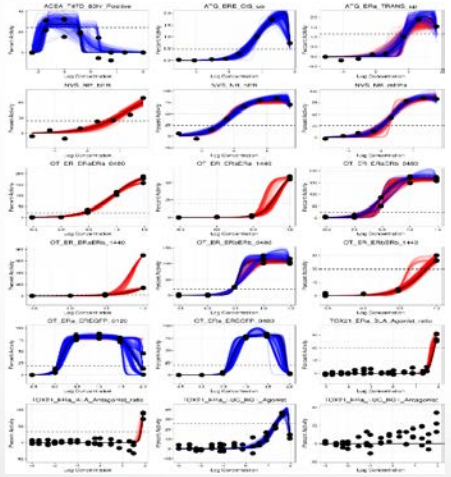
Uncertainty Analysis

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)

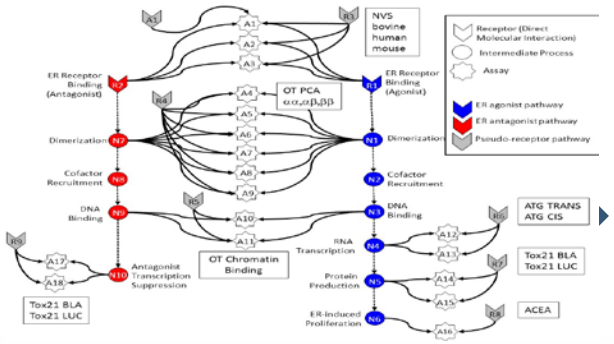
Both are now incorporated into the ER and AR model results through the development of statistical methods have been developed to establish uncertainty bounds around potency and efficacy values. These statistical methods involve resampling the data and refitting the concentration response curves thousands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

Bootstrap Uncertainty in *In Vitro* Potency Values



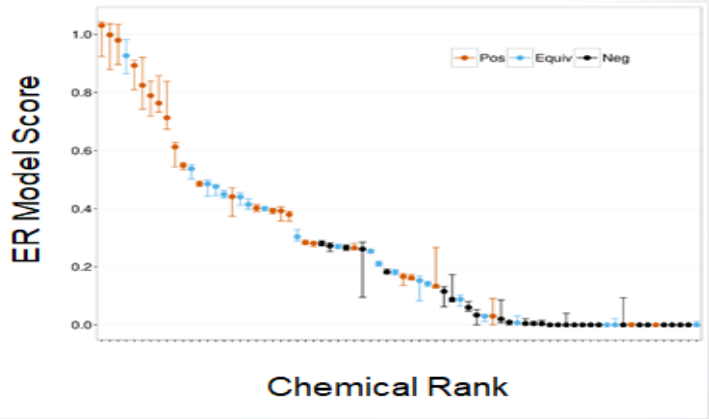
18 ER *In Vitro* Assays

Computational Modeling



ER Pathway Model

Propagation of Uncertainty in Modeling Output



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

CERAPP consensus validation

	Binding		Agonist		Antagonist	
	Training	Validation	Training	Validation	Training	Validation
Sn	0.93	0.58	0.85	0.94	0.67	0.18
Sp	0.97	0.92	0.98	0.94	0.94	0.90
BA	0.95	0.75	0.92	0.94	0.80	0.54

CoMPARA consensus validation

	Binding		Agonist		Antagonist	
	Training	Validation	Training	Validation	Training	Validation
Sn	0.99	0.69	0.95	0.74	1.00	0.61
Sp	0.91	0.87	0.98	0.97	0.95	0.87
BA	0.95	0.78	0.97	0.86	0.97	0.74

Forward Prediction Results

	CERAPP		CoMPARA	
	Active	Inactive	Active	Inactive
Binding	4001	28463	8202	40656
Agonist	2475	29989	1764	47094
Antagonist	2793	29671	9899	38959
Total	4001	28463	10623	47613

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

OXFORD
SOT | Society of Toxicology
www.toxsci.oxfordjournals.org
ToxSci 20 Years

TOXICOLOGICAL SCIENCES, 162(2), 2018, 509–534
doi: 10.1093/toxsci/kfx274
Advance Access Publication Date: December 1, 2017
Research Article

High-Throughput H295R Steroidogenesis Assay: Utility as an Alternative and a Statistical Approach to Characterize Effects on Steroidogenesis

Derik E. Haggard,*,† Agnes L. Karmaus,*,†,1 Matthew T. Martin,†,2 Richard S. Judson,† R. Woodrow Setzer,† and Katie Paul Friedman†,3

Regulatory Toxicology and Pharmacology 109 (2019) 104510

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Contents lists available at ScienceDirect

Regulatory Toxicology and Pharmacology

journal homepage: www.elsevier.com/locate/yrtph

Development of a prioritization method for chemical-mediated effects on steroidogenesis using an integrated statistical analysis of high-throughput H295R data

Derik E. Haggard[a,b], R. Woodrow Setzer[b], Richard S. Judson[b], Katie Paul Friedman[b,*]

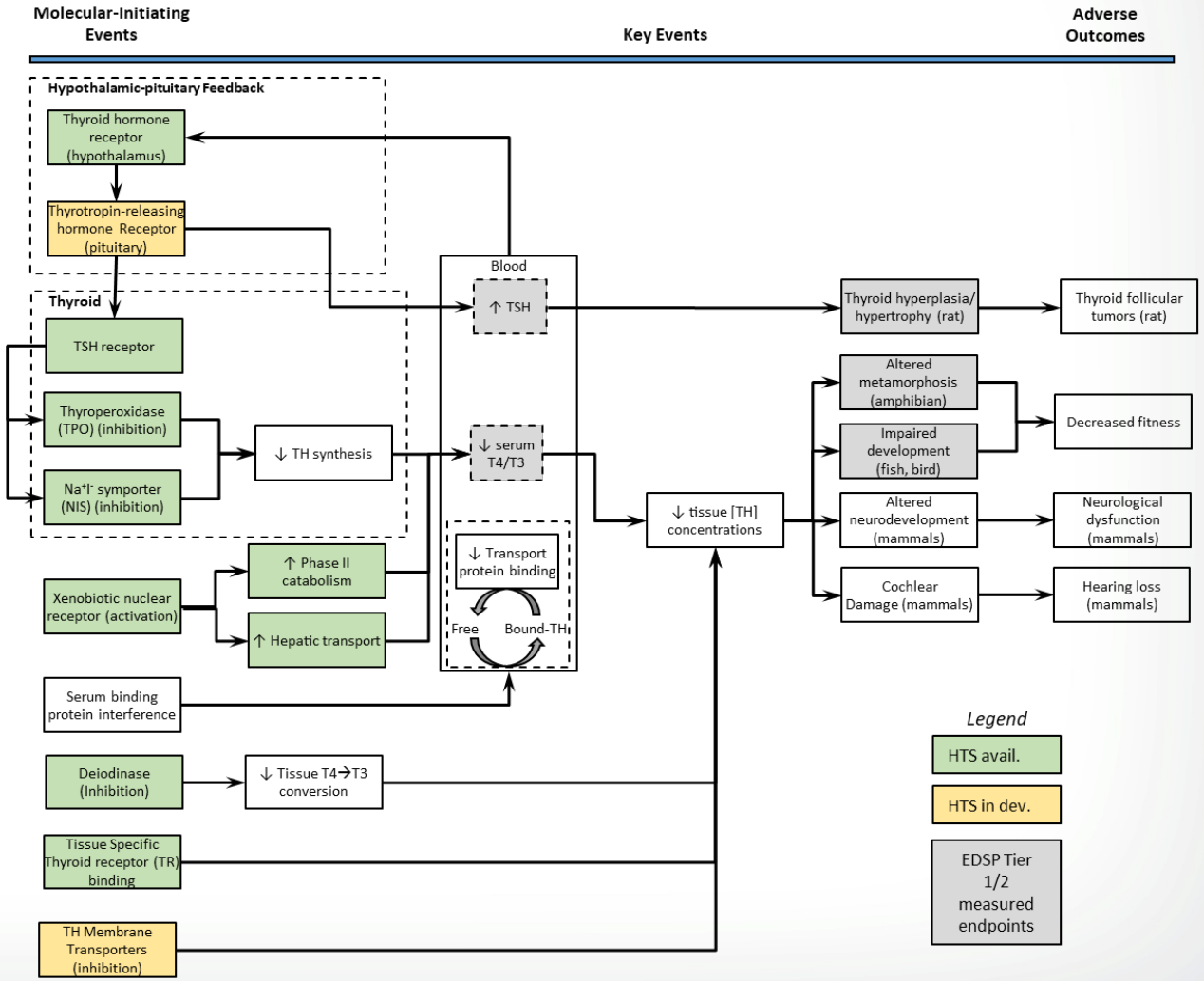
[a] Oak Ridge Institute for Science and Education, 100 ORAU Way, Oak Ridge, TN, 37830, USA
[b] National Center for Computational Toxicology, Office of Research and Development, U.S. Environmental Protection Agency, Research Triangle Park, NC, 27711, USA

- 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.

Haggard et al., 2018 doi: 10.1093/toxsci/kfx274.; Haggard et al., 2019 doi: 10.1016/j.yrtph.2019.104510.

Making Progress on Thyroid

- 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



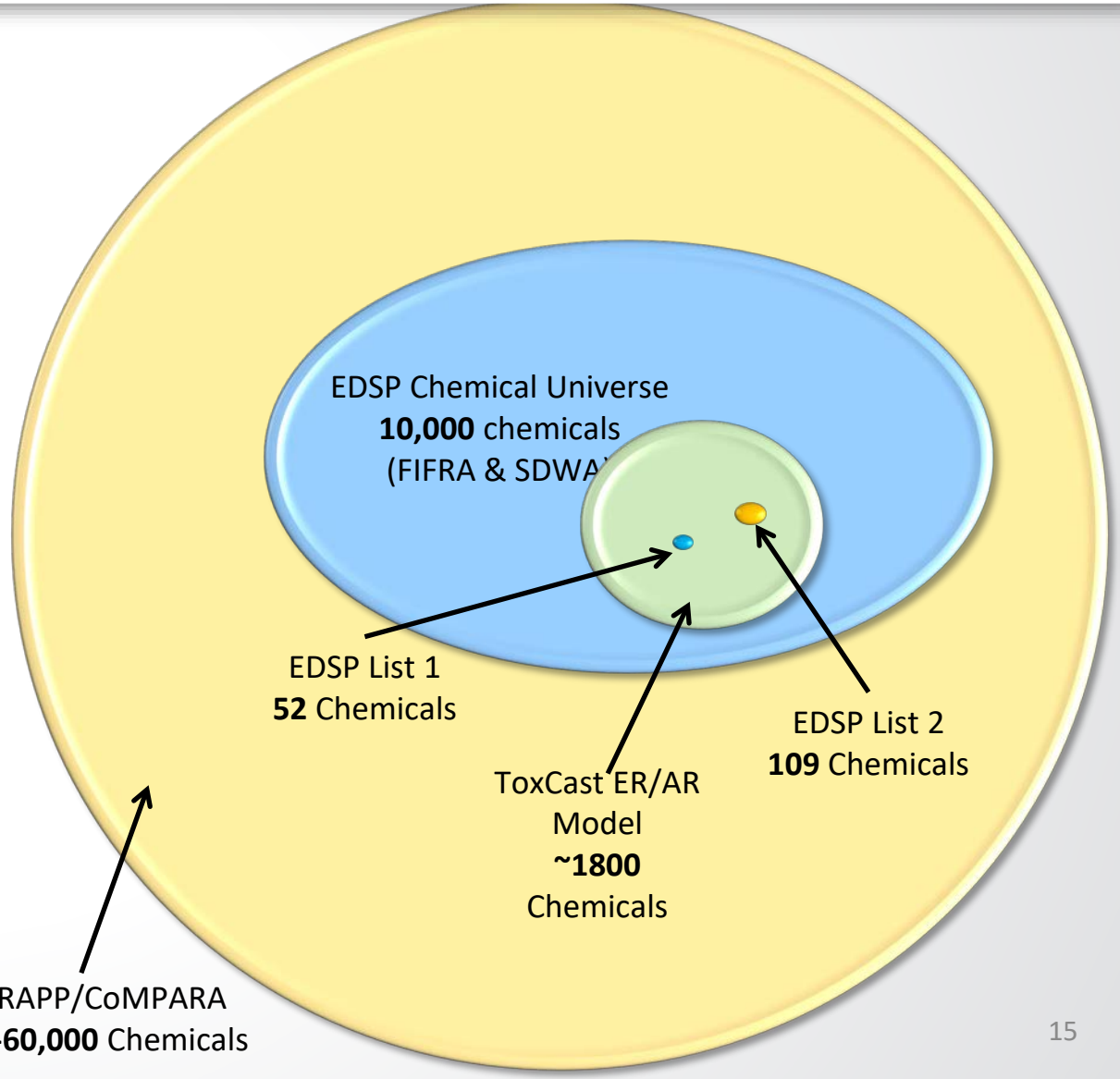
Paul-Friedman et al., 2016 doi: 10.1093/toxsci/kfw034; Noyes *et al.*,2019 doi: 10.1289/EHP5297

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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Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM)
Unilever

Center for Computational Toxicology and Exposure (CCTE)
Office of Research and Development (ORD)
US Environmental Protection Agency

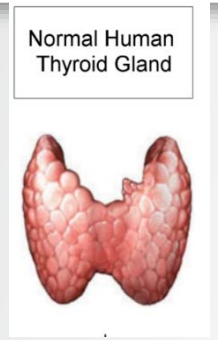
Additional Slides

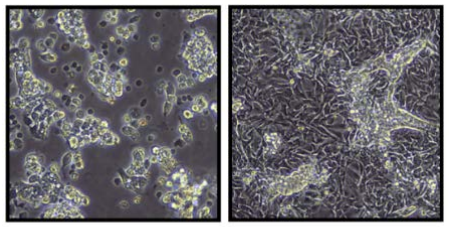
Developing Alternative EDSP Assays

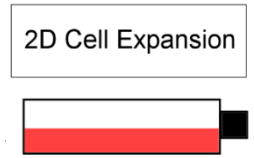
EDSP Tier 1 Battery of Assays (current)	High Throughput Assays and Computational Model Tier 1 Battery Alternatives
Estrogen Receptor (ER) Binding	**ER Model (alternative)**
Estrogen Receptor Transactivation (ERTA)	**ER Model (alternative)**
Uterotrophic	**ER Model (alternative)**
Androgen Receptor (AR) Binding	AR Model
Hershberger	AR Model
Aromatase	STR Model
Steroidogenesis (STR)	STR Model
Female Rat Pubertal	**ER**, STR , THY Models
Male Rat Pubertal	AR, STR , THY Models
Fish Short Term Reproduction	**ER**, AR, STR Models
Amphibian Metamorphosis	THY Model
EDSP Tier 2 Tests	**High Throughput Assays and Computational Model Tier 2 Battery Alternatives**
Rat 2-gen/EOGRT	**ER**, AR, STR, THY
Medaka Extended 1-Gen Reproduction	**ER**, AR, STR
Larval Amphibian Growth & Development	THY
Avian Multi-Generation Reproduction	**ER**, AR, STR, THY

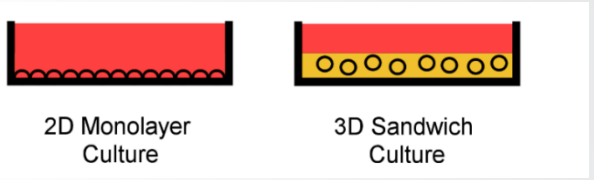
ER = estrogen receptor; AR = androgen receptor; STR = steroidogenesis; THY = thyroid

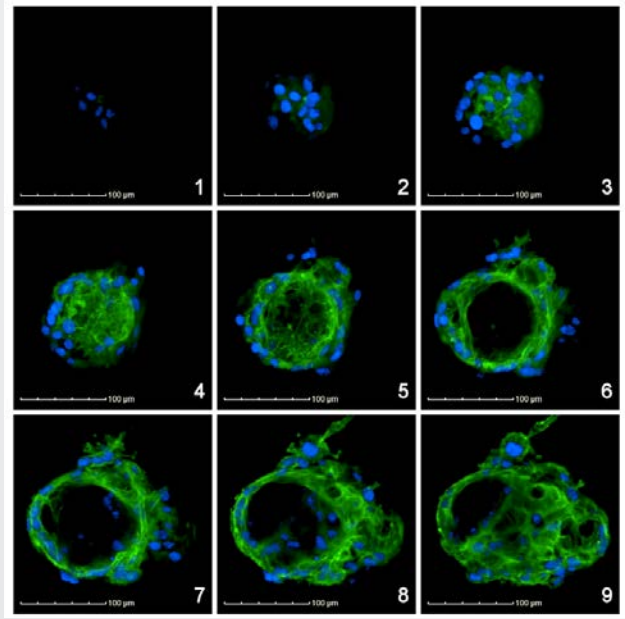
Developing Organotypic Culture Models to Identify Tissue/Organ Effects



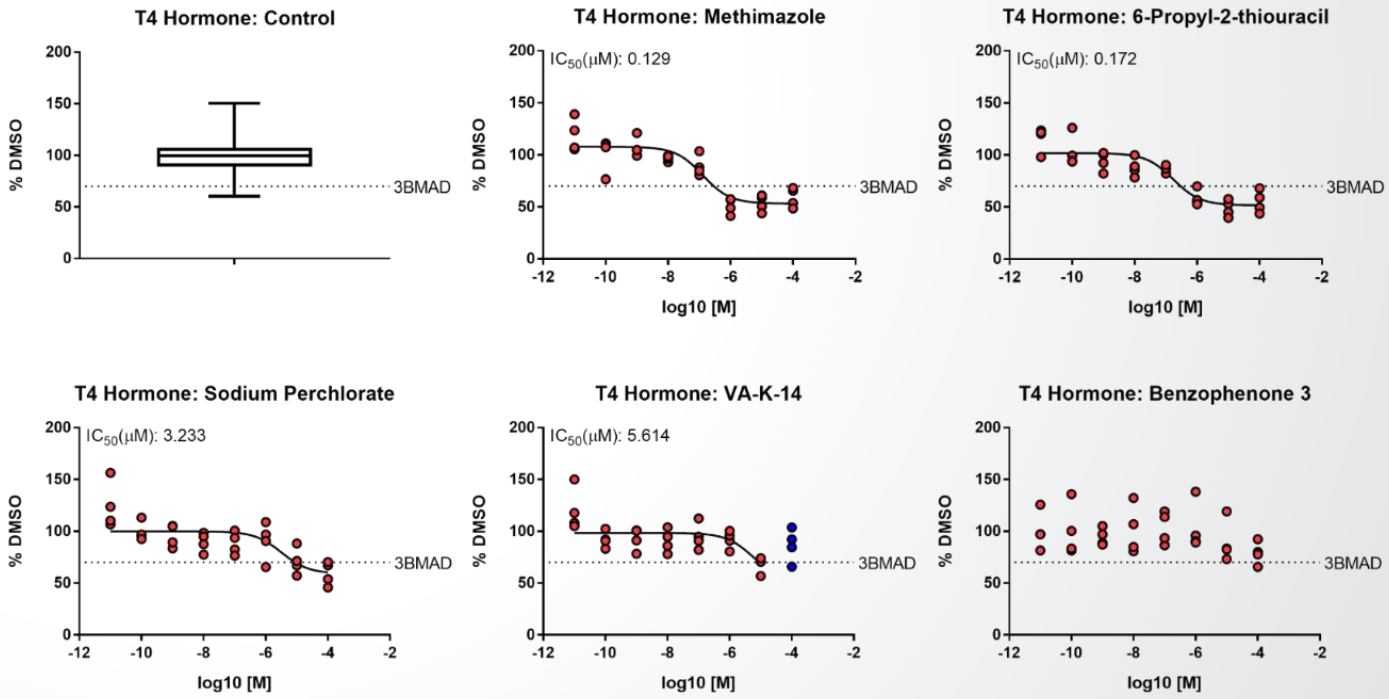








Blue, Hoechst 33342 /DNA
Green, Phalloidin/Actin



C. Deisenroth, In Review

High-Throughput Toxicokinetic Component

EPA ToxCast Phase I and II Chemicals

Human Liver Metabolism

Human Plasma Protein Binding

Population-Based IVIVE Model

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

- Currently evaluated ~700 ToxCast Phase I and II chemicals
- Models available through '"httk" R package (https://cran.r-project.org/web/packages/httk/)

In Vitro Potency Value

Plasma Concentration

Exposure Route

Reverse Dosimetry

Administered Dose Required to Achieve Steady State Plasma Concentrations Equivalent to *In Vitro* Bioactivity

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