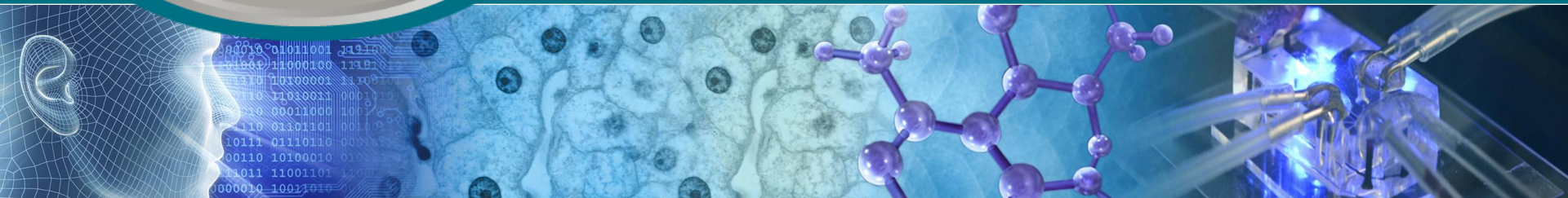




# Interagency Coordinating Committee on the Validation of Alternative Methods

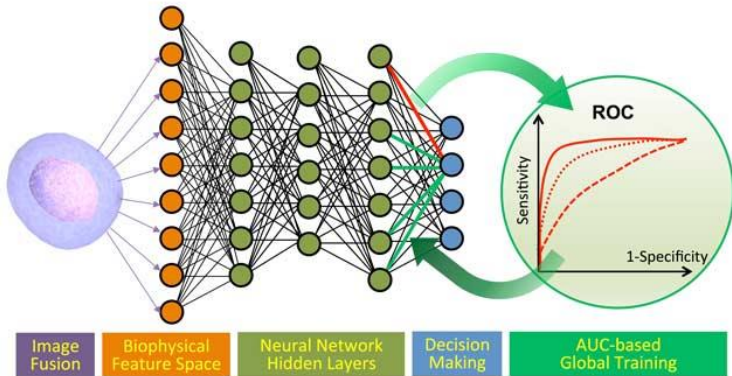
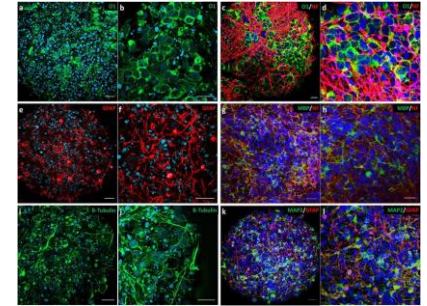
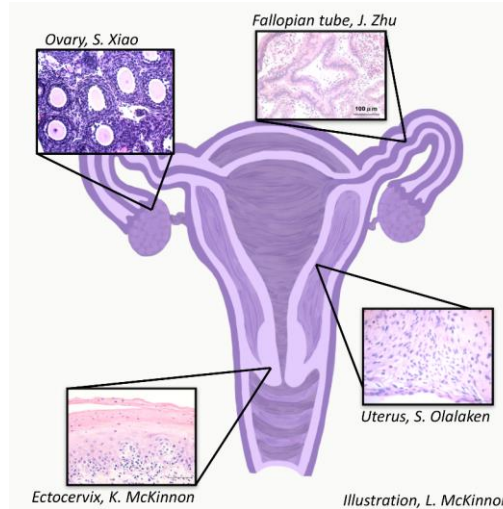


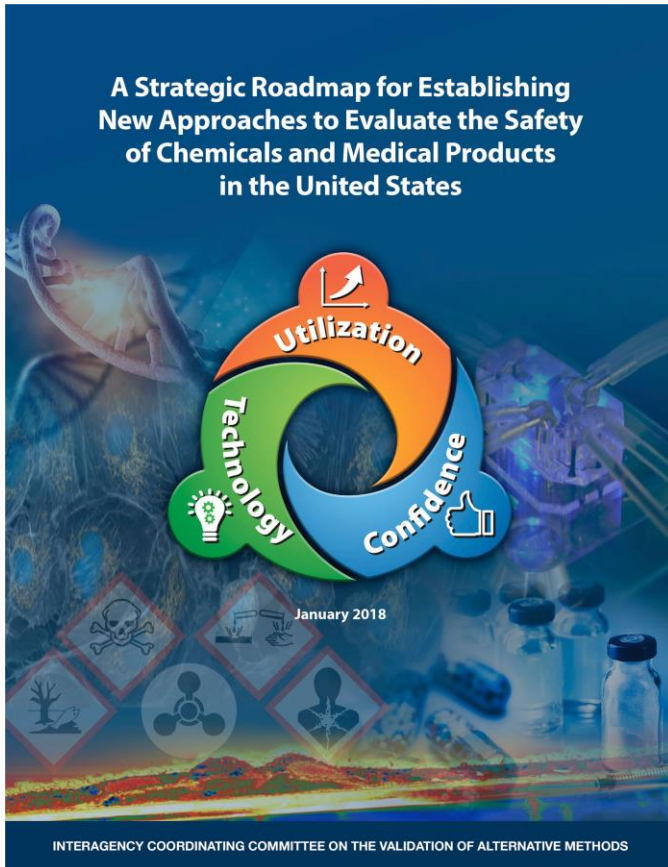
## Computational tools and alternative methods in developmental toxicology

Nicole Kleinstreuer, PhD  
Deputy Director, NICEATM  
14<sup>th</sup> 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

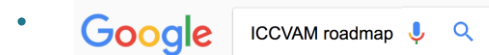
# It is difficult for evolving INSTITUTIONAL PRACTICES to keep pace with revolutionary advances in science and technology

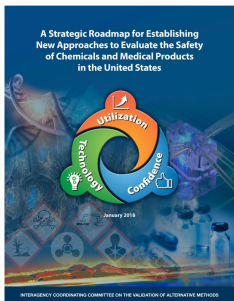




**“Federal agencies and stakeholders will work together to build a new framework to develop, establish confidence in, and encourage use of new approaches to toxicity testing that improve human health relevance and reduce or eliminate the need for testing in animals.”**

- Published Jan 30, 2018
- <https://ntp.niehs.nih.gov/go/natl-strategy>



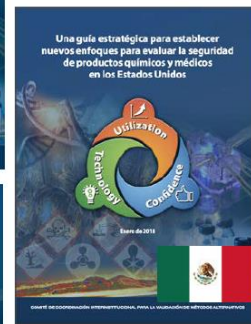


Encourage the adoption and use of new methods and approaches by federal agencies and regulated industries

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



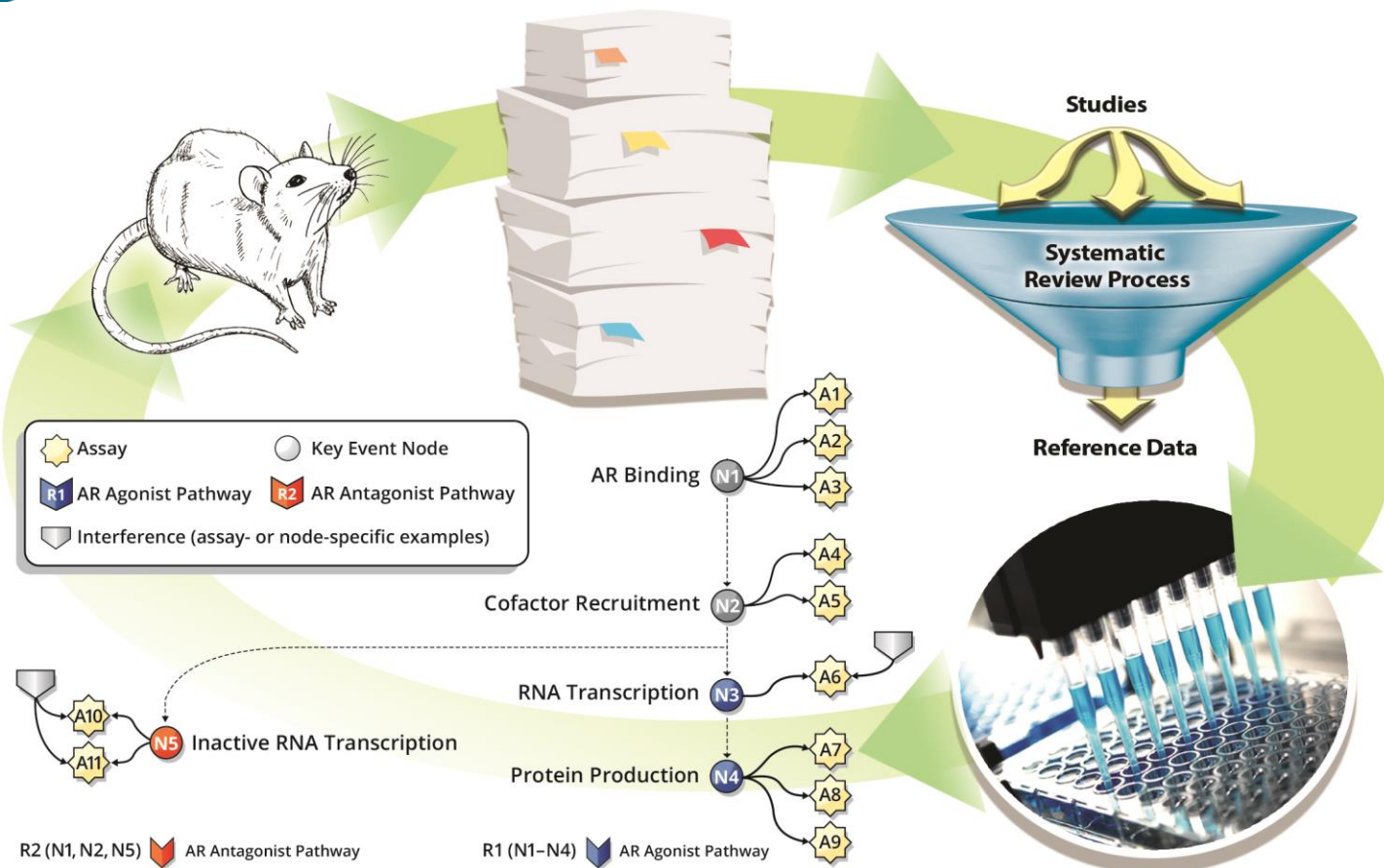
Foster the use of efficient, flexible, and robust practices to establish confidence in new methods





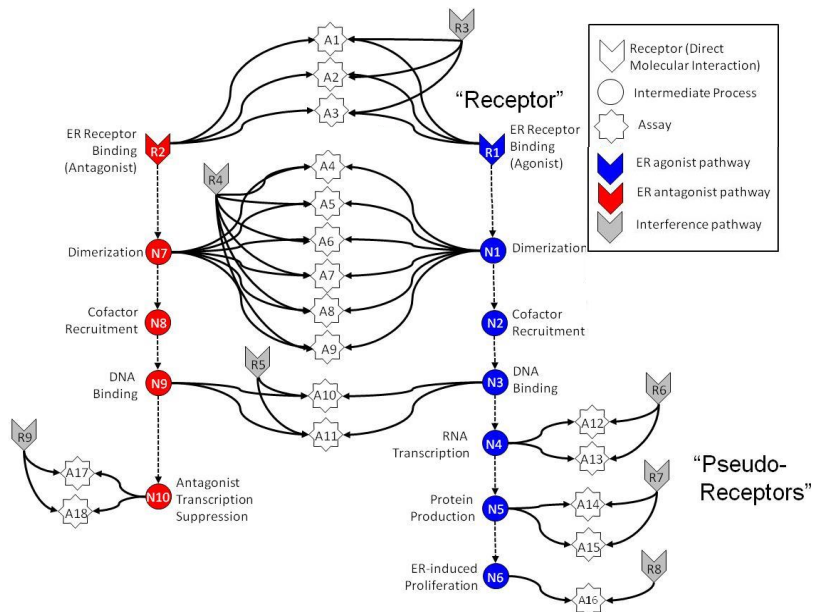
## Federal ICCVAM DART WG: Scope and Charge

- 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



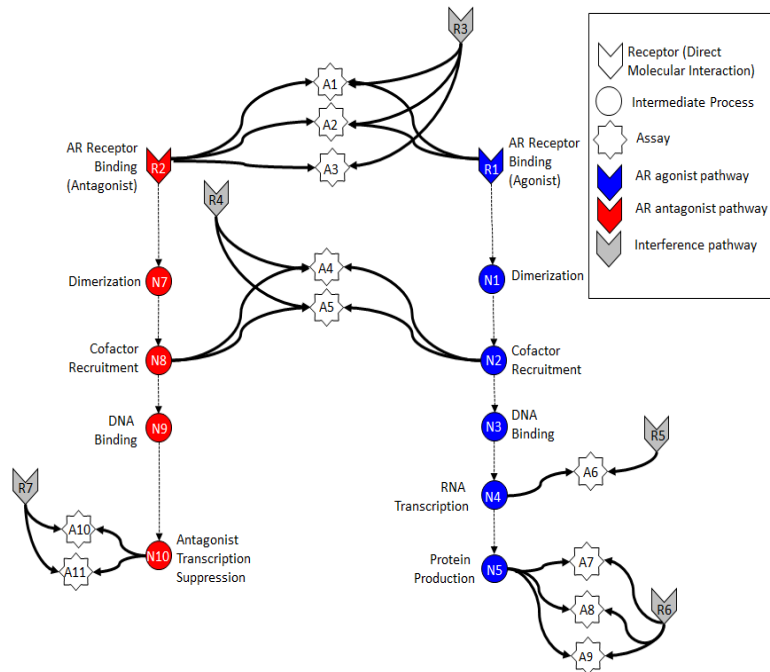
# Tox21/ToxCast Endocrine Pathway Models

## ER Pathway Model



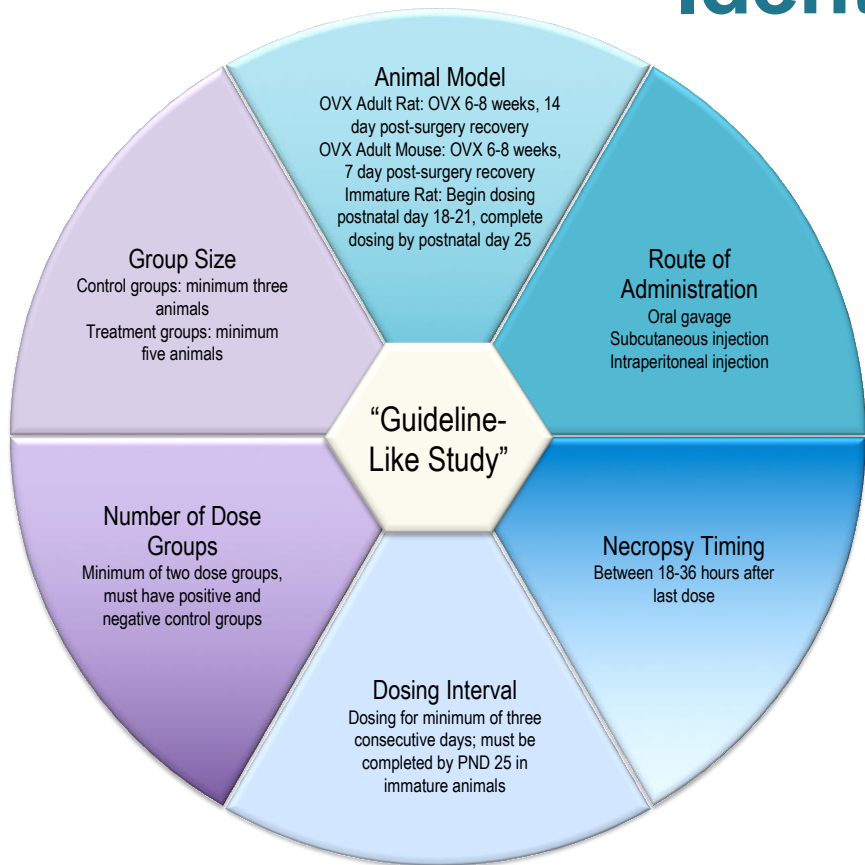
Judson et al *Toxicol. Sci.* (2015)

## 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  $\geq 2$  independent studies
  - Inactive chemicals verified in  $\geq 2$  independent studies (with no positive results in any study)



# 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*)

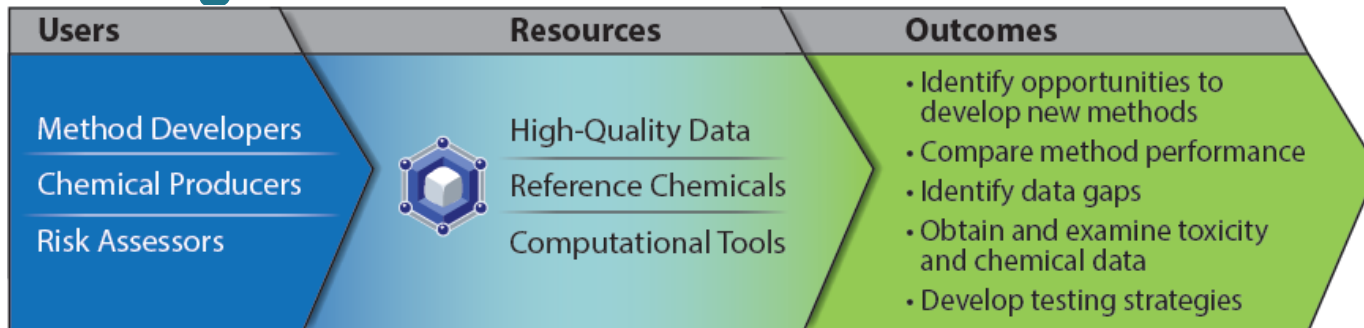
## ER Agonist

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



- 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

*Bell et al. 2017 EHP*

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

- Workflows:

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

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	68-28-8	DTXSID3003550		Inactive

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**

### Endocrine Call Breakdown

Model	Active	Inactive	Inconclusive	Total
ER Pathway Model, Agonist	92	1409	311	1812
ER Pathway Model, Antagonist	18	1643	151	1812
AR Pathway Model, Agonist	33	1751	71	1855
AR Pathway Model, Antagonist	160	1442	253	1855

■ active   
 ■ inactive   
 ■ inconclusive

# Global QSAR Modeling Collaboration

## CERAPP

Collaborative **E**strogen Receptor  
Activity **P**rediction **P**roject

*Mansouri et al. EHP (2017)*

## CoMPARA

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

*Mansouri et al. in prep (2018)*

# Global QSAR Modeling Collaboration

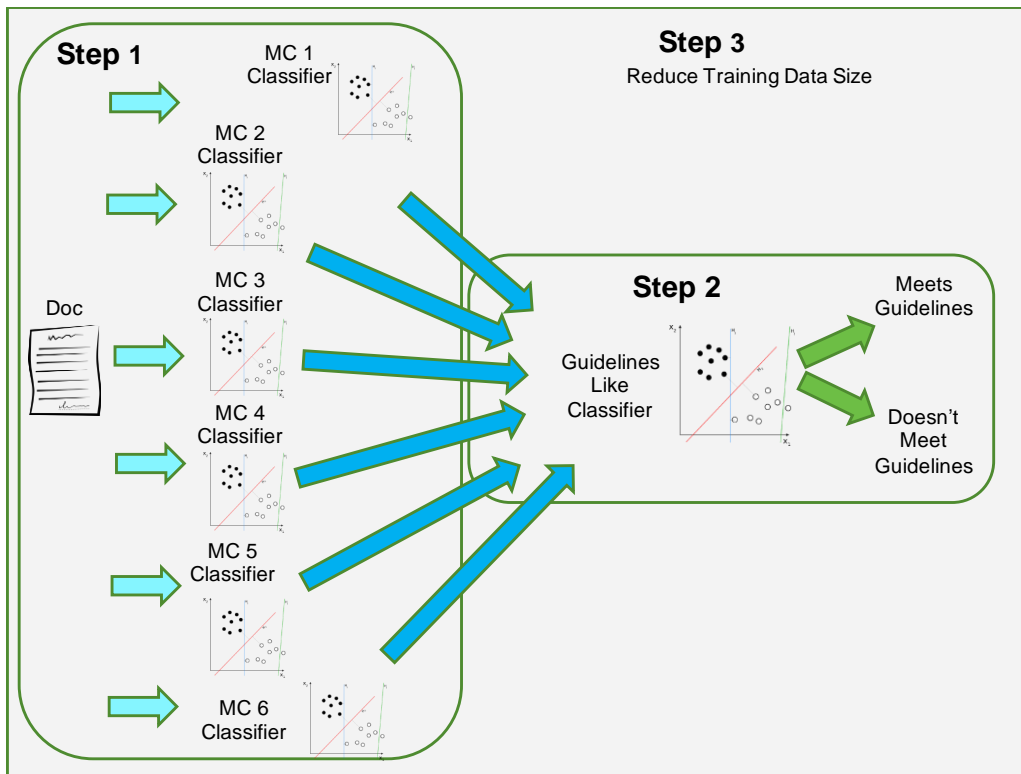


<https://github.com/kmansouri/OPERA>

OPERA is a suite of property predictions from the National Center for Computational Toxicology at the US Environmental Protection Agency. OPERA was derived from curated data (An automated curation procedure for addressing chemical errors and inconsistencies in public datasets used in QSAR modelling).

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

# Automating Reference Data Identification

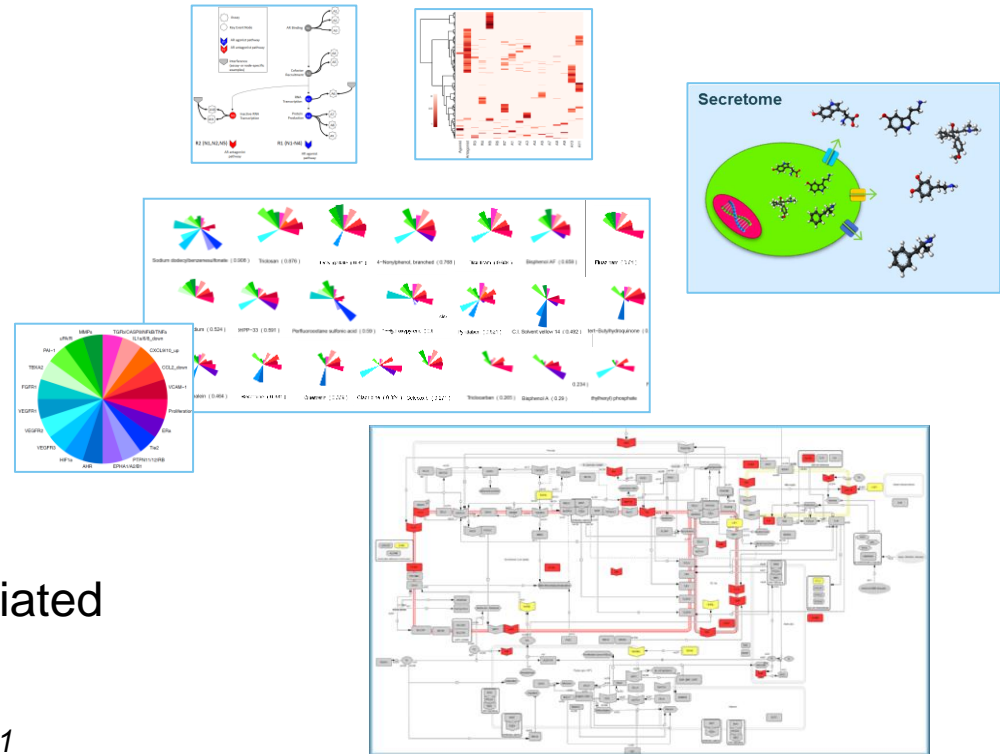


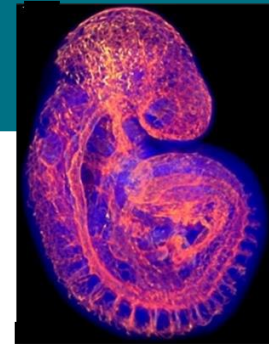
- Project with Oak Ridge National Labs (ORNL) to apply text-mining (NLP) approaches & ML to identify high-quality data
- Semi-automated retrieval and evaluation of published literature (trained on uterotrophic database)
- Apply to developmental toxicity studies

# 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





# 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>

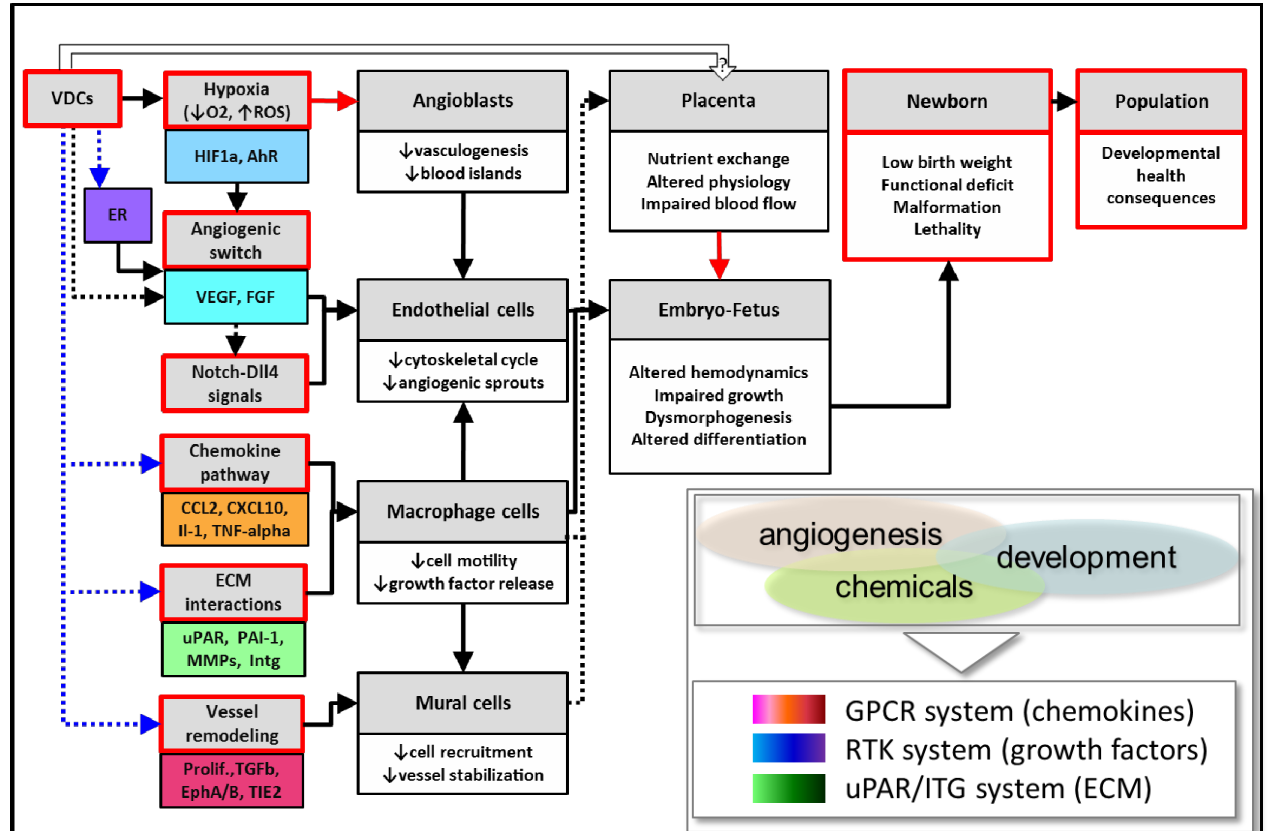
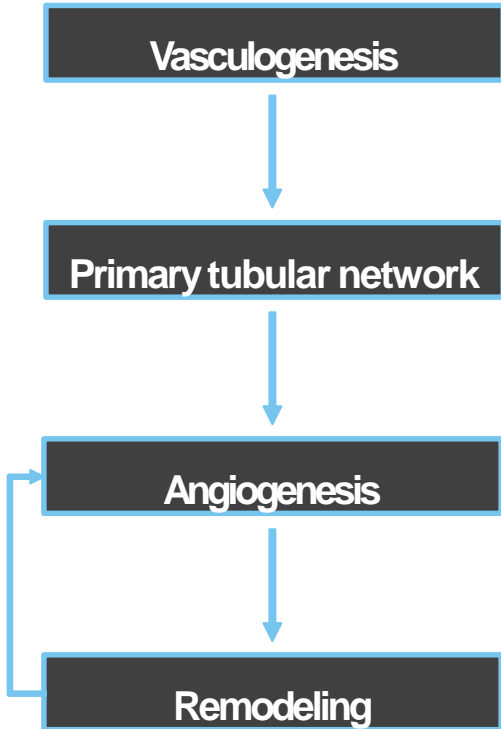
Welcome to the Collaborative Adverse Outcome Pathway Wiki (AOP-Wiki)

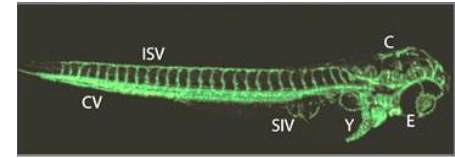
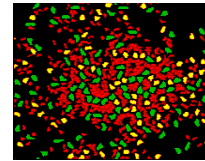
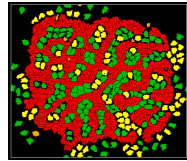
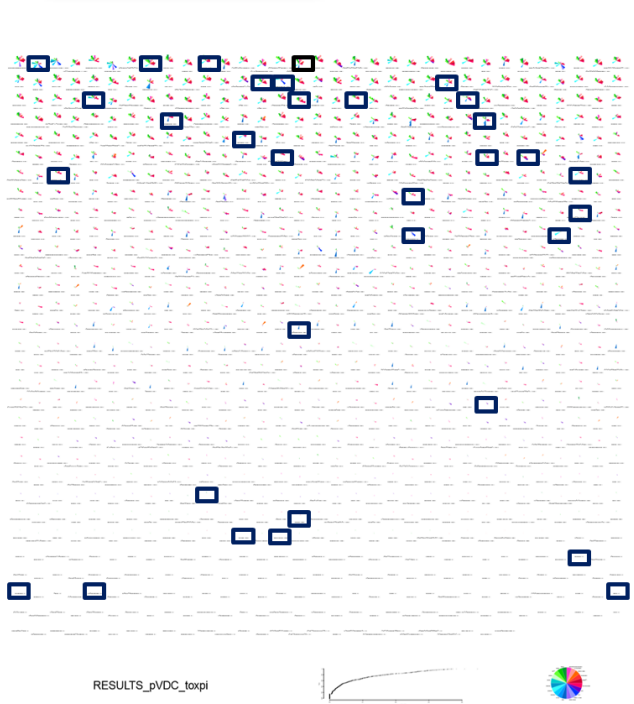
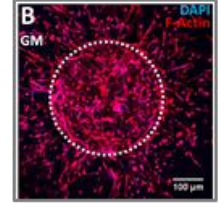
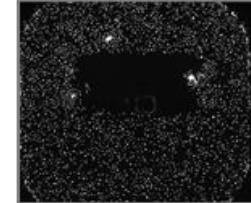
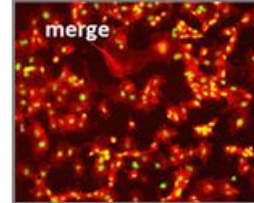
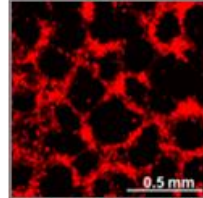
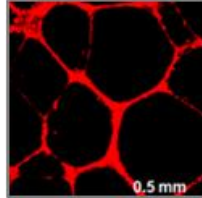
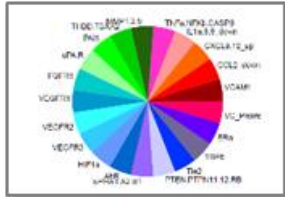


Aop: 43  
**Disruption of VEGFR Signaling Leading to  
Developmental Defects**

Short name:  
Developmental Vascular Toxicity







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

- Virtual vascular plexus simulation [*Kleinstreuer et al. (2013) PLoS Comp Bio*]
- 3D angiogenic sprouting [*Belair et al. (2016) Acta Biomater*]
- 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*]

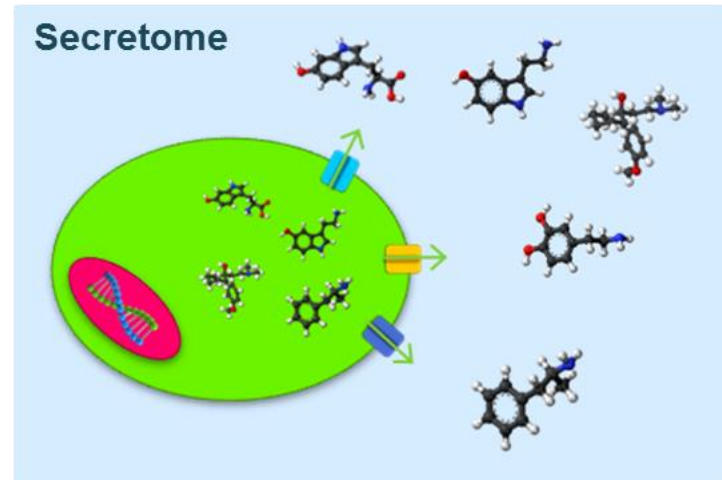
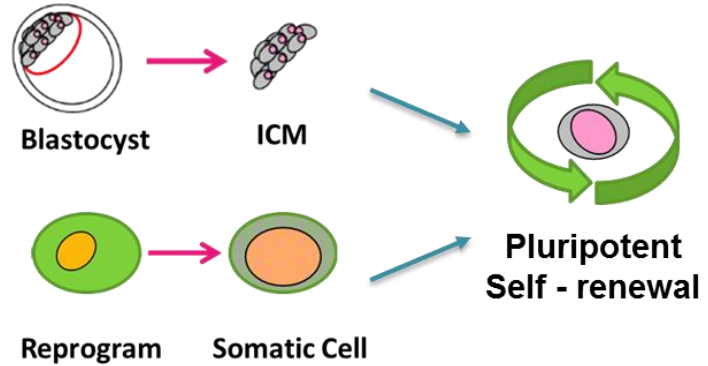
	ToxCast pVDC	FICAM	tubulogenesis synthetic	tubulogenesis Matrigel	tubulogenesis nuCTNB	EC Migration	Sprouting UWIsc	ZF-TG embryo	ZF hyaloid	VALA	tubulogenesis ANY
Decane	0	0	0	0	0	0	0	0	0	0	0
1,2,3-Trichloropropane	0	0	0	0	0	0	0	0	0	0	0
Pymetrozine	0	0	0	0	0	0	0	0	0	0	0
Methimazole	0	0	0	0	0	0	0	0	0	0	0
Imazamox	0	0	0	0	0	0	0	0	0	0	0
D-Mannitol	0	0	0	0	0	0	0	0	0	0	0
Methylparaben	0	0	0	0	0	0	0	0	0	0	0
Valproic acid	0	0	0	0	0	0	0	0	0	0	0
Tris(2-ethylhexyl) phosphate	0	0	0	0	0	0	0	0	0	0	0
PFOS	0	0	0	0	0	0	0	0	0	0	0
1,2,4-Trichlorobenzene	0	0	0	0	0	0	0	0	0	0	0
TNP-470	1	1	1	1	1	1	1	1	1	1	1
Reserpine	1	1	1	1	1	1	1	1	1	1	1
Sodium dodecylbenzenesulfonate	1	1	1	1	1	1	1	1	1	1	1
4-Nonylphenol, branched	1	1	1	1	1	1	1	1	1	1	1
Tris(2-chloroethyl) phosphate	1	1	1	1	1	1	1	1	1	1	1
2,4-Diaminotoluene	1	1	1	1	1	1	1	1	1	1	1
Tris(1,3-dichloro-2-propyl)phosphate	1	1	1	1	1	1	1	1	1	1	1
Oxytetracycline dihydrate	1	1	1	1	1	1	1	1	1	1	1
Celecoxib	1	1	1	1	1	1	1	1	1	1	1
Quercetin	1	1	1	1	1	1	1	1	1	1	1
C.I. Solvent Yellow 14	1	1	1	1	1	1	1	1	1	1	1
Triclosan	1	1	1	1	1	1	1	1	1	1	1
Bisphenol AF	1	1	1	1	1	1	1	1	1	1	1
Docusate sodium	1	1	1	1	1	1	1	1	1	1	1
tert-Butylhydroquinone	1	1	1	1	1	1	1	1	1	1	1
Haloperidol	1	1	1	1	1	1	1	1	1	1	1
Cladribine	1	1	1	1	1	1	1	1	1	1	1
Triclocarban	1	1	1	1	1	1	1	1	1	1	1
Pyridaben	1	1	1	1	1	1	1	1	1	1	1
1-Hydroxypyrene	1	1	1	1	1	1	1	1	1	1	1
Disulfiram	1	1	1	1	1	1	1	1	1	1	1
Fluazinam	1	1	1	1	1	1	1	1	1	1	1
Bisphenol A	1	1	1	1	1	1	1	1	1	1	1
Phenolphthalein	1	1	1	1	1	1	1	1	1	1	1
Octylgallate	1	1	1	1	1	1	1	1	1	1	1
SHPP-33	1	1	1	1	1	1	1	1	1	1	1

### 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)

# 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

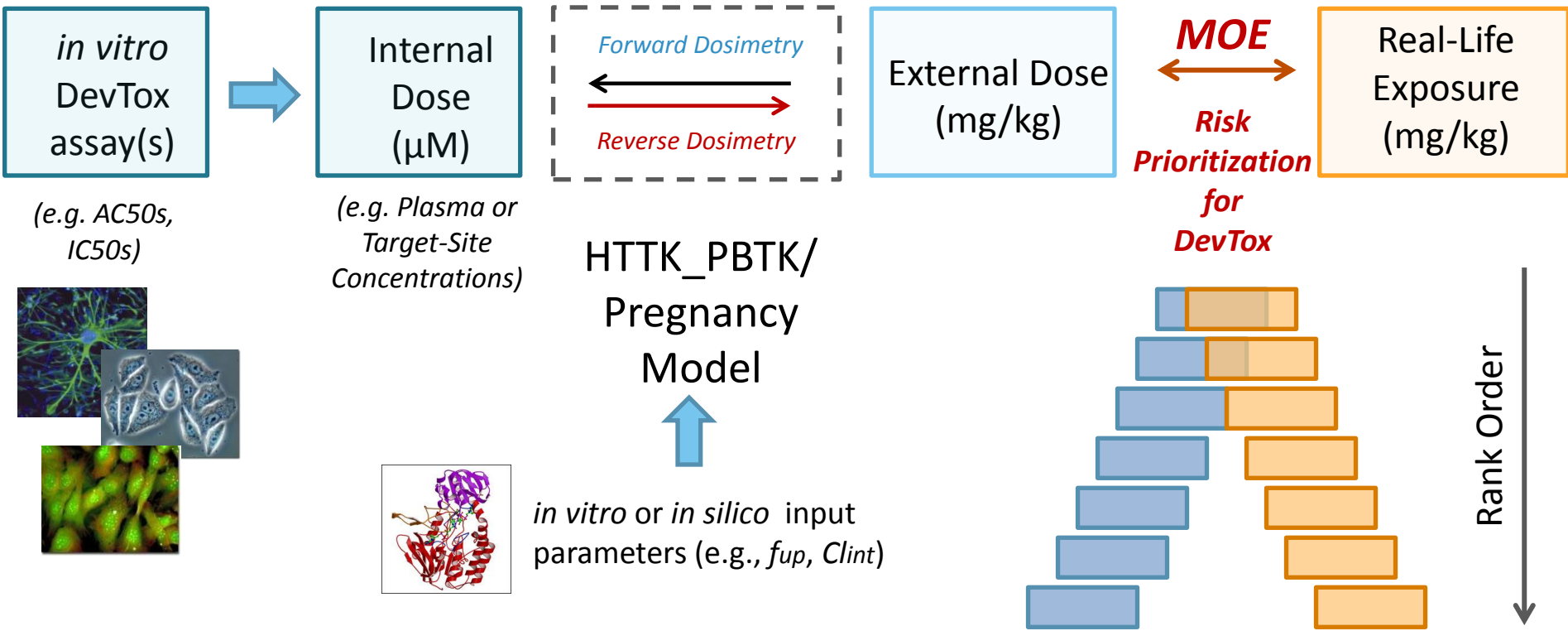
S5(R3)

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 (L/R)
Sotalol	3930-20-9	Channel Modulator	1
Hydrochlorothiazide	58-93-5	Channel Modulator	0
Almokalant	12895-10-2	Channel Modulator	1
Chlorbutolone	77-36-1	Channel Modulator	0
Diltiazem	42399-41-7	Channel Modulator	1
Topiramate	97240-79-4	Channel Modulator	1
Trimetadione	127-48-0	Channel Modulator	1
Phenytol (Diphenylhydantoin)	57-41-0	Channel Modulator	1
Carbamazepine	298-46-4	Channel Modulator	1
Cyclophosphamide	6055-19-3	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	36142-04-8	Enzyme Modulator	0
Enalapril	75847-79-3	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	90565-93-3	Hormone/Steroid	1
Progesterone	57-83-0	Hormone/Steroid	0
Afinib	850140-72-6	Kinase Modulator	1
Centinib	102300-25-6	Kinase Modulator	1
Dabrafenib	1195765-45-7	Kinase Modulator	1
Dasatinib	302962-49-8	Kinase Modulator	1
Icotinib	396049-46-1	Kinase Modulator	1
Pazopanib	444731-53-6	Kinase Modulator	1
Tacrolimus	104987-11-3	Kinase Modulator	1
Imatinib	22027-57-1	Kinase Modulator	1
Cytarabine	147-94-4	Nucleoside Modulator/Central metabolite inhibitor	1
5-Fluorouracil	56377-80-1	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	38794-04-5	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
Amisulpride (Amisulprazine)	8885-63-0	Other	1
Clarithromycin	81103-11-9	Other	1
Doxycycline	564-25-0	Other	1
Fluconazole	86369-73-4	Other	1
Pamidolamide	19121-94-8	Other	1
Tafamidis	594839-88-0	Other	1
Telavancin	372151-71-8	Other	1
Thalidomide	50-15-1	Other	1
Valproic acid	99-66-1	Other	1
Amoxicillin	26787-78-0	Other	0
Clopidogrel	18523-44-9	Other	0
Cyclosporine	6202-23-9	Other	0
Erythromycin	114-07-8	Other	0
Sulfasalazine	599-79-3	Other	0
Boceprevir	147536-07-8	Receptor Modulator	1
Clozapem	22116-47-8	Receptor Modulator	1
Fingolimod	162359-55-9	Receptor Modulator	1
Pleiotar	130078-46-1	Receptor Modulator	1
Sumatriptan	10028-46-2	Receptor Modulator	1
Certirazine	83881-152-1	Receptor Modulator	0
Cyproheptadine	129-03-1	Receptor Modulator	0
Dexamethasone	562-10-7	Receptor Modulator	0
Maraviroc	376348-65-1	Receptor Modulator	0
Mefenorexipamide	364-62-5	Receptor Modulator	0
Nizatidine	78563-41-2	Receptor Modulator	0
Theophylline	58-55-9	Second Messenger	1
Acetretin	55079-83-9	Transcription Modulator	1
Isothretinoin (13-cis-retinoic acid)	4729-48-1	Transcription Modulator	1
Vismodegib	87905-55-9	Transcription Modulator	1

# Risk Prioritization for DevTox



# Acknowledgments

- ILS/NICEATM group
- ICCVAM agencies
- Tom Knudsen (EPA/NCCT)
- Patience Browne (OECD)
- Robert Patton (ORNL)
- Annie Lumen (FDA)

Questions?

