NICEATM Update

Warren Casey, PhD, DABT
Director, NICEATM

Division of the National Toxicology Program
National Institute of Environmental Health Sciences

SACATM
September 16, 2014

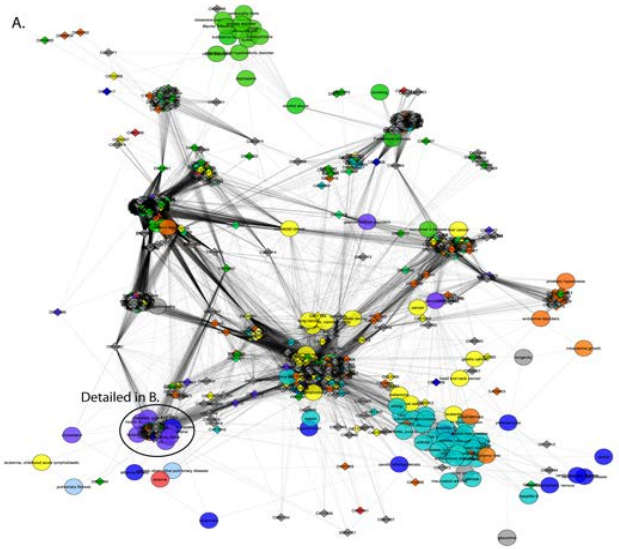
NICEATM

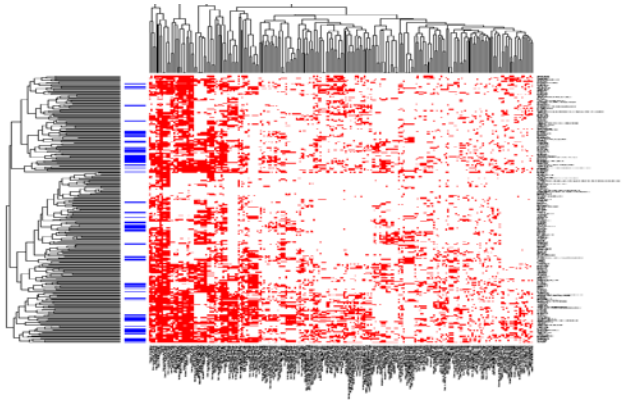
NTP Interagency Center for the Evaluation of Alternative Toxicological Methods (NICEATM), organized as an office under the NTP Division, part of NIEHS

National Toxicology Program
NTP

Tox21 Goals

- Prioritize compounds for more extensive toxicological evaluation
- Develop predictive models for biological response in humans
- Reduce reliance on animal models





Science. 2008 February 15; 319(5865): 906–907. doi:10.1126/science.1154619.

Transforming Environmental Health Protection

Francis S. Collins[1,*,†], **George M. Gray**[2,*], and **John R. Bucher**[3,*]

1*Director, National Human Genome Research Institute (NHGRI), National Institutes of Health, Bethesda, MD 20892*

2*Assistant Administrator for the Office of Research and Development, U.S. Environmental Protection Agency, Washington, DC 20460*

3*Associate Director, U.S. National Toxicology Program, National Institute of Environmental Health Sciences (NIEHS), Research Triangle Park, NC 27709, USA.*

Tox21

NICEATM Focus Areas

- Validation
  - Fit for purpose
  - Retrospective
- Curating high quality reference data
- Analysis and validation of Tox21 HTS Data
- Integrated testing and decision strategies (ITDS)
- *In vitro* to *In vivo* extrapolation (IVIVE)
- Development and validation of QSAR and QSPR models
- Alternative model systems

National Institute of Environmental Health Sciences
Your Environment. Your Health.

Endocrine Disruptors

Four Points about endocrine disruption:

- **Low dose matters**
- **Wide range of health effects**
- **Persistence of biological effects**
- **Ubiquitous exposure**

What are endocrine disruptors?

Endocrine disruptors are naturally occurring compounds or man-made substances that may mimic or interfere with the function of hormones in the body. Endocrine disruptors may turn on, shut off, or modify signals that hormones carry, which may affect the normal functions of tissues and organs. Many of these substances have been linked with developmental, reproductive, neural, immune, and other problems in wildlife and laboratory animals.

Some research suggests that these substances are also adversely affecting human health in similar ways, resulting in reduced fertility and increased incidences or progression of some diseases, including obesity, diabetes, endometriosis, and some cancers.

Collaboration

- NICEATM
- EPA NCCT
- EPA OSCP (responsible for EDSP21)

Objectives

- Characterize the relationship between *in vitro* ER-pathway activity measured using Tox21 HTS assays (human) and outcomes in uterotrophic animal studies (rodent).

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Objectives

- Characterize the relationship between *in vitro* ER-pathway activity measured using Tox21 HTS assays (human) and outcomes in uterotrophic animal studies (rodent).
- Validate a HTS approach incorporating *in silico*, *in vitro*, and alternative animal models that can be used to test 1000's of chemicals in order to prioritize, or possibly exclude from uterotrophic testing

Developing a DB of Uterotrophic Sudies

Literature Searches

- PubMatrix (keyword searches)
- FDA EDKB, other resources

Data Extraction

- Standardized ontology
- Local PDF repository

Data Quality Review

- Minimum study criteria (MC)
- Chemical/protocol/LEL information

Add to Database

- Data classified as reliable used to evaluate *in silico* and HTS results

Study Design Variables (EPA / OECD)

- Choice of Model
  - Ovariectomized Rat
  - Immature Rat
  - Ovariectomized Mouse

- Duration of dosing; minimum of 3 days, maximum varies with model system

- Dosing route: oral gavage, subcutaneous injection, or i.p. injection

> 40 Descriptors captured for each chem-study-protocol combination

Chemical Name
CASRN
PMID
Author
Year
Class
Study_Type
Assay_Type
Species
Strain
Target
Route of Administration
Age at 1st Dose Administration
Age at OVX
Dose/Response (0 no, 1 yes)
# of doses used
Value
Unit_Response
value_type
LEL
Max_Conc_Tested

Elapsed time between OVX and RX
Dosing Length
# of doses per day
# of animals in estrogen control group
# of animals in RX group
Reference Estrogen
Is there a Vehicle/RX control?
Diet
Indicated that Diet is low-PE?
necropsy time after last dose
Additional_Assay_Info
Source_Name_SID
Chemical_Tested
Chemical_Note
Unit_Max
Response
Response_Note
Remove_From_Analysis
EDSP/OECD Guideline

Developing a DB of Uterotrophic Sudies

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1800 Chemicals

1000's of references

Data Extraction

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- Local PDF repository

> 1000 papers

Data Quality Review

- Minimum study criteria (MC)
- Chemical/protocol/LEL information

686 papers

QC'd x2

Add to Database

- Data classified as reliable used to evaluate *in silico* and HTS results

607 papers

215 chemicals

2270 entries

Developing a DB of Uterotrophic Sudies

Literature Searches

- PubMatrix (keyword searches)
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1200 Chemicals

1000's of references

Data Extraction

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> 1000 papers

Data Quality Review

- Minimum study criteria (MC)
- Chemical/protocol/LEL information

686 papers

QC'd x2

Add to Database

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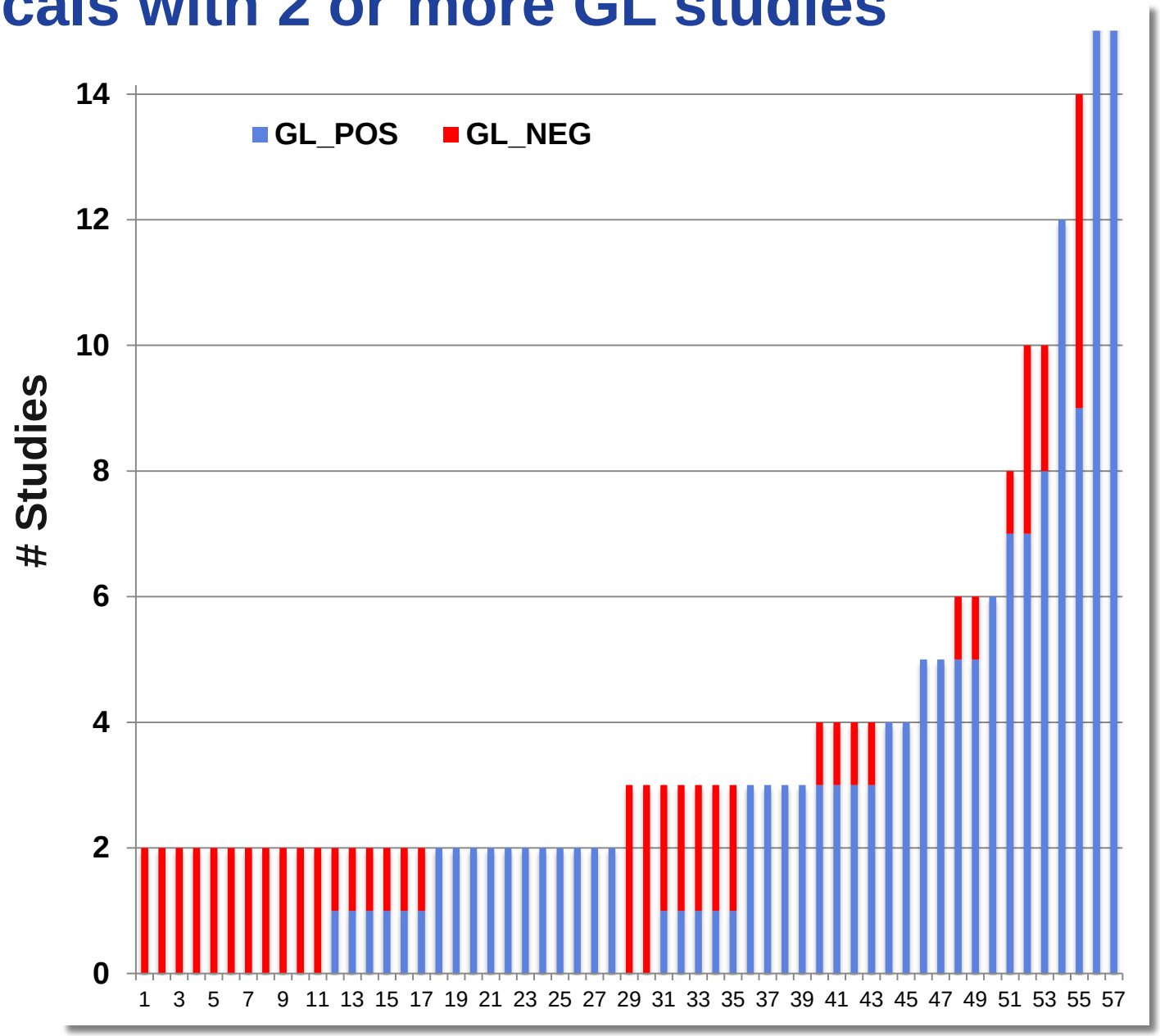
112 Chemicals with guideline-like (GL)studies

Chemicals with 2 or more GL studies



Uterotrophic GL Studies

4-*tert*-octylphenol (CASRN 140-66-9)

PMID	UT-GL	Model	Species	Strain	Doses (mg/kg)	Route	Duration	LEL (mg/kg)
10825675	Pos	Ovx	Rat	Crj:Donryu	6.25, 12.5, 25, 50, 100	SC	14 d	25
10746942	Neg	Immature	Rat	Long Evans	200, 400	Oral	3 d	NA
20391140	Neg	Immature	Rat	SD	125, 250	Oral	3 d	NA

Nonylphenol (CASRN 104-40-5)

PMID	UT-GL	Model	Species	Strain	Doses (mg/kg)	Route	Duration	LEL (mg/kg)
12128099	Pos	Immature	Rat	SD Crl:CD	10, 25, 50, 100, 200	SC	3 d	100
10746942	Pos	Immature	Rat	Long Evans	25, 50, 100, 200	Oral	3 d	50
11750080	Neg	Immature	Rat	SD Crj:CD	2, 20, 200	SC	3 d	NA

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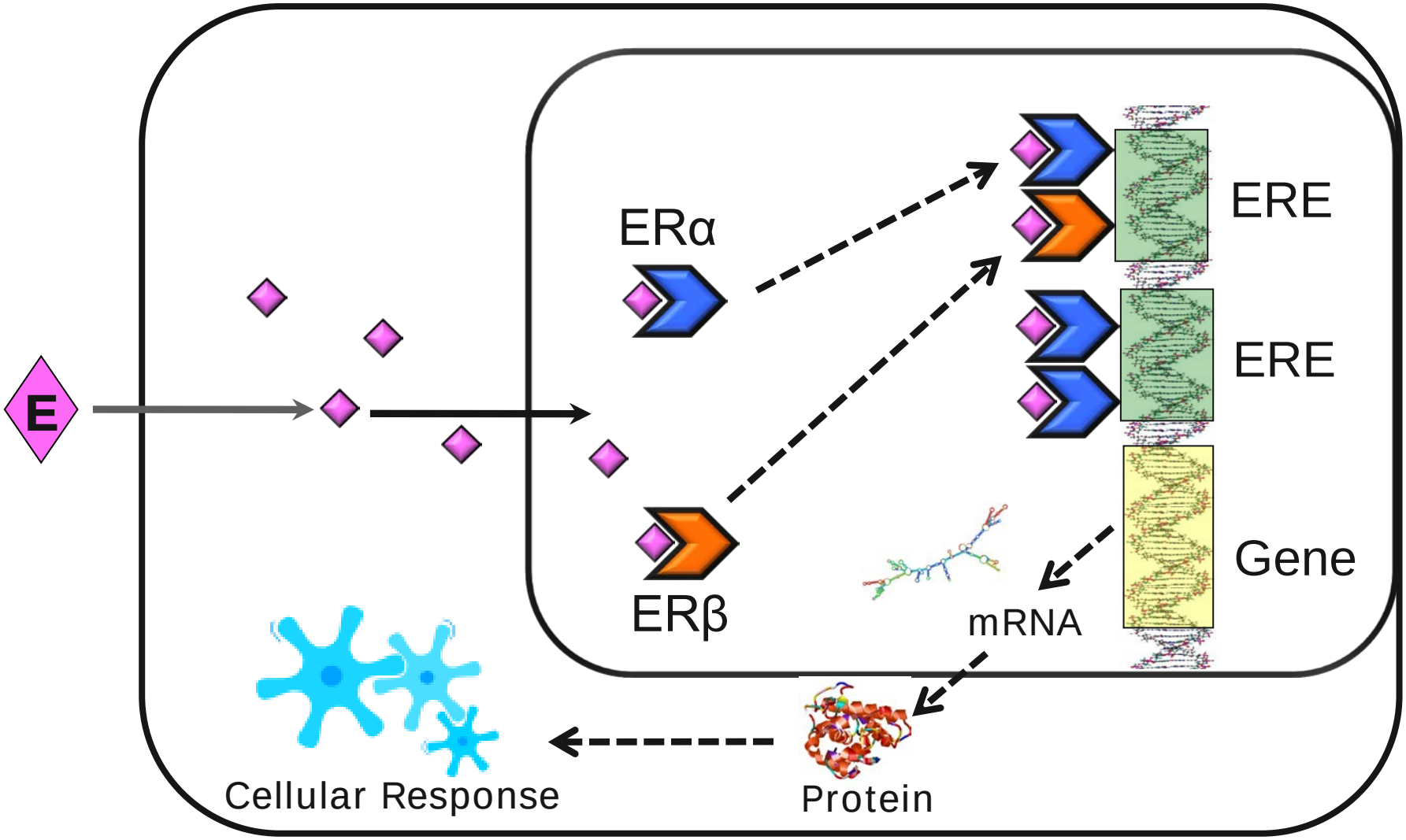
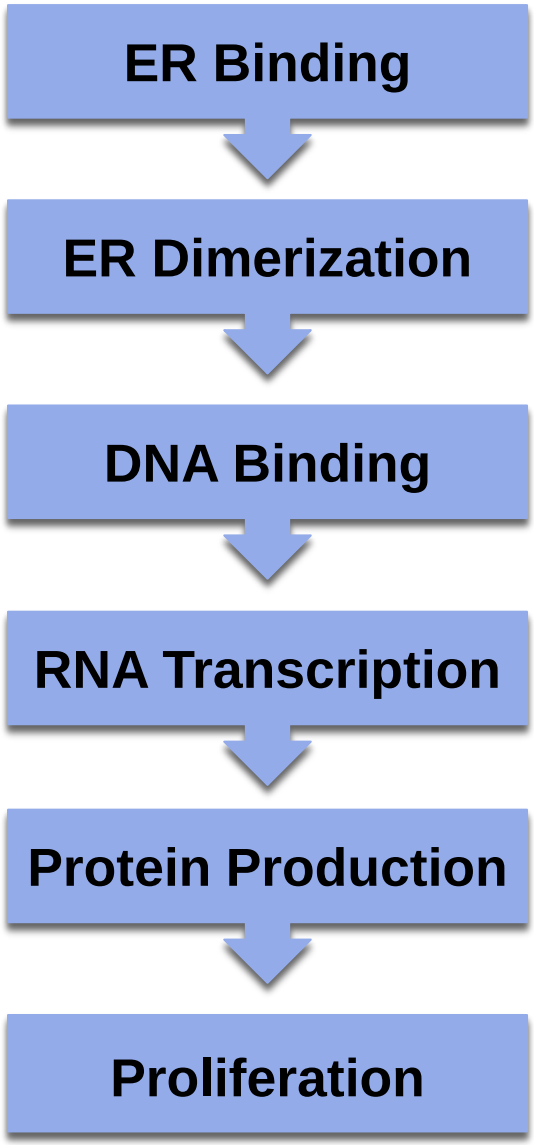
In Vitro Assays



18 ToxCast / Tox21 ER Pathway Assays



(3) Human, Mouse, Bovine

(6) hER-α, hER-β / 8hr, 24 hr

(2) hER-α / 8hr, 24 hr

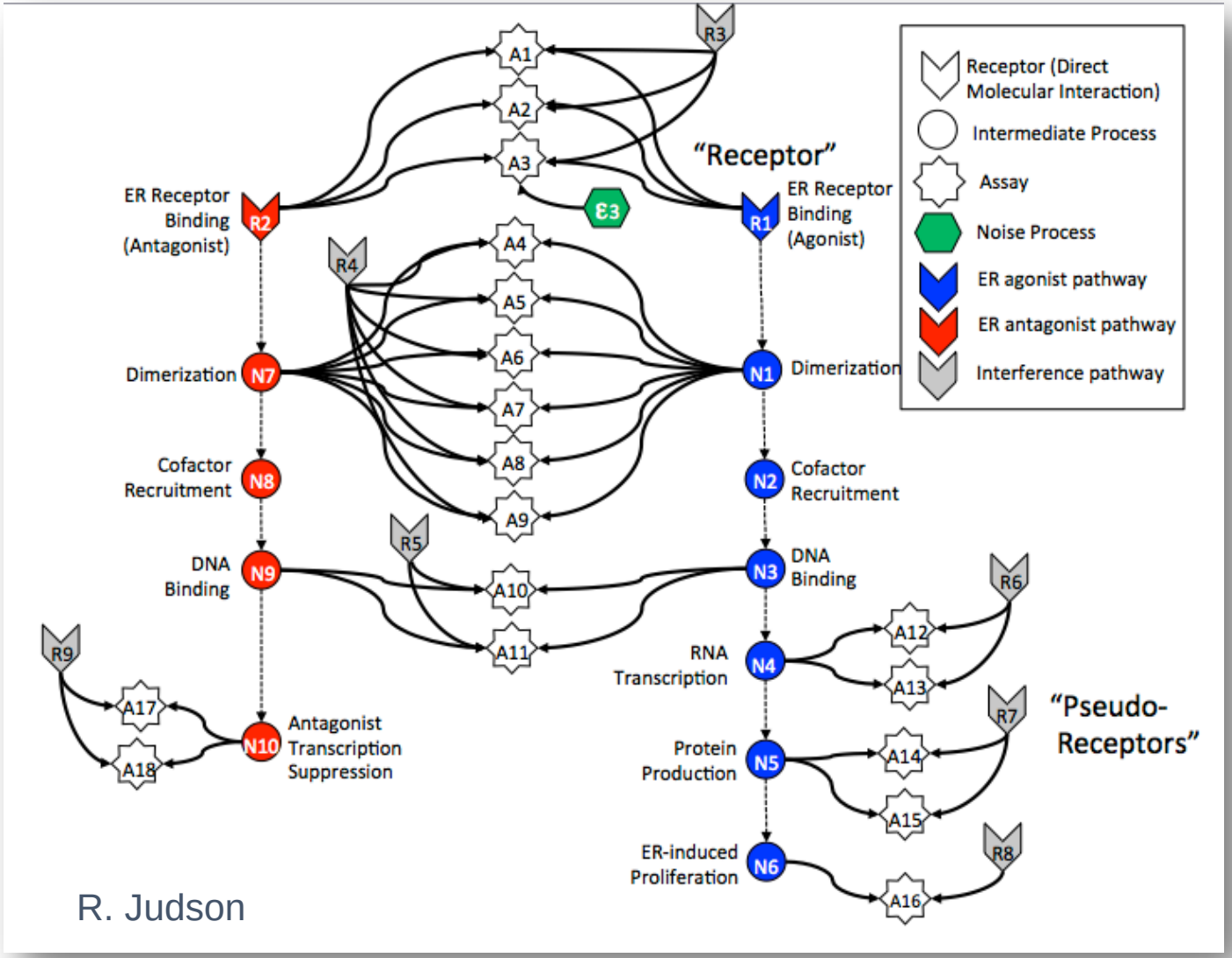
(2) hER-α Trans, ERE Cis

(4) FL hER-α hER-β , LBD hER-α agonist / antagonist mode

(1) T47D proliferation

Rotroff et al., Environ. Sci. Technol. 2014, 48, 8706−8716, doi/10.1021/es502676e

Mathematical Model of ER Pathway, EPA NCCT



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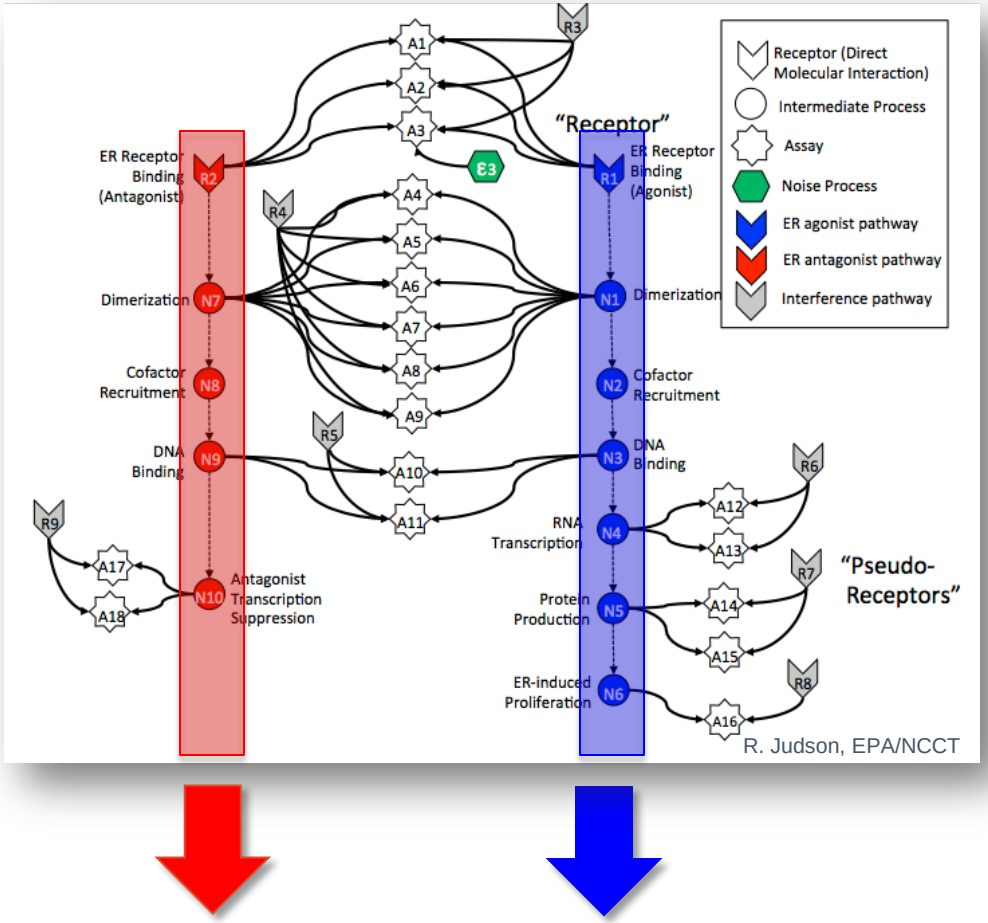
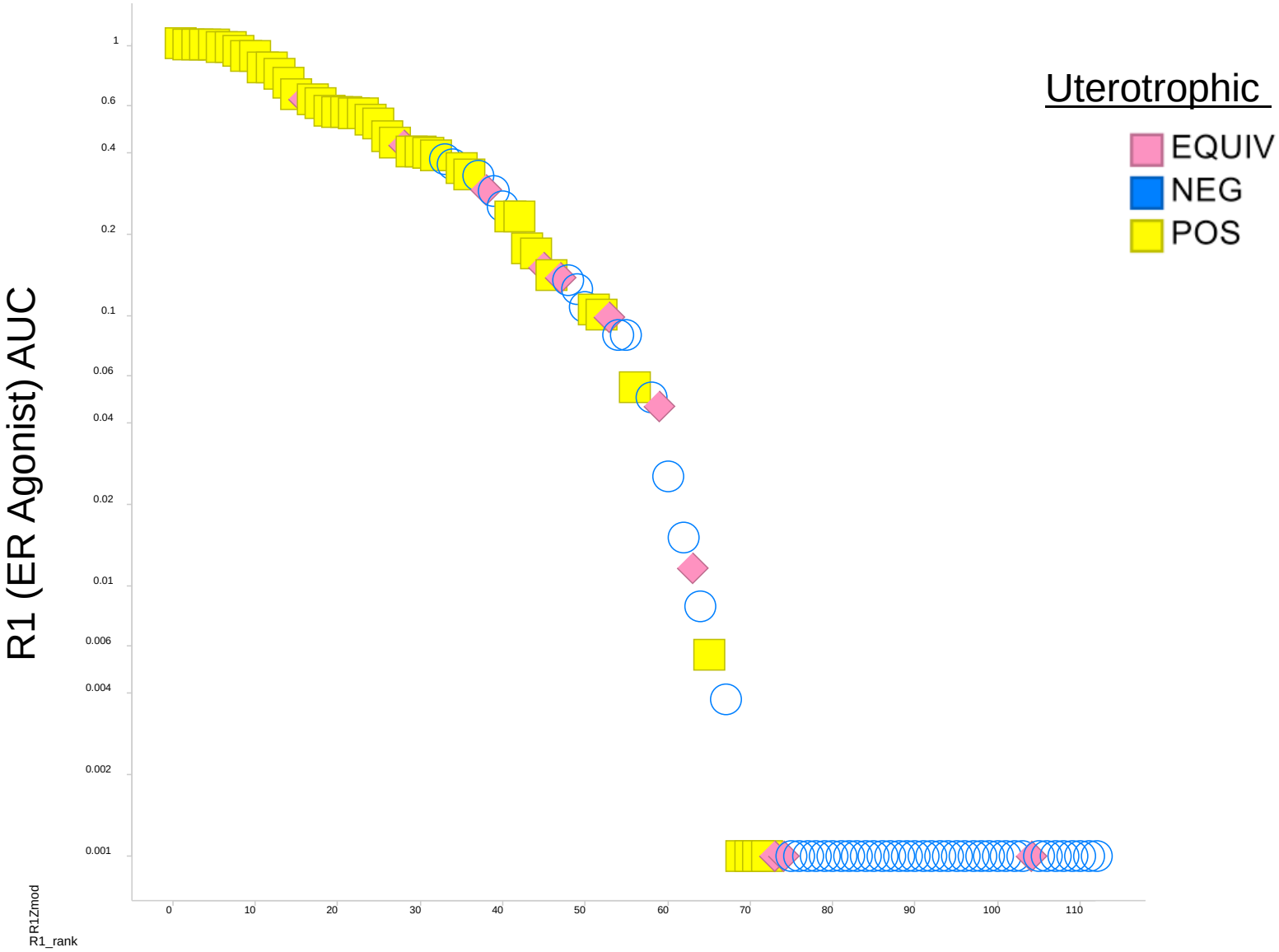


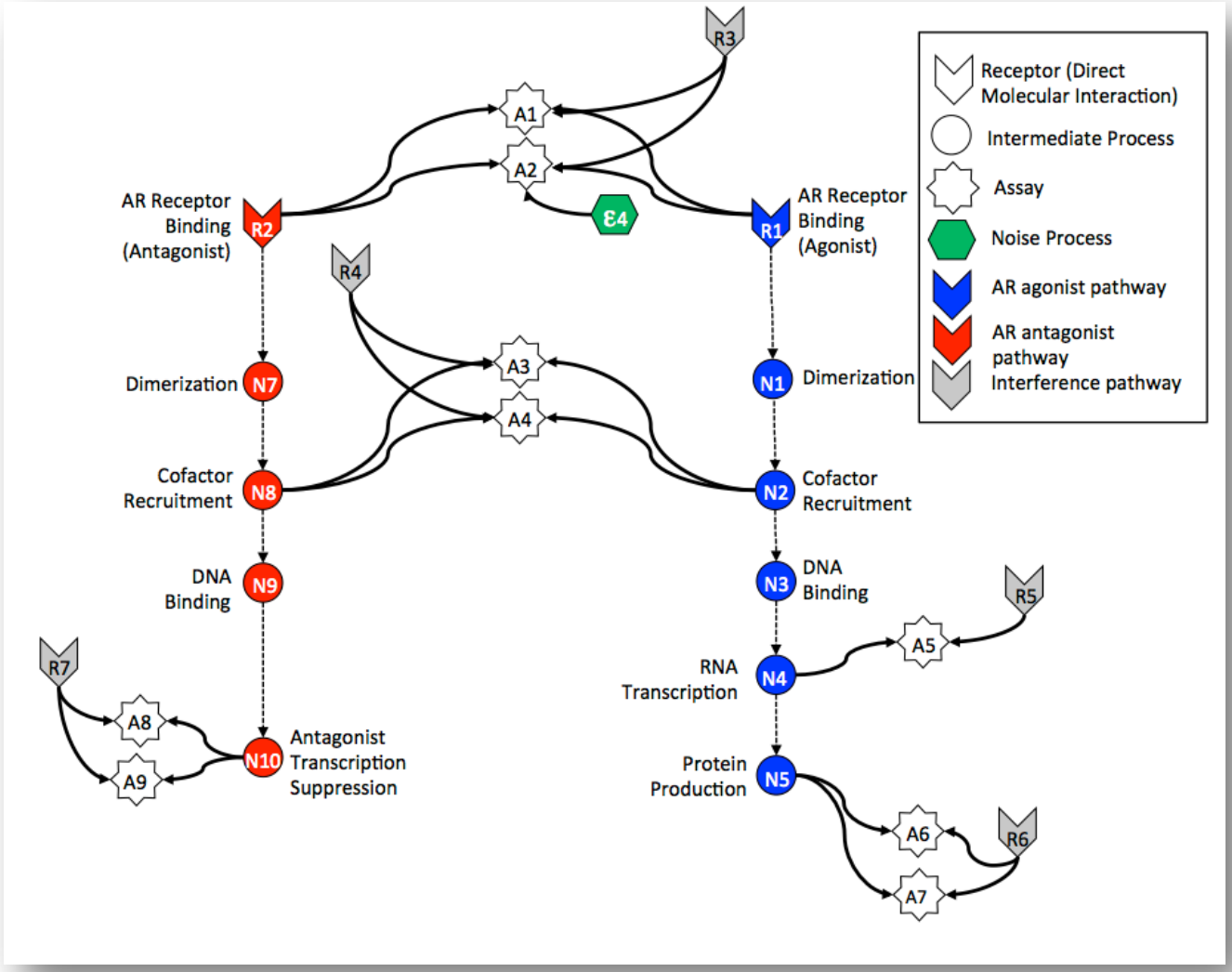
$$AUC_j = \frac{1}{N_{conc}} \sum_{i=1}^{N_{conc}} sign(slope) \times R_j(conc_i)$$

- **AUC summarizes the results from all 18 assays**
- **R (receptor) values range from 0-1**

ER Model Score (Agonist) vs Uterotrophic Outcome



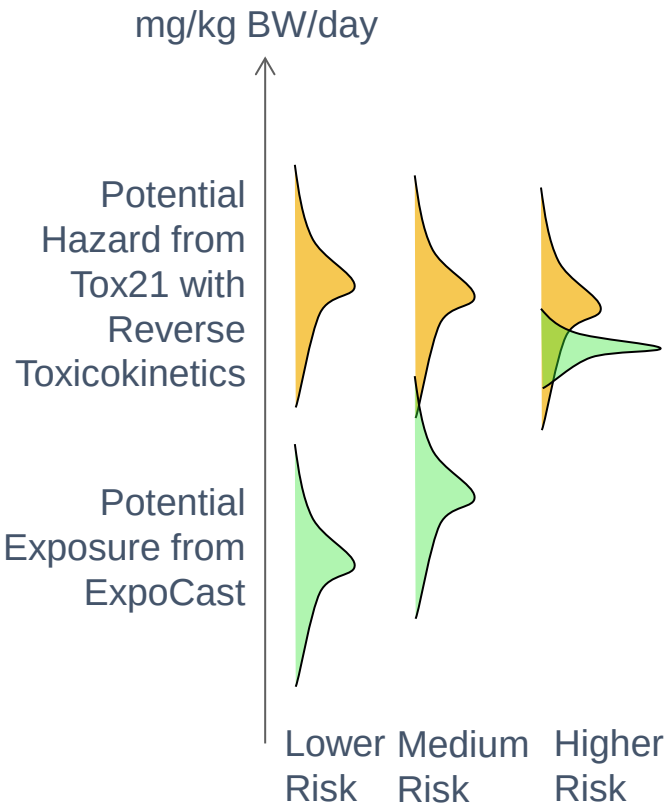
AR Pathway model, NICEATM



Data from 9 ToxCast / Tox21 Assays used to generate a model score and rank compounds

Assessing the Hazard Component of Risk

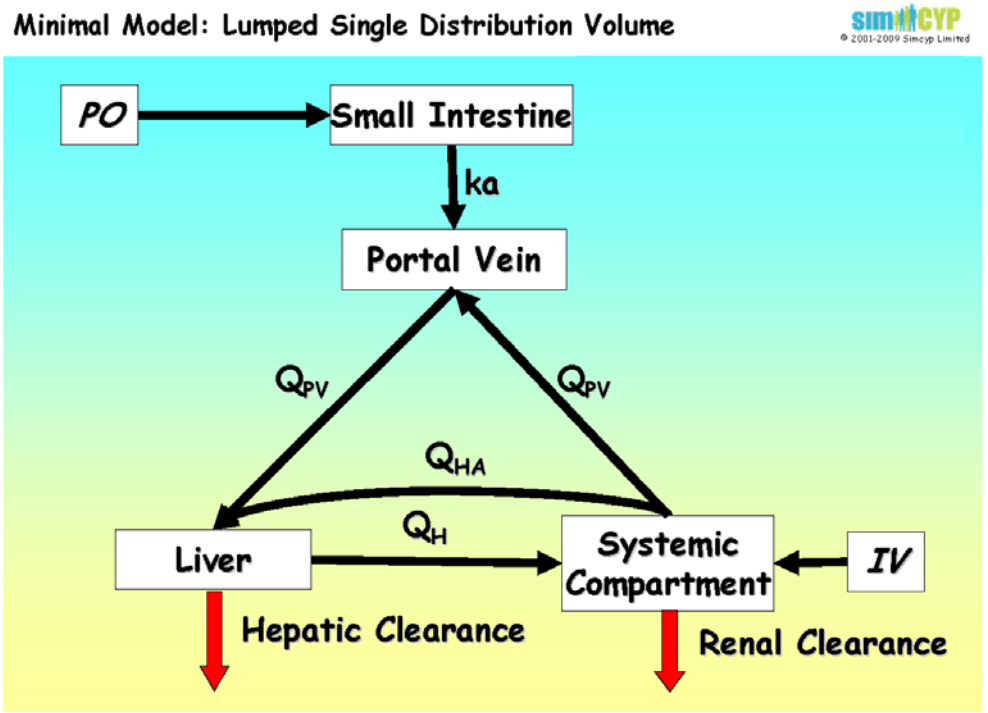
- Risk is the product of hazard and exposure
- Ultimately hope to do a rapid risk prioritization of chemicals with minimal information
- Identify chemicals most in need of additional resources and traditional methodologies



J.Wambaugh, EPA NCCT

Steady-State Plasma Concentrations

- Successful methods have been developed for pharmaceutical compounds to determine high throughput TK (HTTK) from limited in vitro measurements and chemical structure-derived property predictions
- ***In vitro* plasma protein binding and metabolic clearance assays allow approximate hepatic and renal clearances to be calculated**
- At steady state this allows conversion from concentration to administered dose
- No oral absorption/ bioavailability included

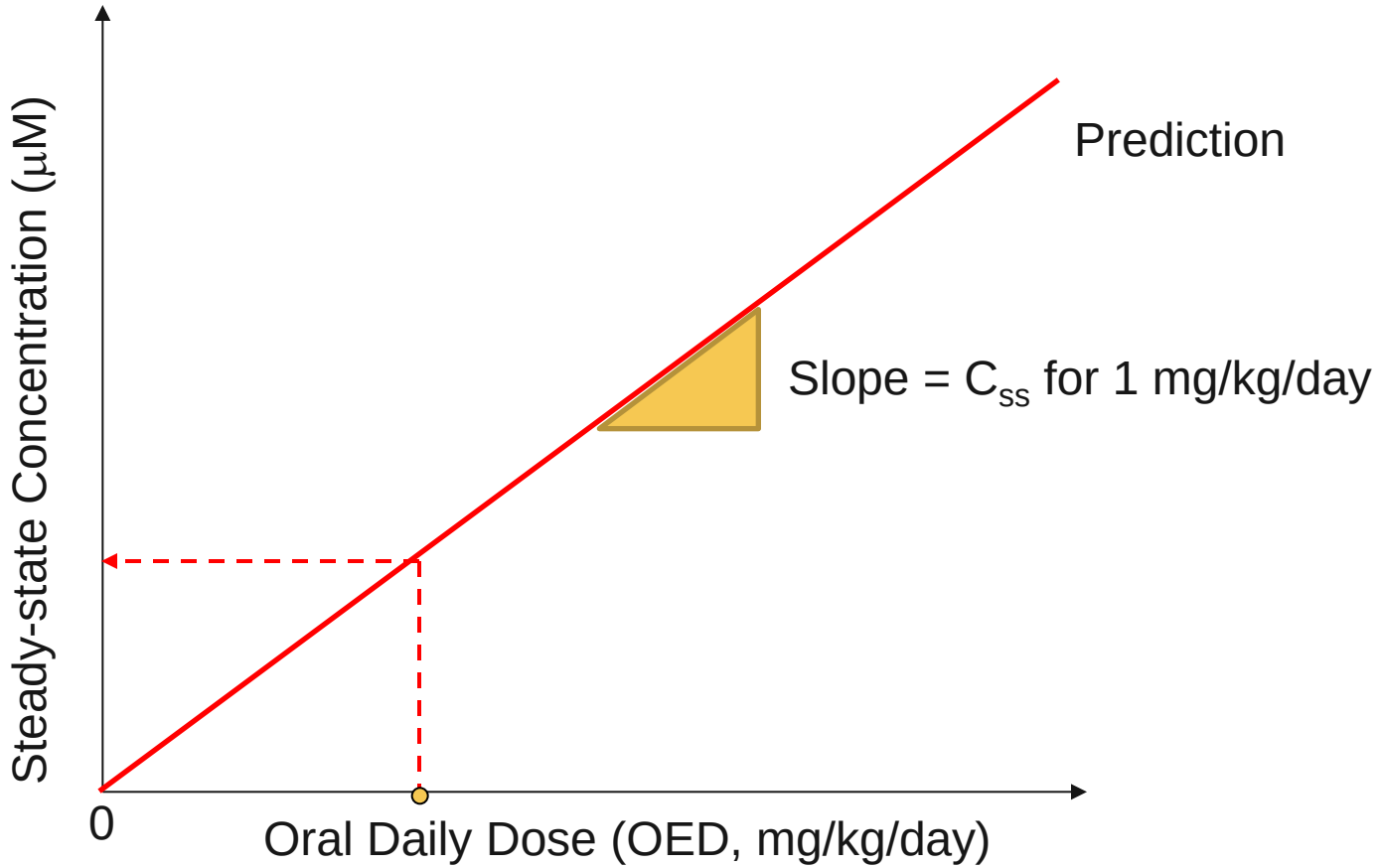


$$C_{ss} = \frac{\text{oral dose rate}}{(\text{GFR} * \text{F}_{ub}) + \left(\text{Q}_{l} * \text{F}_{ub} * \frac{Cl_{int}}{\text{Q}_{l} + \text{F}_{ub} * Cl_{int}}\right)}$$

Oral dose in (mg/kg/day) → Sum of hepatic and renal clearance (mg/kg/day)

J.Wambaugh, EPA NCCT

Steady-State Model is Linear

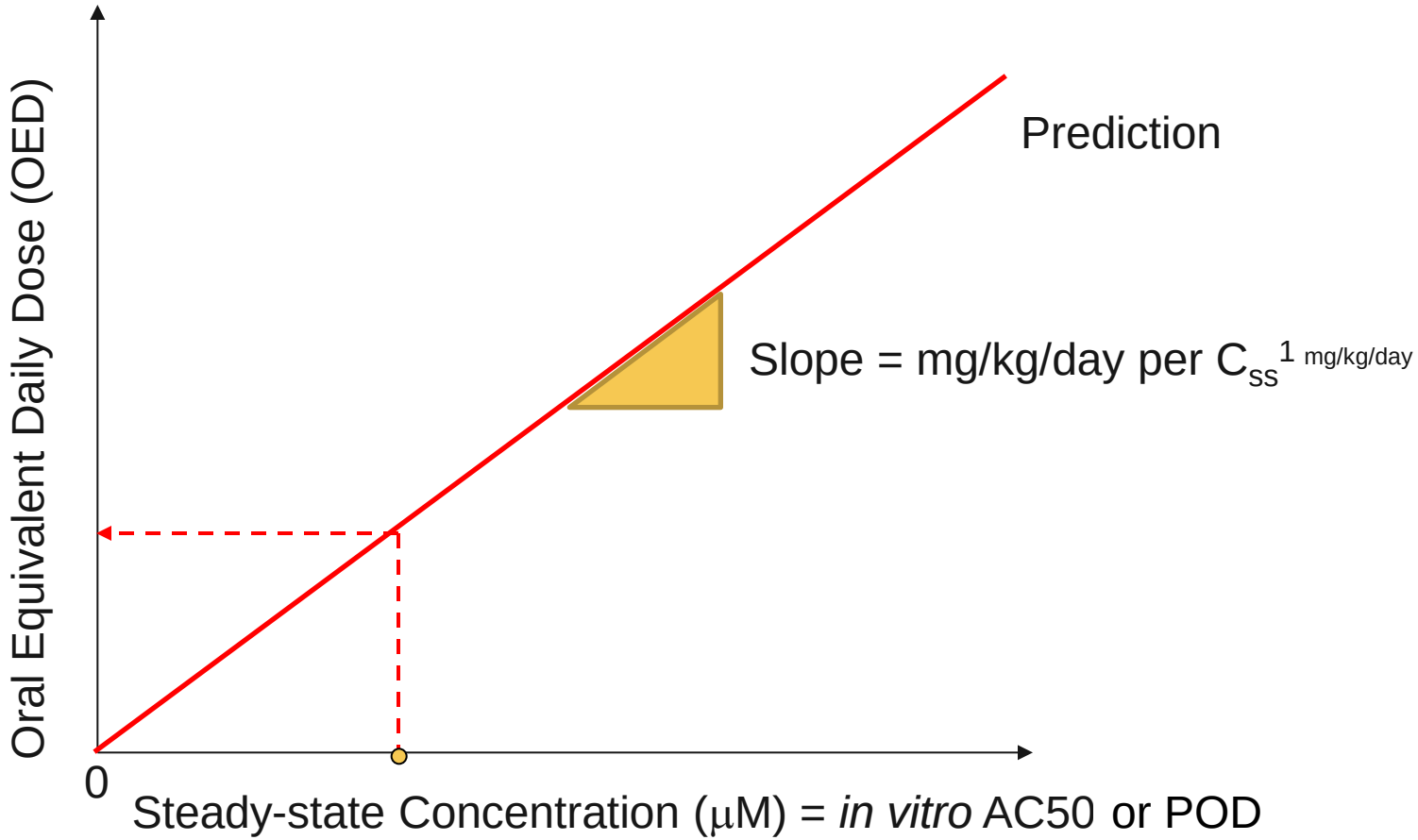


$$C_{ss} = \frac{\text{oral dose rate}}{(\text{GFR} * \text{F}_{ub}) + \left(\text{Q}_l * \text{F}_{ub} * \frac{Cl_{int}}{\text{Q}_l + \text{F}_{ub} * Cl_{int}}\right)}$$

- Can calculate predicted steady-state concentration (C_{ss}) for a 1 mg/kg/day dose and multiply to get concentrations for other doses

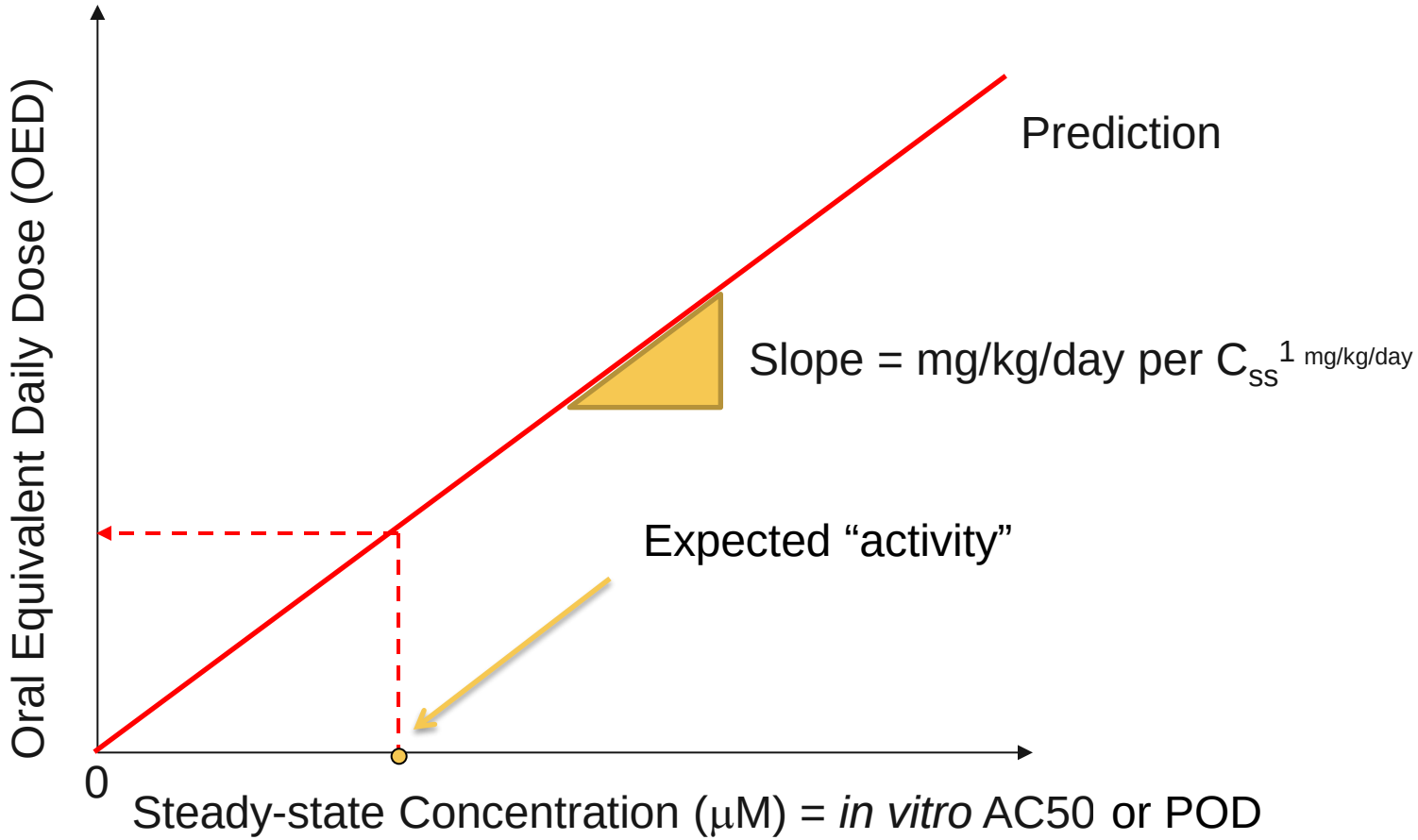
J.Wambaugh, EPA NCCT

Steady-State In Vitro-In Vivo Extrapolation (IVIVE)



- Swap the axes
- Can divide bioactive concentration by C_{ss} for for a 1 mg/kg/day dose to get oral equivalent dose
- POD = Point of Departure

Steady-State In Vitro-In Vivo Extrapolation (IVIVE)



- Swap the axes
- Can divide bioactive concentration by C_{ss} for for a 1 mg/kg/day dose to get oral equivalent dose
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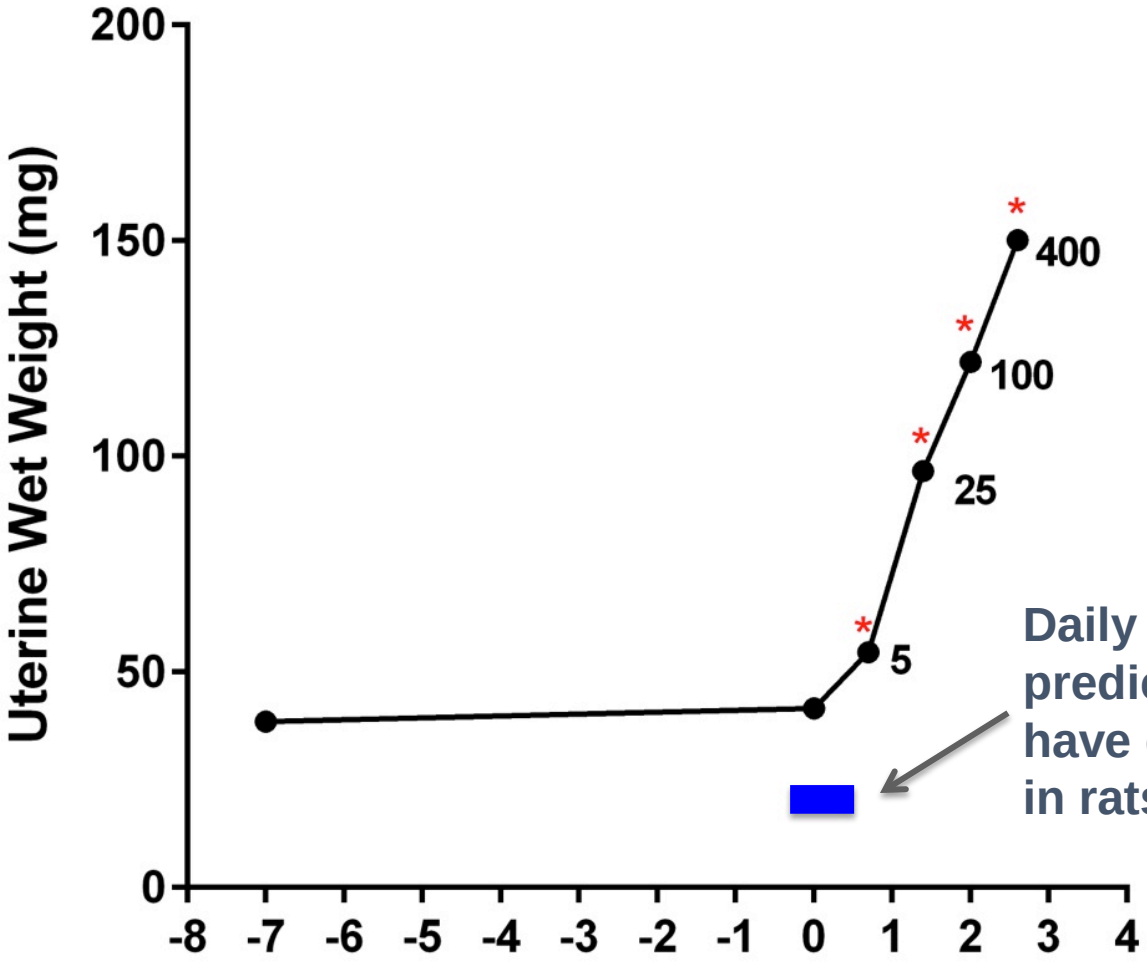
IVIVE vs Rat Uterotrophic data: EE

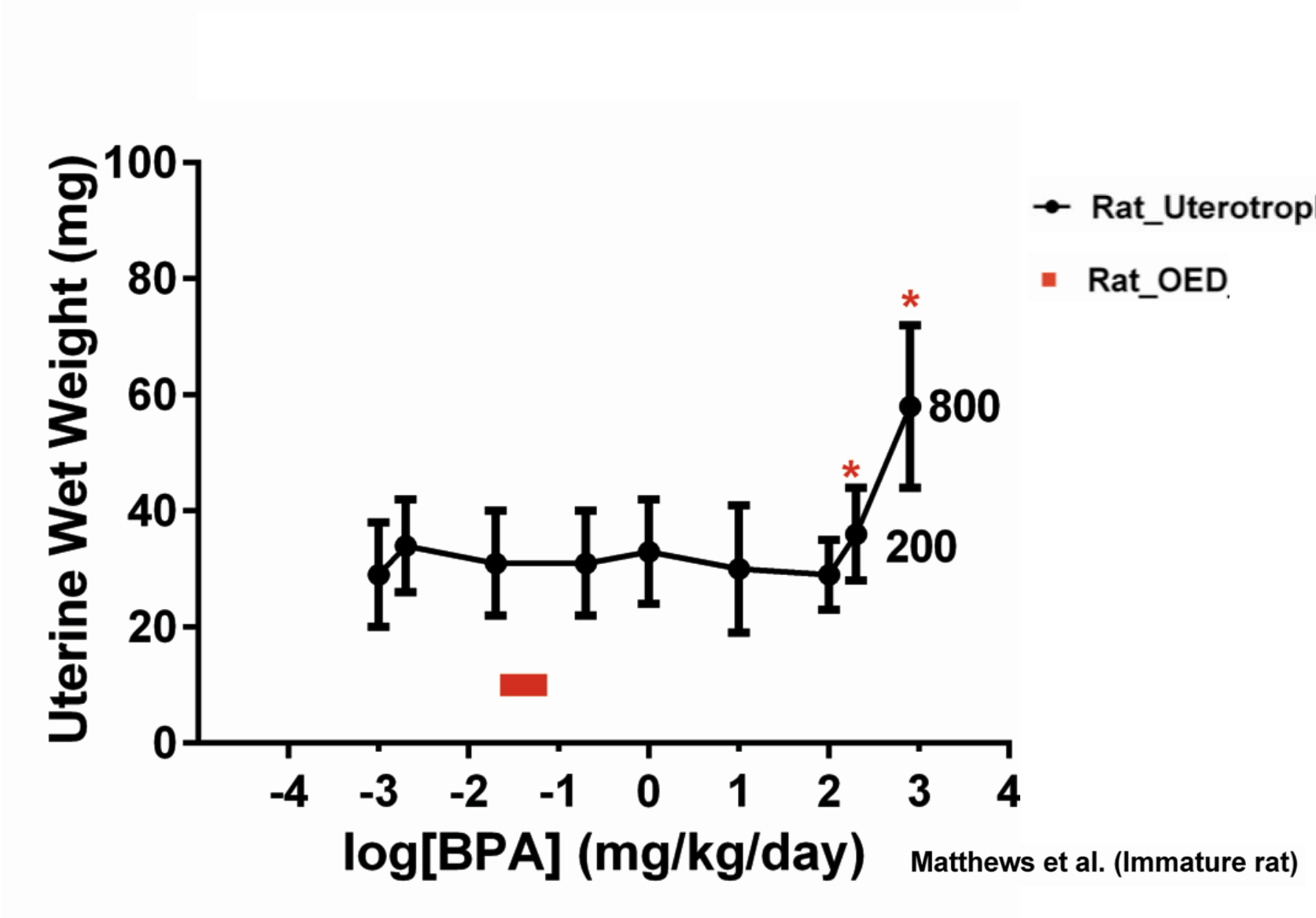


Daily oral dose predicted by IVIVE to have estrogenic activity in rats.

EE= Ethinyl estradiol
POD = Point of Departure
OED = Oral Equivalent Dose

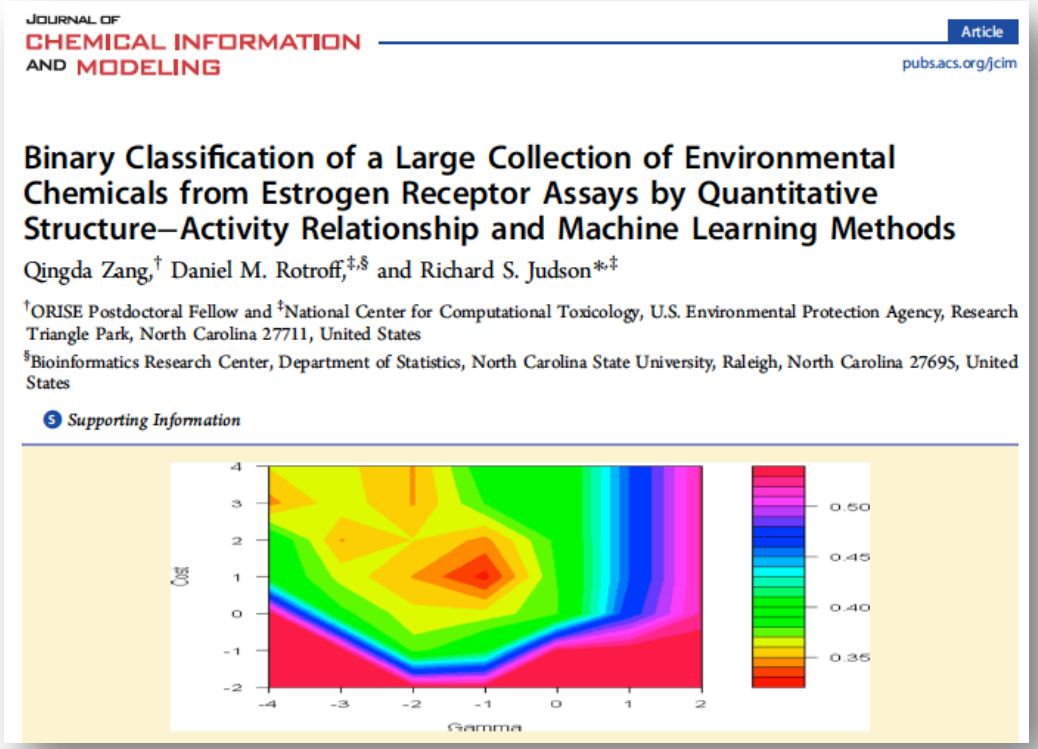
Zhang et al. (immature rat)

IVIVE vs Rat Uterotrophic data: BPA



QSAR

- Quantitative structure–activity relationship models
- ER QSAR model published, being validated for use in prioritization
- Androgen receptor QSAR being developed by NICEATM



JOURNAL OF CHEMICAL INFORMATION AND MODELING

Article

pubs.acs.org/jcim

Binary Classification of a Large Collection of Environmental Chemicals from Estrogen Receptor Assays by Quantitative Structure–Activity Relationship and Machine Learning Methods

Qingda Zang,[†] Daniel M. Rotroff,[‡,§] and Richard S. Judson*[,‡]

[†]ORISE Postdoctoral Fellow and [‡]National Center for Computational Toxicology, U.S. Environmental Protection Agency, Research Triangle Park, North Carolina 27711, United States

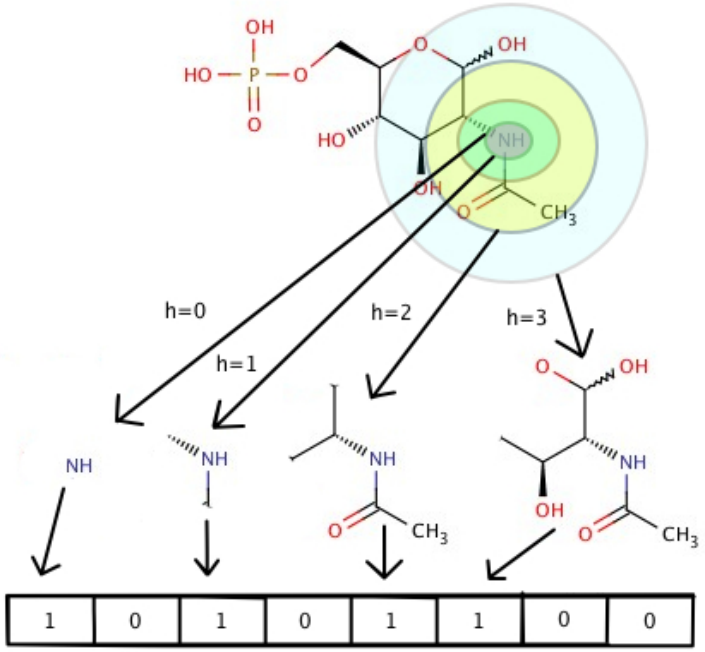
[§]Bioinformatics Research Center, Department of Statistics, North Carolina State University, Raleigh, North Carolina 27695, United States

Supporting Information

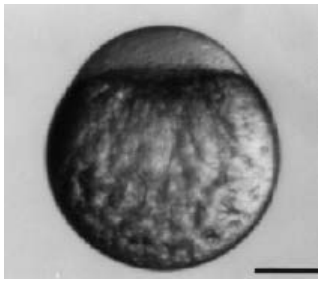
QSPR

- Quantitative Structure–Property Relationship models

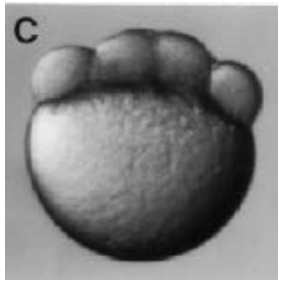
- Being developed for the estimation of physicochemical properties of environmental chemicals:
  - Octanol/water partition coefficient (log P)
  - Water solubility (log S)
  - Boiling point
  - Melting point
  - Vapor pressure
  - Bioconcentration factor (BCF)



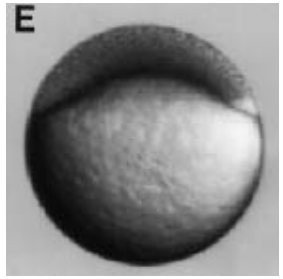
Zebrafish as a Model for Toxicity Testing



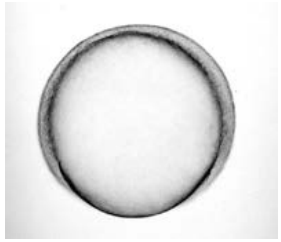
3 min



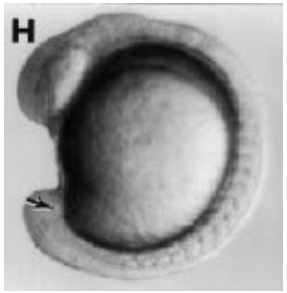
1. 25hr



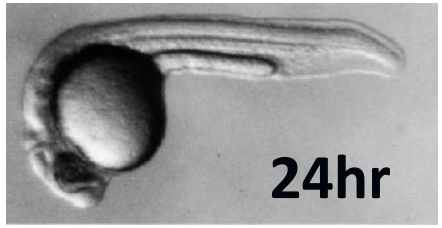
4 hr



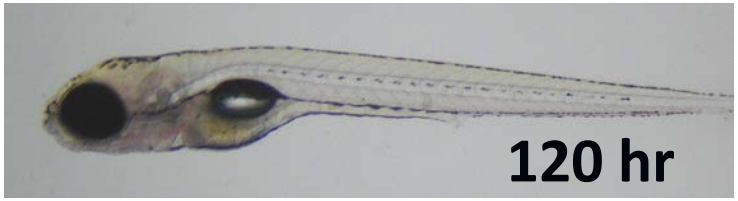
6 hr



19 hr



24hr

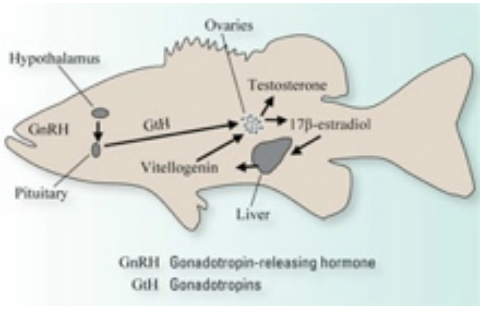


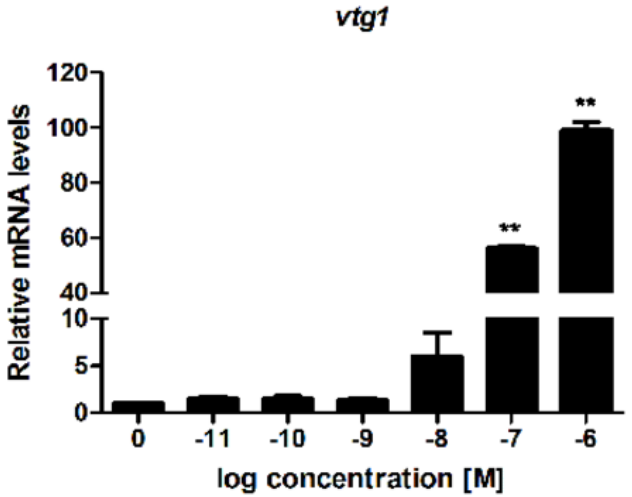
120 hr

48hr

Vitellogenin (Vtg): Response to Estrogens

- Vitellogenin is an egg yolk protein expressed in the females of nearly all oviparous species (fish, amphibians, reptiles, birds, most invertebrates, and monotremes),and is the precursor for most of the protein content of yolk that is a source of nutrients during early development.





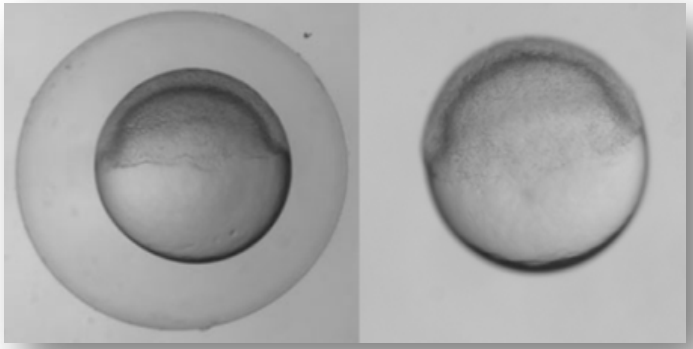
- In the presence of estrogenic endocrine disruptive chemicals (EDCs), juvenile and male fish can express the Vg gene in a dose dependent manner and expression in these populations can be used as a molecular marker of exposure to estrogenic EDCs.

Vtg1 mRNA Pilot Project

- Robert Tanguay, Oregon State University
  - HT Zebrafish screening facility, highly automated
- Stephanie Padilla, EPA NHEERL
  - Medium throughput, manual
- NICEATM coordinating the project, supplying 18 ER reference chemicals to each lab
- 8-point dose response @ 120 hpf

Vtg1 mRNA Pilot Project

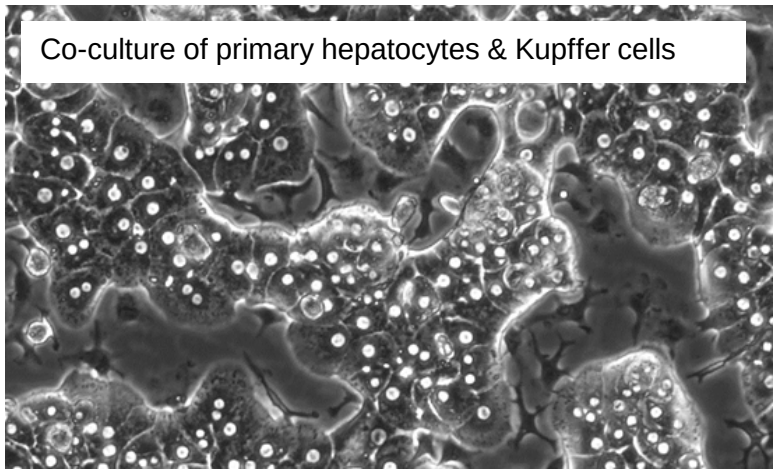
- Establish proof of principal for using zebrafish Vtg1 mRNA as a HT screening tool
- Examine key variables
  - Presence/absence of chorion
  - Single vs. repeat dosing

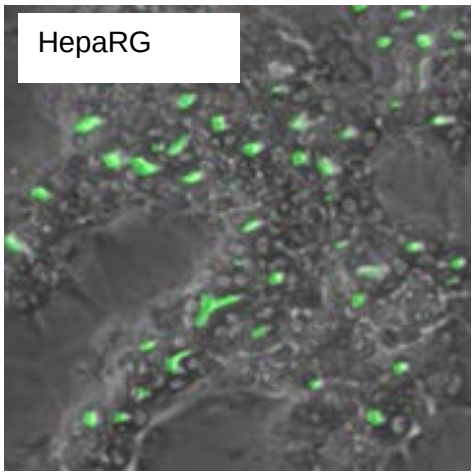


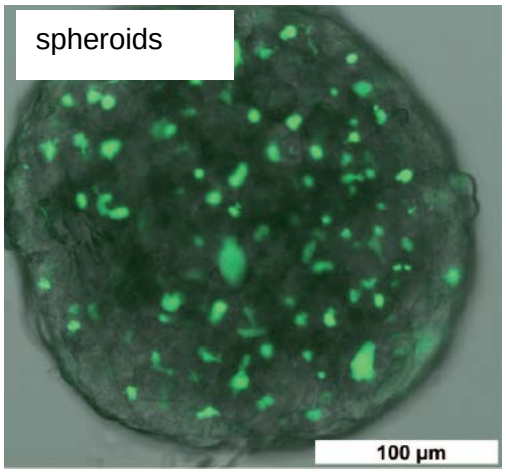
Tox21 Phase III: Metabolism

- Develop more physiologically-relevant *in vitro* models, initial focus on *in vitro* liver models

- Increase the use of computational models to predict metabolism/toxicity







Steve Ferguson, NTP BSB

Toxicity Testing Focus Areas

Endocrine disruptors (ER / AR)

Acute systemic toxicity

In vitro testing of nano materials

Skin sensitization

Reproductive & developmental toxicity

SACATM Charge Question:

Please provide suggestions for future scientific workshops, symposia, or other opportunities related to moving towards ‘fit for purpose’ validation approaches.

NICEATM Criteria for “Guideline Like” (GL)

1) OVX or immature rat, and ovx-adult mouse

2) n >=4 in test group and control

3) Dosing via oral gavage, s.q. or i.p. injection

4) >= 2 dose levels plus positive control and vehicle control

5) Minimum 3 days dosing

6) Necropsy carried out 18-36 hours after the last dose

NICEATM Criteria for “Guideline Like” (GL)

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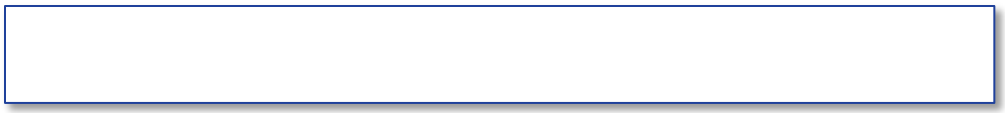
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