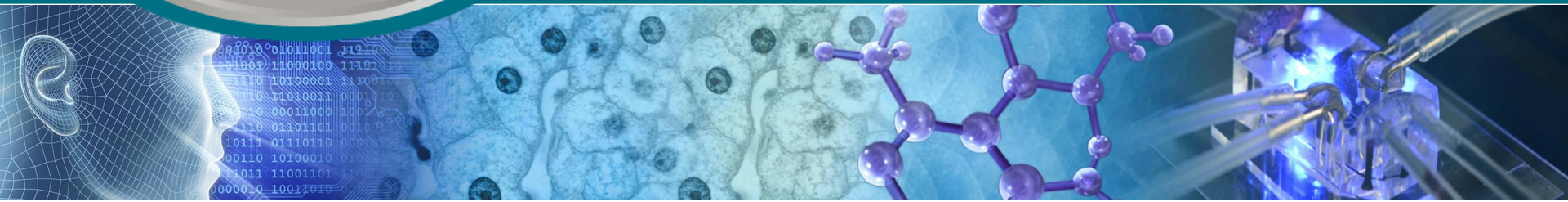
UNITED STATES
ICCVAM
Advancing Alternatives to Animal Testing

Interagency Coordinating Committee on the Validation of Alternative Methods



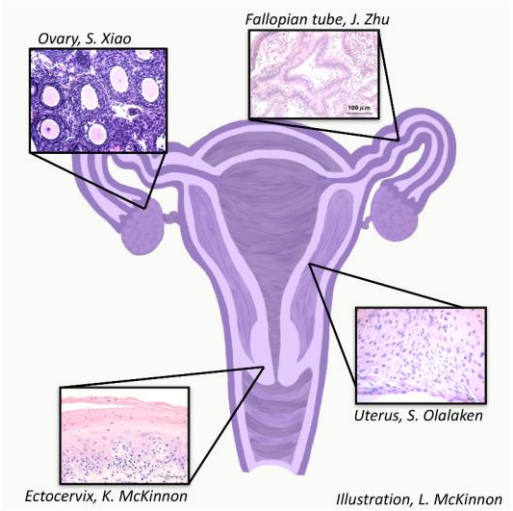
Computational tools and alternative methods in developmental toxicology

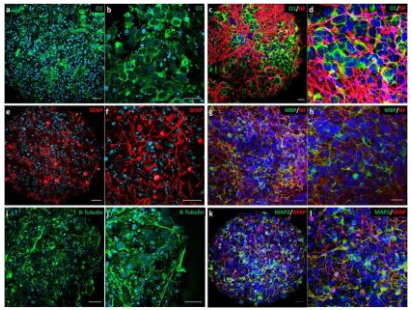
Nicole Kleinstreuer, PhD
Deputy Director, NICEATM

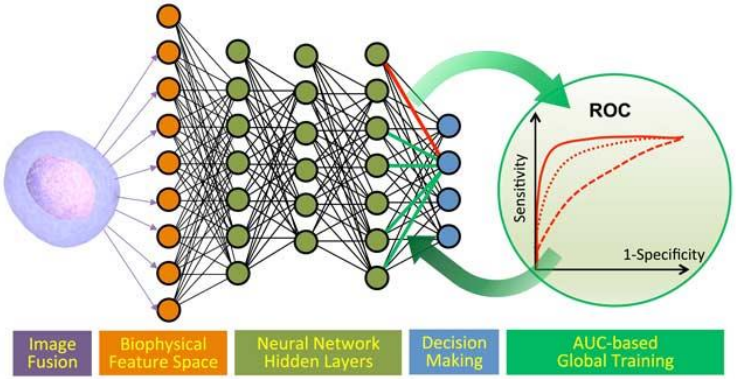
14th September, 2018

Agency for Toxic Substances and Disease Registry • Consumer Product Safety Commission • Department of Agriculture
Department of Defense • Department of Energy • Department of the Interior • Department of Transportation
Environmental Protection Agency • Food and Drug Administration • National Institute for Occupational Safety and Health
National Institute of Standards and Technology • National Institutes of Health • National Cancer Institute • National Library of Medicine
National Institute of Environmental Health Sciences • Occupational Safety and Health Administration

It is difficult for evolving INSTITUTIONAL PRACTICES to keep pace with revolutionary advances in science and technology

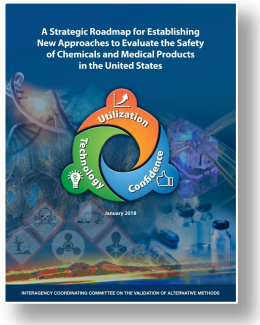






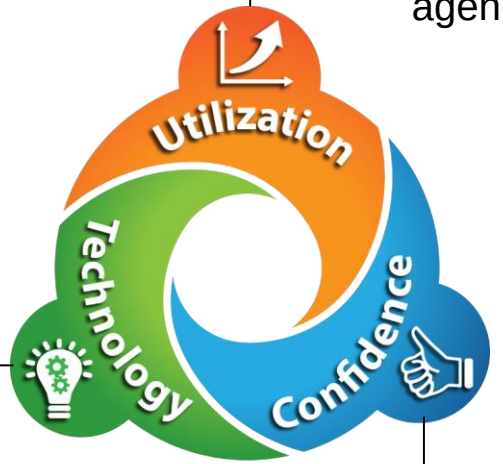
"Federal agencies and stakeholders will work together to build a new framework to develop, establish confidence in, and encourage use of new approaches to toxicity testing that improve human health relevance and reduce or eliminate the need for testing in animals."

- **Published Jan 30, 2018**
- **https://ntp.niehs.nih.gov/go/natl-strategy**
- Google ICCVAM roadmap



Encourage the adoption and use of new methods and approaches by federal agencies and regulated industries

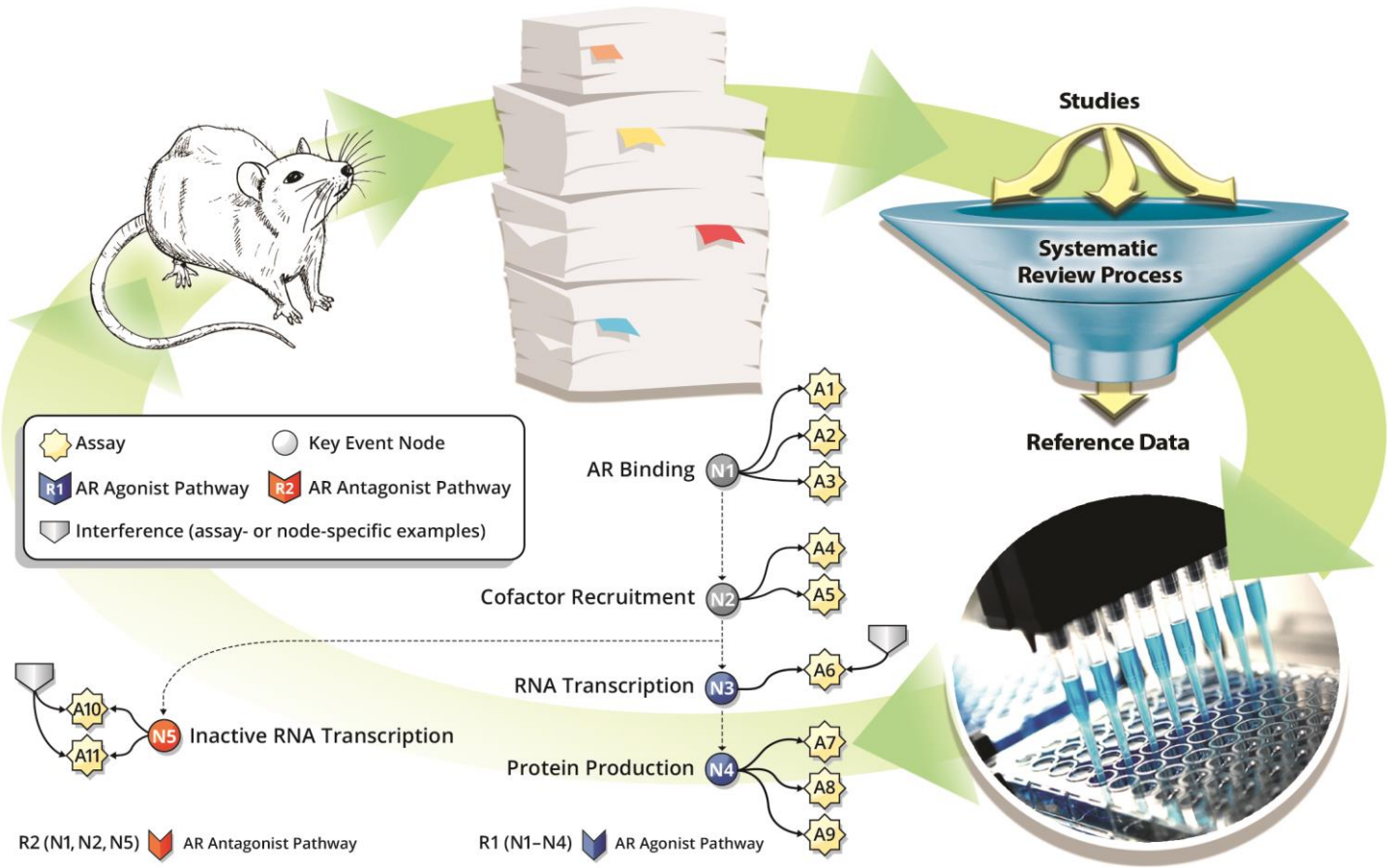
Help end-users guide the development of the new tools needed to support their needs



Foster the use of efficient, flexible, and robust practices to establish confidence in new methods

Federal ICCVAM DART WG: Scope and Charge

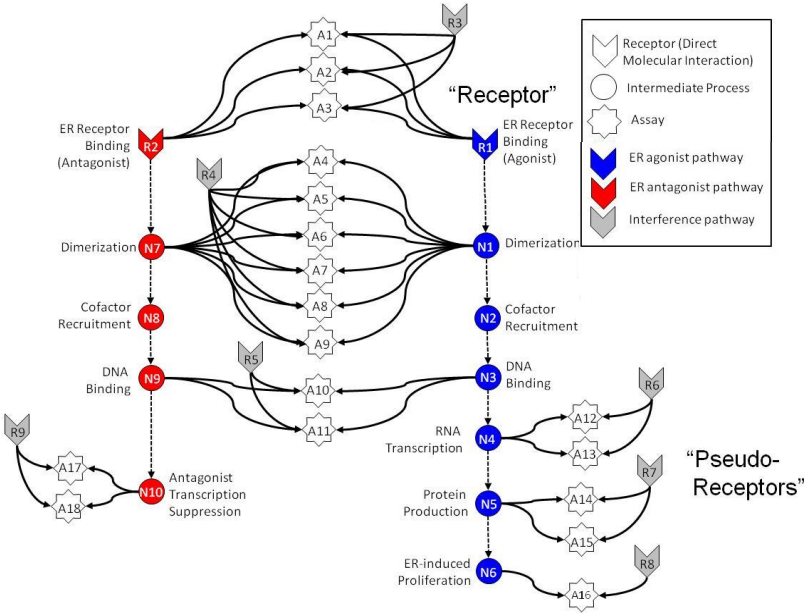
- Identify agency needs for assessment of adverse developmental effects
- Work with international partners to identify global regulatory requirements for developmental toxicity testing
- Identify endpoints needed by each federal agency, and commonalities and differences between agencies
- Examine the importance of specific endpoints and study types to research product development and regulatory decision-making
- Create a catalog of existing and emerging technologies, map the endpoints measured by those technologies to known mechanisms of developmental toxicity, and assess their potential to fulfill regulatory testing requirements
- Establish a stakeholder group of both government and non- government scientists to coordinate efforts towards developing and implementing integrated strategies for developmental toxicity testing



Kleinstreuer et al. RTX (2018) in press

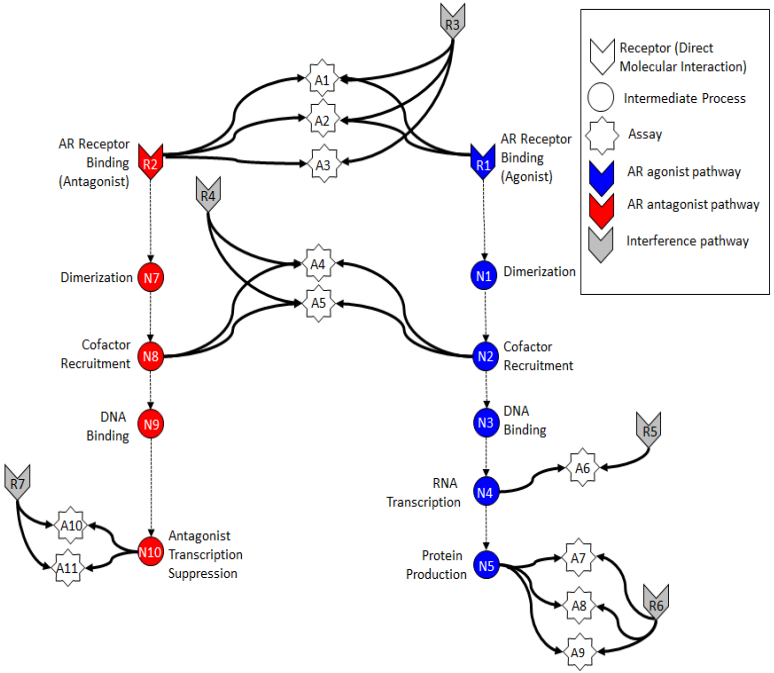
Tox21/ToxCast Endocrine Pathway Models

ER Pathway Model



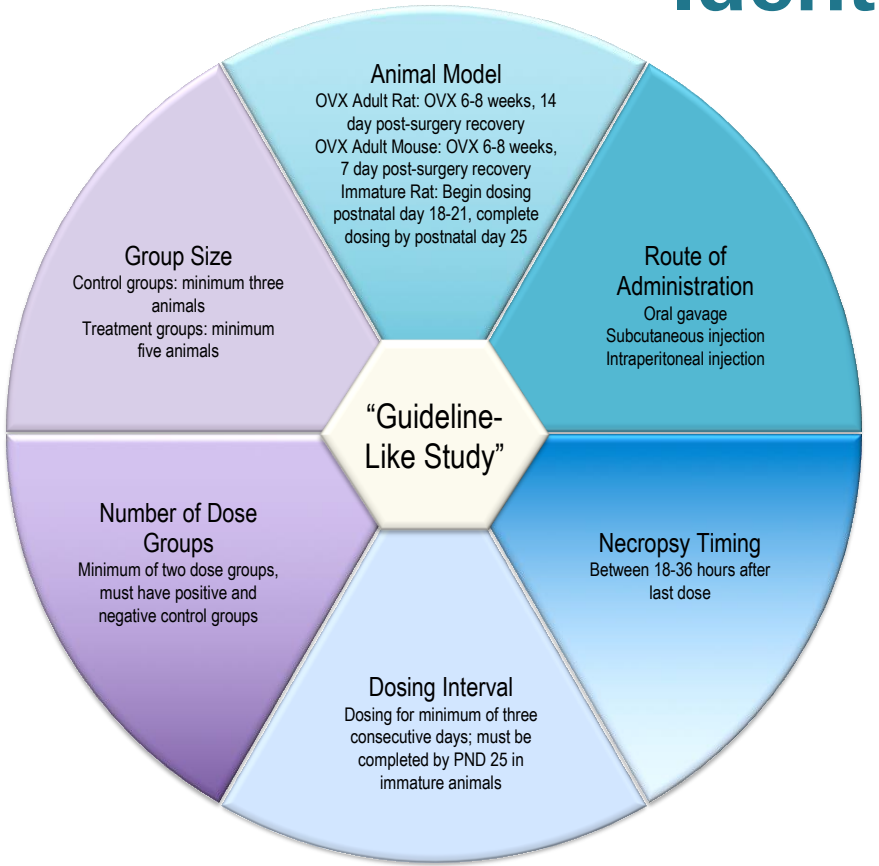
Judson et al Toxicol. Sci. (2015)

AR Pathway Model



Kleinstreuer et al. Chem Res Tox (2017)

Identifying Reference Data



- Systematic literature search of publically available data (e.g. PubMed)
- 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. EHP (2015)

ER/AR Pathway Model Performance

- Reference chemicals identified from validation studies, regulatory guideline submissions, and literature reviews *(Kleinstreuer et al. 2015, Kleinstreuer et al. 2017, Browne et al. 2018, Kleinstreuer et al. 2018)*

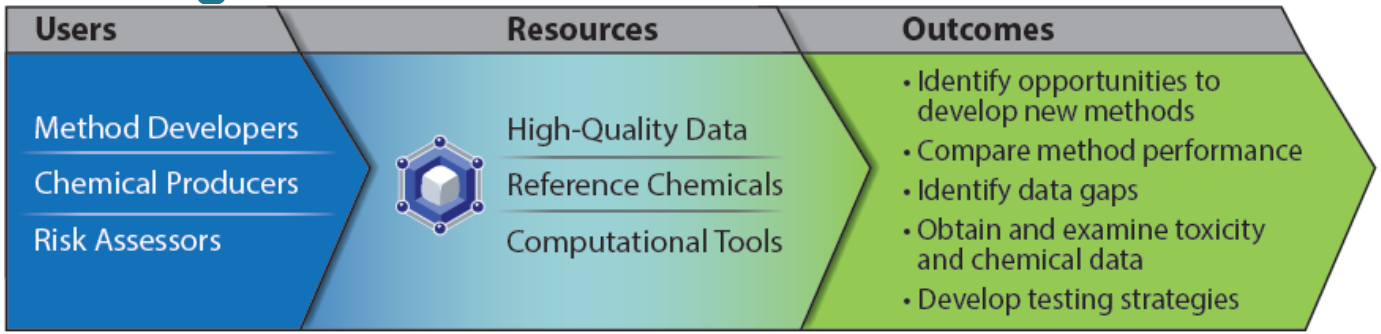
ER Agonist

Performance Metrics	Value
# True Pos	29
# True Neg	46
# False Pos	1
# False Neg	1
Accuracy	0.97
Sensitivity	0.97
Specificity	0.97

AR Antagonist

Performance Metrics	Value
# True Pos	19
# True Neg	8
# False Pos	0
# False Neg	1
Accuracy	0.975
Sensitivity	0.95
Specificity	1.00

Integrated Chemical Environment: ICE



Bell et al. 2017 EHP

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

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

- Workflows:
  - IVIVE, Chemical space characterization, Machine learning, AOP mapping

Run Search | Clear

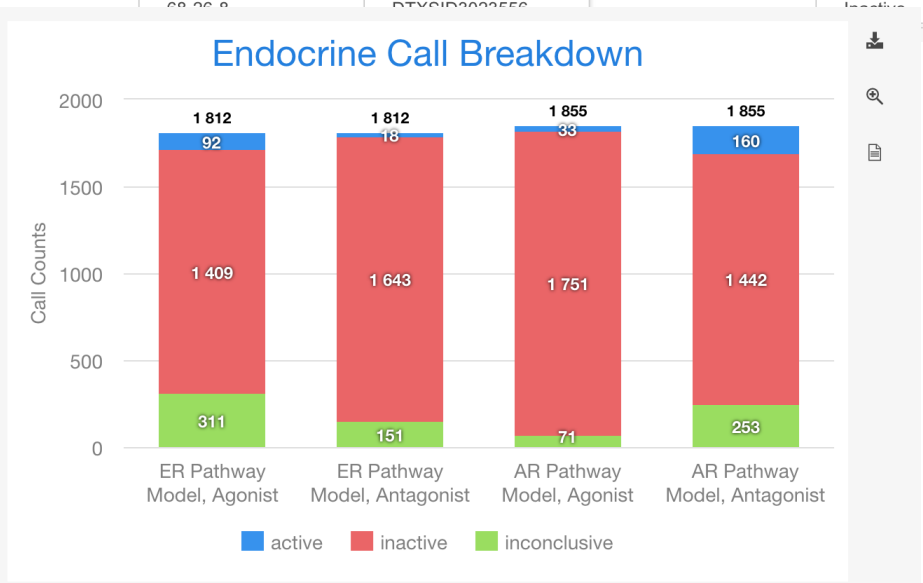
Select Assays

Select Assay Target

- Acute Oral Toxicit
- Skin Sensitization
- Skin Irritation
- Eye Irritation
- **Endocrine**
  - **Androgen**
    - in vitro
    - in silico
  - **Estrogen**
    - in vivo
    - in vitro
    - in silico
- in vitro (all)
- **in silico**

Download | Query Formulations | Number of chemicals = 1856. Showing 8 Endpoints.

Substance Name	CASRN	DSSTOXID	ER Pathway Mode…	ER Pathway
Propofol	2078-54-8	DTXSID6023523		Inactive
Diisopropyl phthalate	605-45-8	DTXSID2040731	33.053	Inconclusive
Triamcinolone	124-94-7	DTXSID1040742	30.386	Inconclusive
Pyridoxine	65-23-6	DTXSID4023541		Inactive
17alpha-Hydroxyp…	68-96-2	DTXSID6040747	0.365	Active
Fandosentan pota…	221246-12-4	DTXSID5047249		Inactive
Retinol				



Global QSAR Modeling Collaboration

CERAPP

Collaborative Estrogen Receptor Activity Prediction Project

Mansouri et al. EHP (2017)

CoMPARA

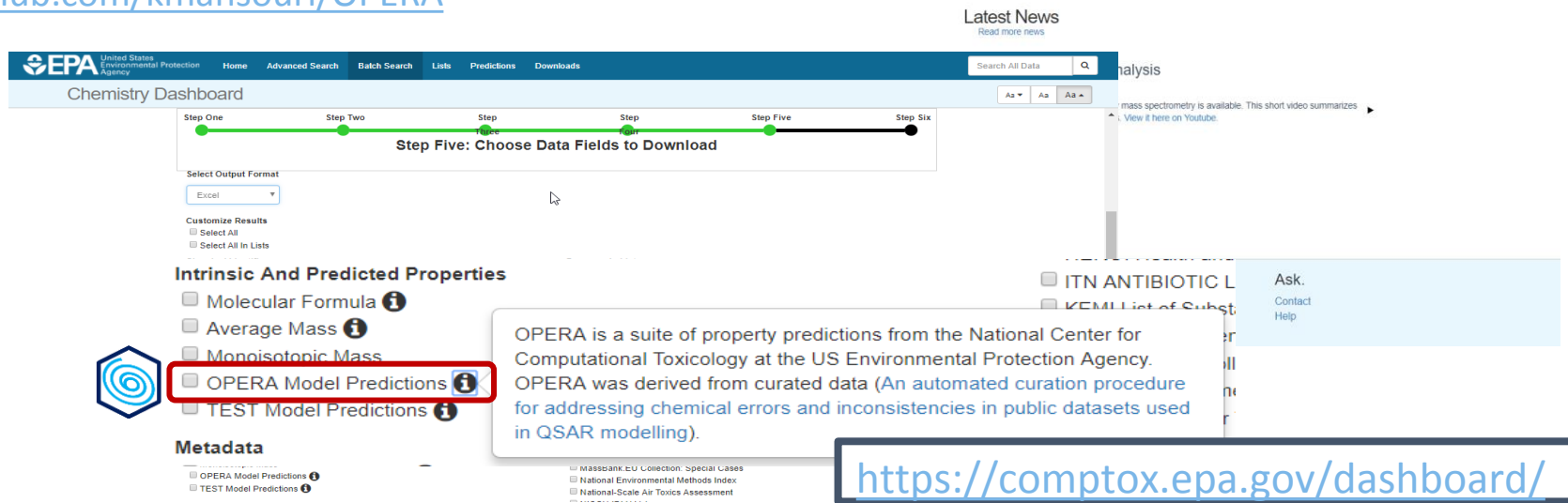
Collaborative **M**odeling **P**roject for **A**ndrogen **R**eceptor **A**ctivity

Mansouri et al. in prep (2018)

Global QSAR Modeling Collaboration

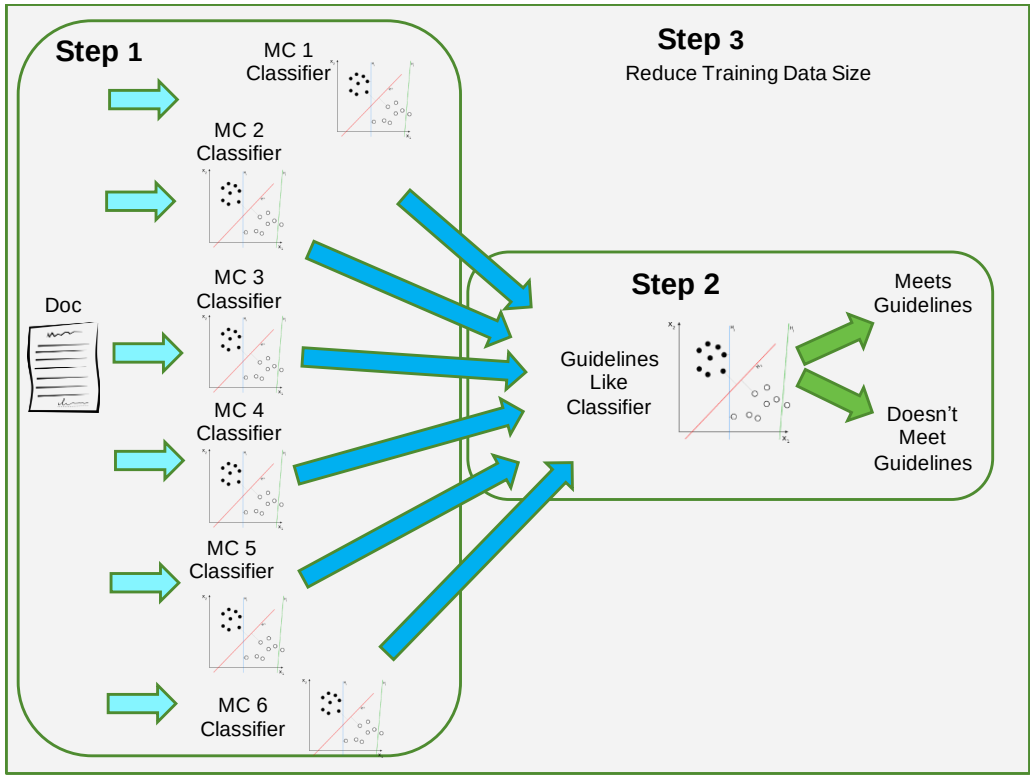
OPERA
OPEn (q)saR App

https://github.com/kmansouri/OPERA



https://comptox.epa.gov/dashboard/

Automating Reference Data Identification

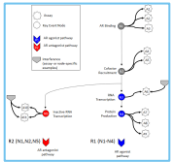


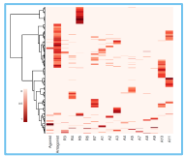
- Project with Oak Ridge National Labs (ORNL) to apply text-mining (NLP) approaches & ML to identify high-quality data
- Semi-automated retrieval and evaluation of published literature (trained on uterotrophic database)
- Apply to developmental toxicity studies

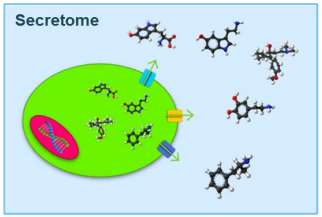
Mechanistic Mapping of HTS Assays

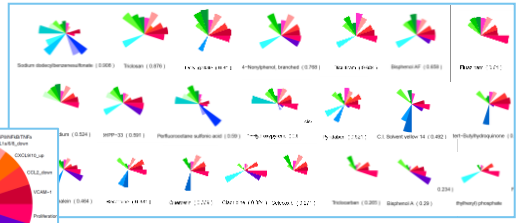
Human Teratogenic Mechanisms

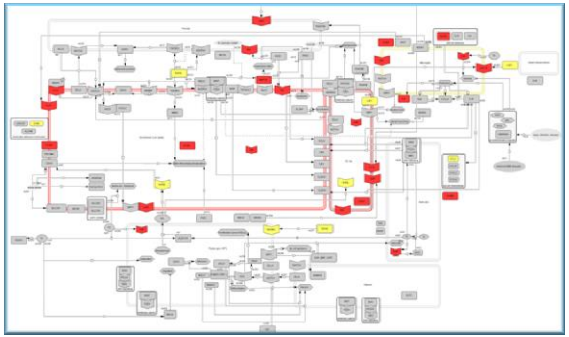
- Endocrine disruption
- Oxidative stress
- Vascular disruption
- Folate antagonism
- Neural crest cell disruption
- Specific receptor- or enzyme-mediated



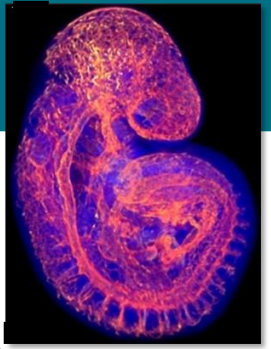






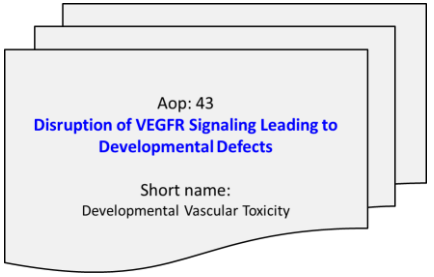


Van Gelder et al. 2010; Knudsen and Kleinstreuer 2011



Vascular Development & Disruption

- Blood vessel development is essential to the embryo (cardiovascular first functioning organ system in vertebrate species).
- Vascular insufficiency is tied to many disease processes (teratogenesis, stroke, diabetes, pre-eclampsia, neonatal respiratory distress, osteoporosis, Alzheimer's, …).
- AOP43: one of 28 AOPs included in the OECD work plan with status 'open for citation & comment' https://aopwiki.org/wiki/index.php/Aop:43

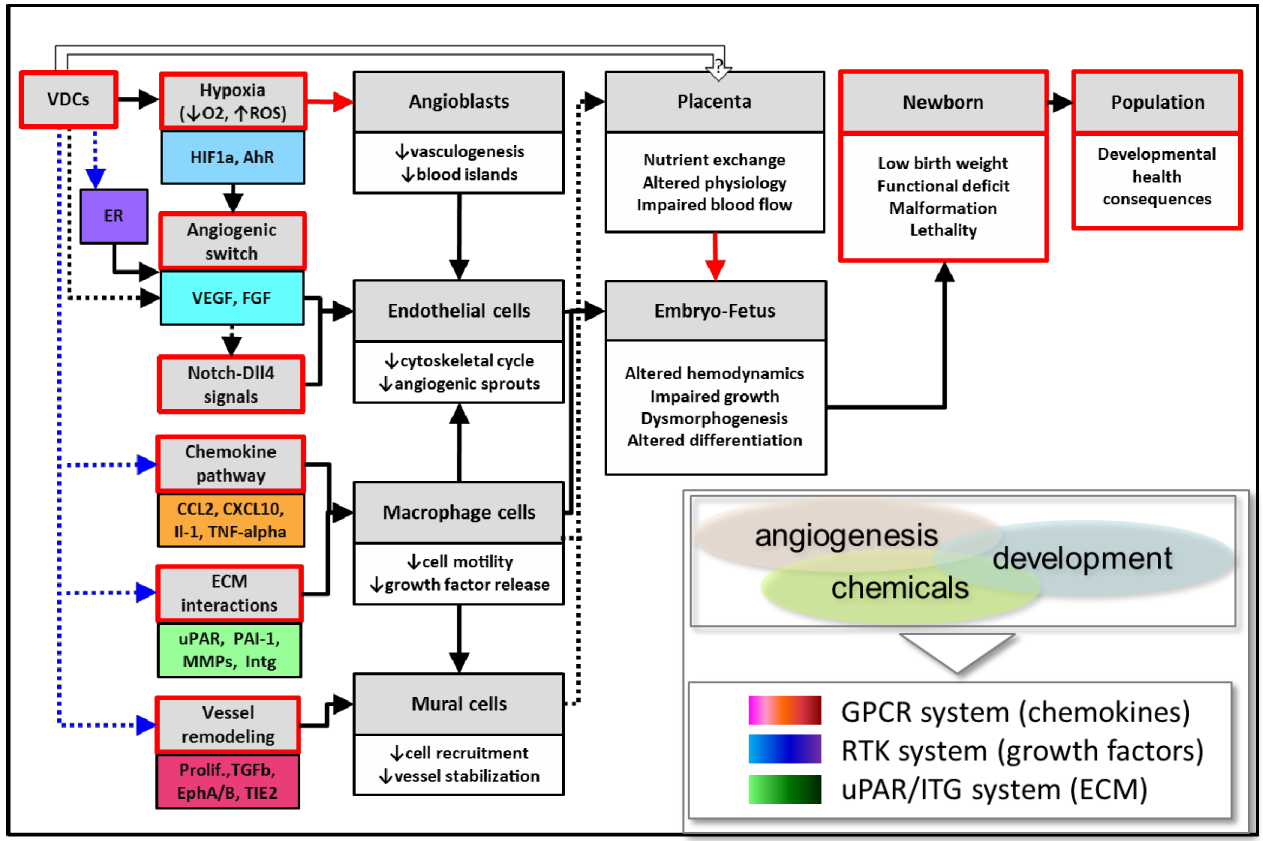


Vasculogenesis

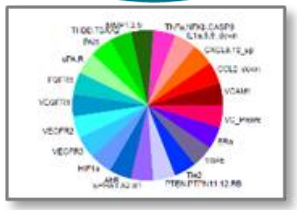
Primary tubular network

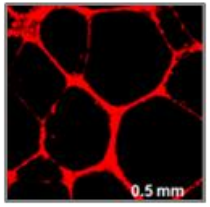
Angiogenesis

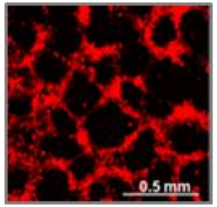
Remodeling

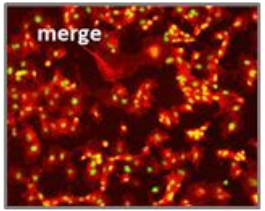


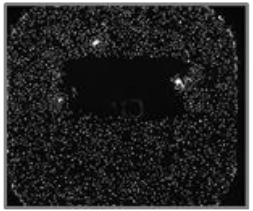
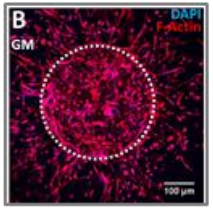
Knudsen and Kleinstreuer (2011) Birth Defects Res

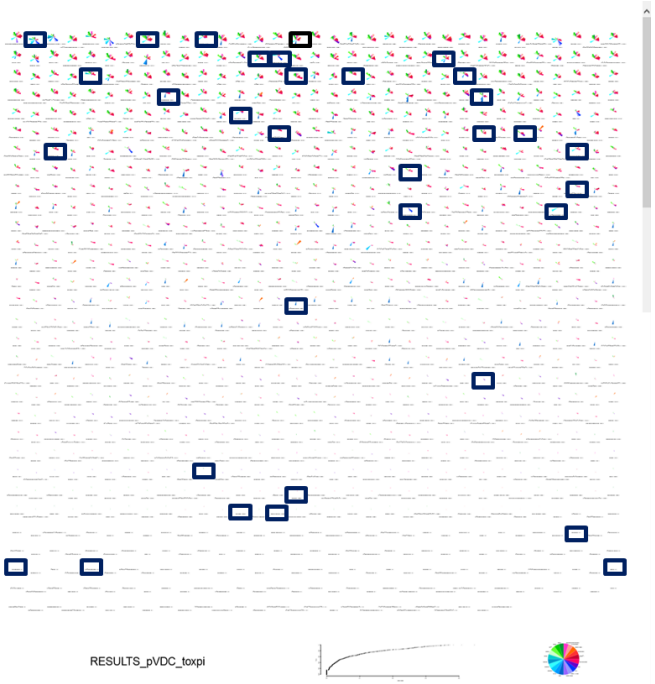


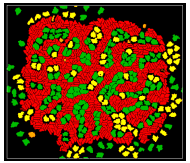
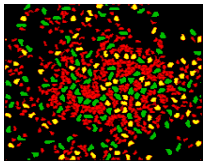
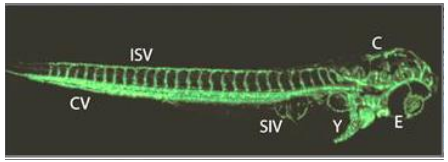







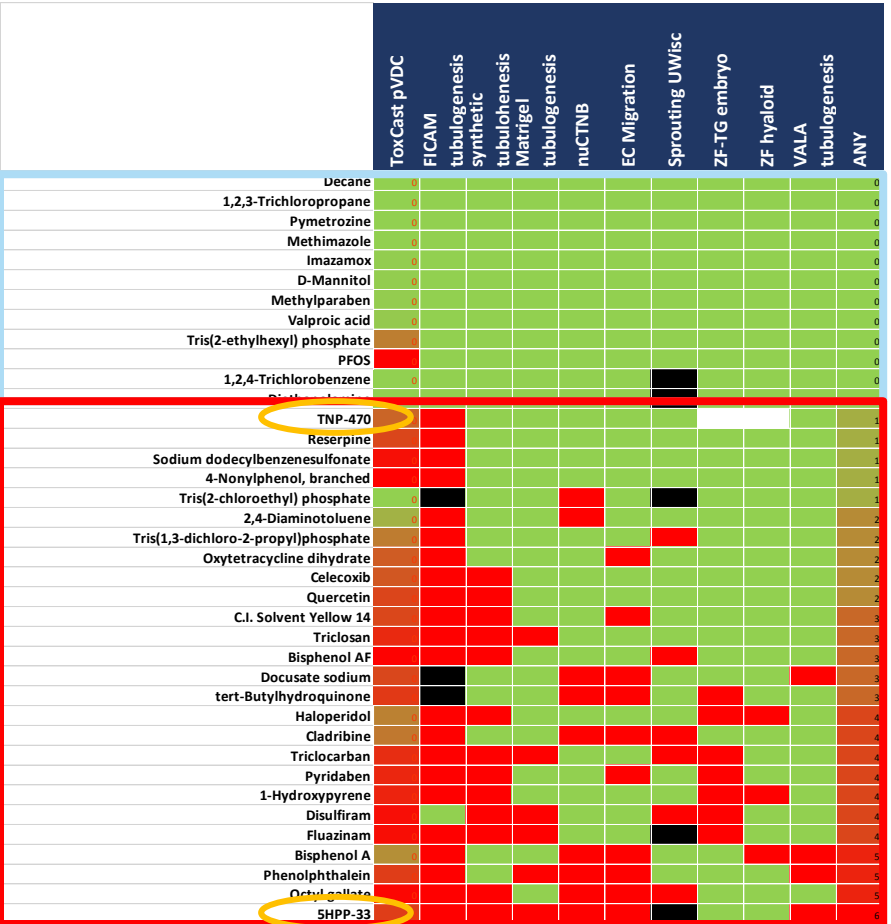





How well does ToxCast do in predicting disruption of angiogenesis across different endothelial platforms?

- Virtual vascular plexus simulation [*Kleinstreuer et al. (2013) PLoS Comp Biol*]
- 3D angiogenic sprouting [*Belair et al. (2016) Acta Biomat*]
- engineered matrices [*Nguyen et al. (2017) Nature Bioeng*]
- EC-reporter zebrafish embryos [*Tal et al. (2017) RTX*]
- nuCTNB and endothelial migration [*manuscript in prep*]
- tubulogenesis (FICAM, VALA) [*manuscript in prep*]



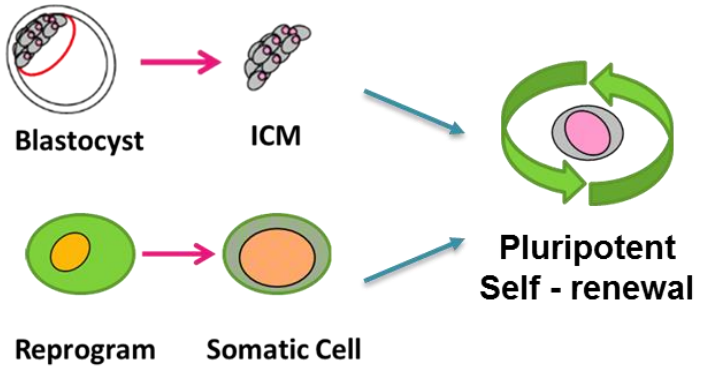
38 chemical test set: qualification of pVDC ToxPi across 9 endothelial behaviors

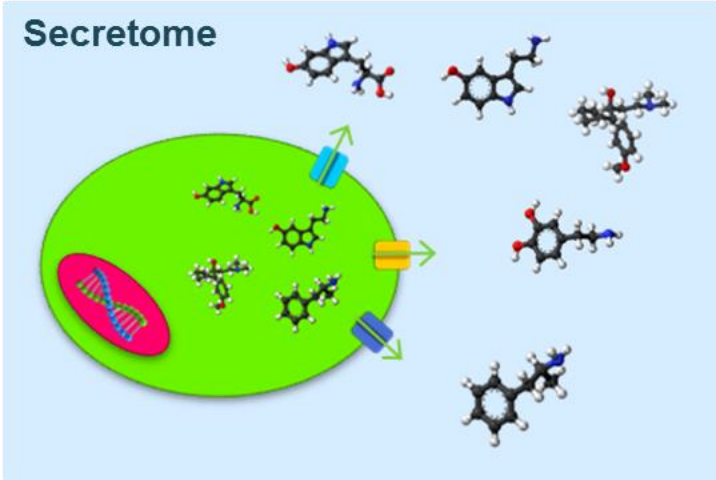
A pVDC score from ToxCast dataset (ToxPi)
B HUVEC tubulogenesis (FICAM)
C tubulogenesis in synthetic matrices
D tubulogenesis in Matrigel
E nuCTNB biomarker (EndMT)
F endothelial cell migration
G sprouting assay (iPSC-derived endothelial cells)
H reporter zebrafish (ISV outgrowth)
I reporter zebrafish (hyaloid vascular network)
J HUVEC tubulogenesis (VALA)
K ANY (B to J)

Tom Knudsen, EPA/NCCT

Disruption of Stem Cell Metabolism

- Biomarker-based human pluripotent stem cell assay for developmental toxicity screening
- Assay performed with human pluripotent stem cells
- Measured changes in **secreted** and **consumed** metabolites following chemical exposure using LC-MS





Testing with Reference Compounds

INTERNATIONAL COUNCIL FOR HARMONISATION OF TECHNICAL REQUIREMENTS FOR PHARMACEUTICALS FOR HUMAN USE

DRAFT ICH HARMONISED GUIDELINE

DETECTION OF TOXICITY TO REPRODUCTION FOR HUMAN PHARMACEUTICALS

S5(R3)

Current *Step 2* draft version
dated 5 July 2017

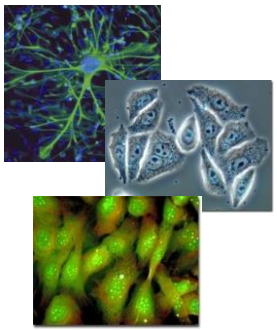
- ICH S5 list used as a starting point to select reference compounds
- Also considered NTP compounds with extensive animal data
- Testing 80 compounds in the stem cell platform (and others to come)

Chemical	CASRN	Category	Positive/Negative (1/0)
Sotalol	3930-20-9	Channel Modulator	1
Hydrochlorothiazide	58-93-5	Channel Modulator	0
Almokalant	123955-10-2	Channel Modulator	1
Chlorthalidone	77-36-1	Channel Modulator	0
Diltiazem	42399-41-7	Channel Modulator	1
Topiramate	97240-79-4	Channel Modulator	1
Trimethadione	127-48-0	Channel Modulator	1
Phenytoin (Diphenylhydantoin)	57-41-0	Channel Modulator	1
Carbamazepine	298-46-4	Channel Modulator	1
Cyclophosphamide	6055-19-2	DNA Modifiers	1
Busulfan	55-98-1	DNA Modifiers	1
Cisplatin	15663-27-1	DNA Modifiers	1
Thiotepa	52-24-4	DNA Modifiers	1
Aspirin	50-78-2	Enzyme Modulator	1
Captopril	62571-86-2	Enzyme Modulator	1
Saxagliptin	361442-04-8	Enzyme Modulator	0
Enalapril	75847-73-3	Enzyme Modulator	1
Vildagliptin	274901-16-5	Enzyme Modulator	0
Methimazole (Thiamazole)	60-56-0	Enzyme Modulator	1
Dexamethasone	50-02-2	Hormone/Steroid	1
Fluticasone	90566-53-3	Hormone/Steroid	1
Progesteorone	57-83-0	Hormone/Steroid	0
Afatinib	850140-72-6	Kinase Modulator	1
Ceritinib	1032900-25-6	Kinase Modulator	1
Dabrafenib	1195765-45-7	Kinase Modulator	1
Dasatinib	302962-49-8	Kinase Modulator	1
Ibrutinib	936563-96-1	Kinase Modulator	1
Pazopanib	444731-52-6	Kinase Modulator	1
Tacrolimus	104987-11-3	Kinase Modulator	1
Imatinib	220127-57-1	Kinase Modulator	1
Cytarabine	147-94-4	Nucleoside Modulator/Central metabolite inhibitor	1
5-Fluorouracil	56177-80-1	Nucleoside Modulator/Central metabolite inhibitor	1
Hydroxyurea	127-07-1	Nucleoside Modulator/Central metabolite inhibitor	1
Methotrexate	59-05-2	Nucleoside Modulator/Central metabolite inhibitor	1
Ribavirin	36791-04-5	Nucleoside Modulator/Central metabolite inhibitor	1
Teriflunomide	163451-81-8	Nucleoside Modulator/Central metabolite inhibitor	1
Warfarin	81-81-2	Nucleoside Modulator/Central metabolite inhibitor	1
Artesunate /amodiaquine	88495-63-0	Other	1
Clarithromycin	81103-11-9	Other	1
Doxycycline	564-25-0	Other	1
Fluconazole	86386-73-4	Other	1
Pomalidomide	19171-19-8	Other	1
Tafamidis	594839-88-0	Other	1
Telavancin	372151-71-8	Other	1
Thalidomide	50-35-1	Other	1
Valproic acid	99-66-1	Other	1
Amoxicillin	26787-78-0	Other	0
Clindamycin	18323-44-9	Other	0
Cyclobenzaprine	6202-23-9	Other	0
Erythromycin	114-07-8	Other	0
Sulfasalazine	599-79-1	Other	0
Bosentan	147536-97-8	Receptor Modulator	1
Clobazam	22316-47-8	Receptor Modulator	1
Fingolimod	162359-55-9	Receptor Modulator	1
Pleriaxfor	110078-46-1	Receptor Modulator	1
Sumatriptan	103628-46-2	Receptor Modulator	1
Cetirizine	83881-52-1	Receptor Modulator	0
Cyproheptadine	129-03-3	Receptor Modulator	0
Doxylamine	562-10-7	Receptor Modulator	0
Maraviroc	376348-65-1	Receptor Modulator	0
Metoclopramide	364-62-5	Receptor Modulator	0
Nizatidine	76963-41-2	Receptor Modulator	0
Theophylline	58-55-9	Second Messenger	1
Acitretin	55079-83-9	Transcription Modulator	1
Isotretinoin (13-cis-retinoic acid)	4759-48-2	Transcription Modulator	1
Vismodegib	879085-55-9	Transcription Modulator	1

Risk Prioritization for DevTox

in vitro DevTox assay(s)

(e.g. AC50s, IC50s)



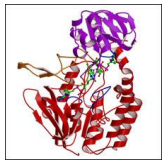
Internal Dose (µM)

(e.g. Plasma or Target-Site Concentrations)

Forward Dosimetry

Reverse Dosimetry

HTTK_PBTK/ Pregnancy Model



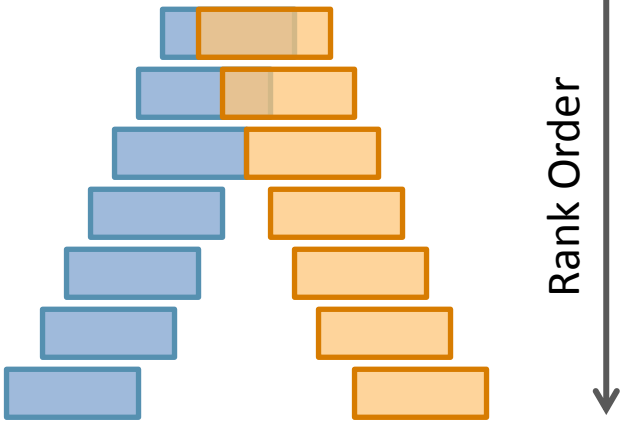
in vitro or *in silico* input parameters (e.g., f_{up}, Cl_{int})

External Dose (mg/kg)

MOE

Risk Prioritization for DevTox

Real-Life Exposure (mg/kg)



Annie Lumen, FDA

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Questions?