Chemical Prioritization Methods for Nuclear Receptor Modulators at the U.S. EPA

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November 9, 2018

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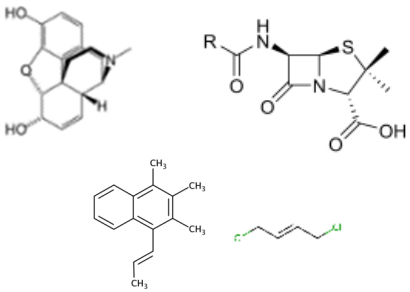
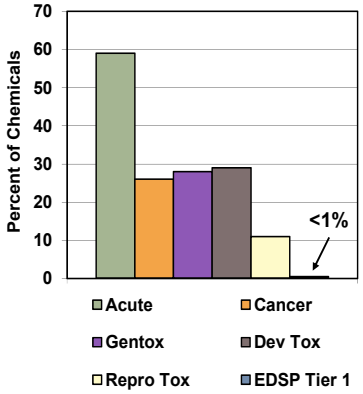
Regulatory Agencies Make a Broad Range of Decisions on Chemicals…

Number of Chemicals /Combinations



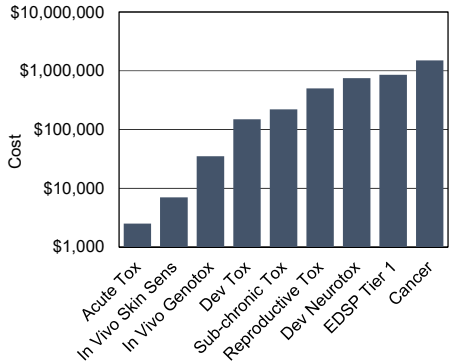
Lack of Data



Modified from Judson *et al.*, EHP 2010

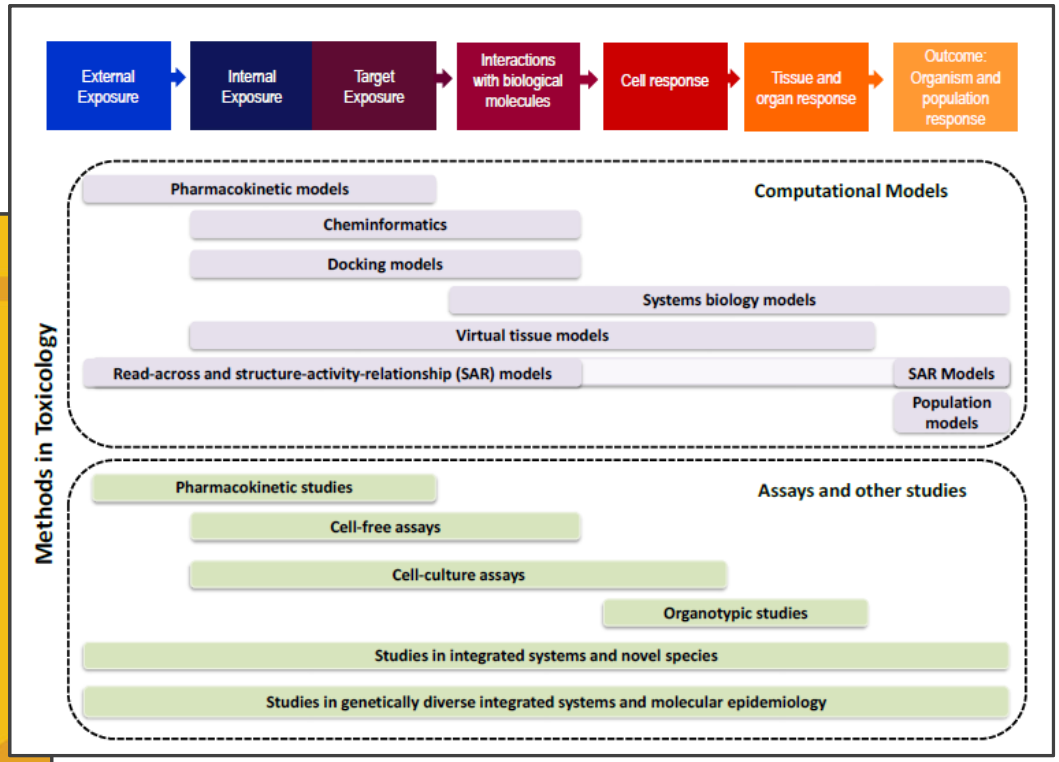
Ethics/Relevance Concerns

Economics

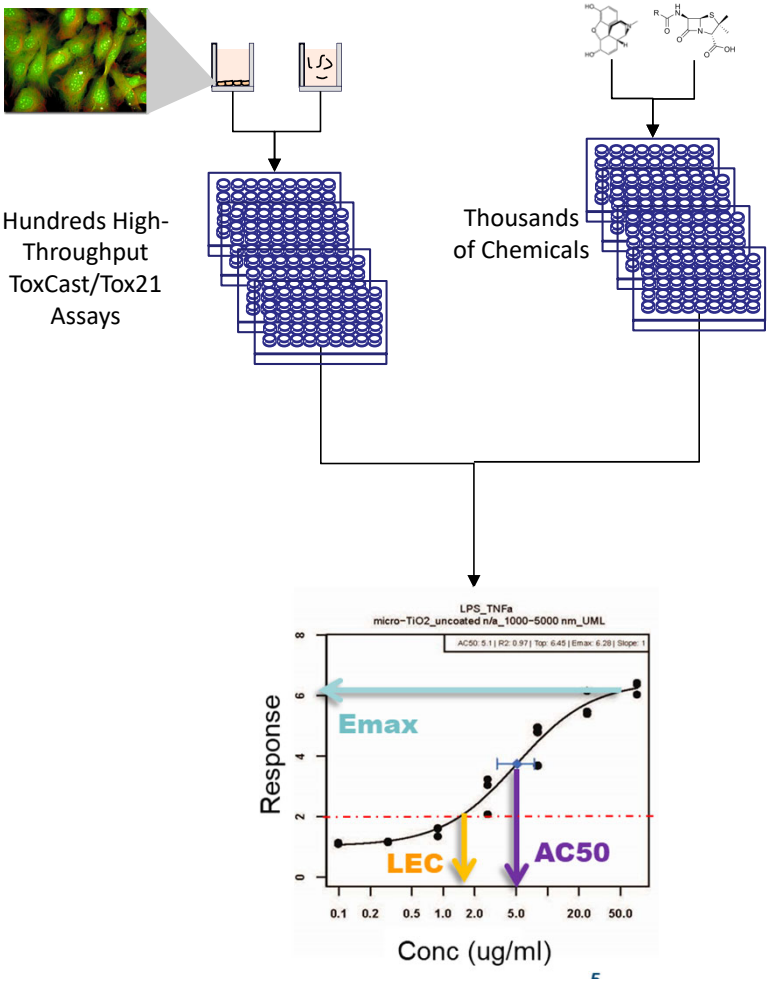


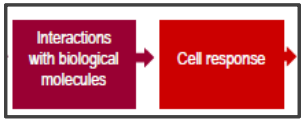
- Number of chemicals and combinations of chemicals is extremely large (>20,000 substances on active TSCA inventory)
- Due to historical regulatory requirements, most chemicals lack traditional toxicity testing data
- Traditional toxicology testing is expensive and time consuming
- Traditional animal-based testing has issues related to ethics and relevance

Toxicology Moving to Embrace 21st Century Methods



High-Throughput Assays Used to Screen Chemicals for Potential Toxicity

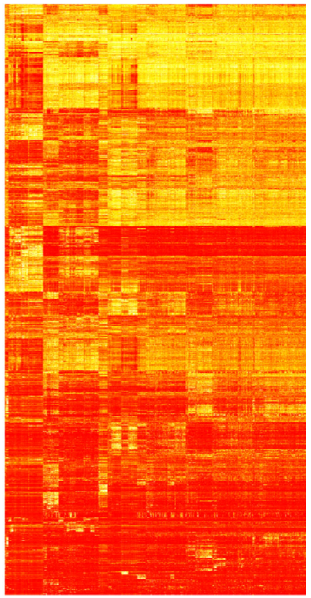




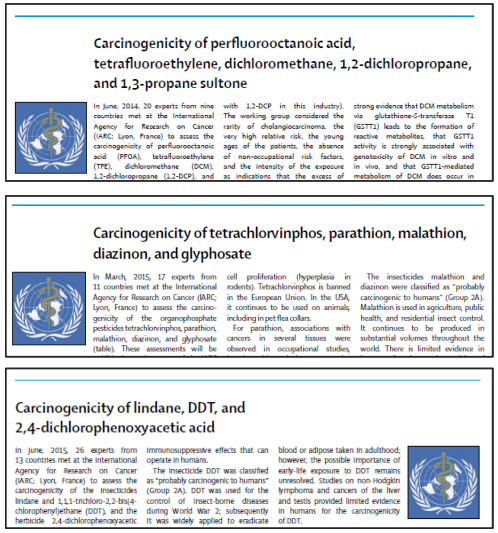
- Understanding of what cellular processes/pathways may be perturbed by a chemical
- Understanding of what amount of a chemical causes these perturbations

Broad Success Derived from High-Throughput Screening Approaches

Group Chemicals by Similar Bioactivity and Predictive Modeling



Provide Mechanistic Support for Hazard ID



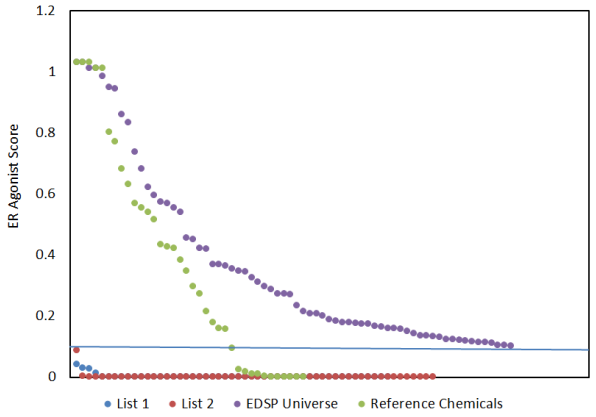
Carcinogenicity of perfluorooctanoic acid, tetrafluoroethylene, dichloromethane, 1,2-dichloropropane, and 1,3-propane sultone

Carcinogenicity of tetrachlorvinphos, parathion, malathion, diazinon, and glyphosate

Carcinogenicity of lindane, DDT, and 2,4-dichlorophenoxyacetic acid

IARC Monographs

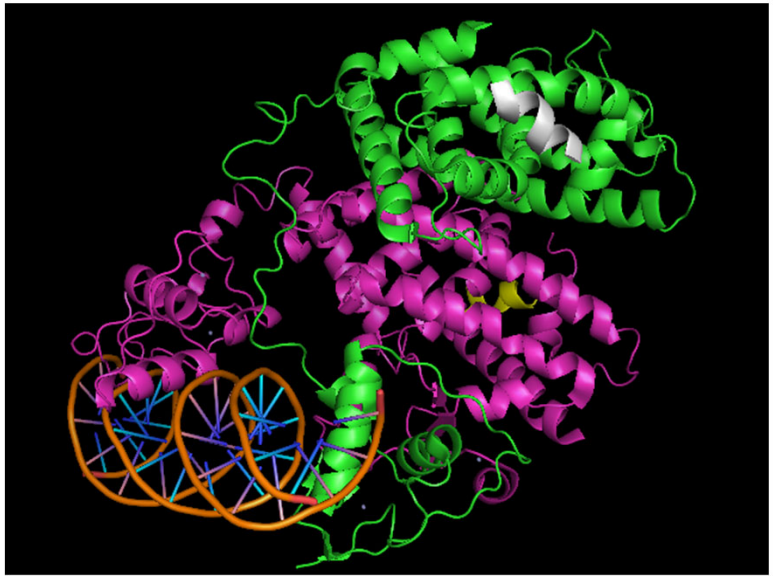
Prioritization of Chemicals for Further Testing



FIFRA SAP, Dec 2014

Focus on Nuclear Receptors and Xenobiotics

- Family of ligand-regulated nuclear transcription factors (48 human)
- Conserved, modular domains
  - DNA-binding domain
  - Ligand-binding domain
    - Binds lipophilic, small molecules
    - Endogenous ligands: steroid hormones, fatty acids
- Regulates genes for key physiological processes: endocrine system, growth and differentiation, metabolism
- Endogenous ligand physicochemical properties consistent with cell permeable qualities
- Good focus for **selective xenobiotic effects**



http://proteopedia.org/wiki/index.php/Image:3dzy2.png

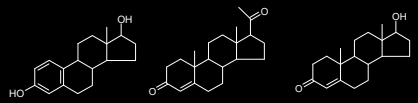
Ligands for Nuclear Hormone Receptors

From the EPA's Endocrine Disruptor Screening Management Plan:

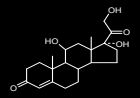
"Examine effects of these chemicals on estrogen, androgen and thyroid hormone-related processes"

Sex Steroids — *ER*, *PR*, *AR*

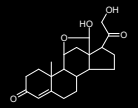
Estrogens, Progestins, Androgens



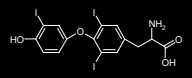
Glucocorticoids — *GR*



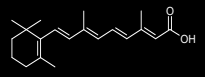
Mineralocorticoids — *MR*



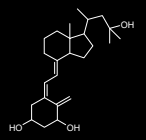
Thyroid Hormones — *TR*



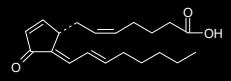
Retinoids — *RARs*, *RXRs*



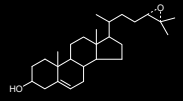
Vitamin D — *VDR*



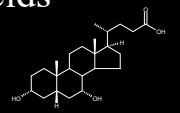
Lipids — *PPARs*



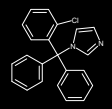
Oxysterols — *LXRs*



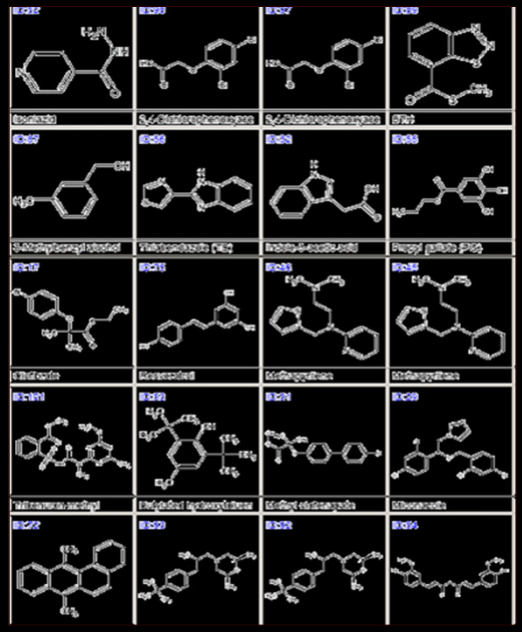
Bile Acids — *FXR*



Xenobiotics — *PXR*, *CAR*



Unknowns

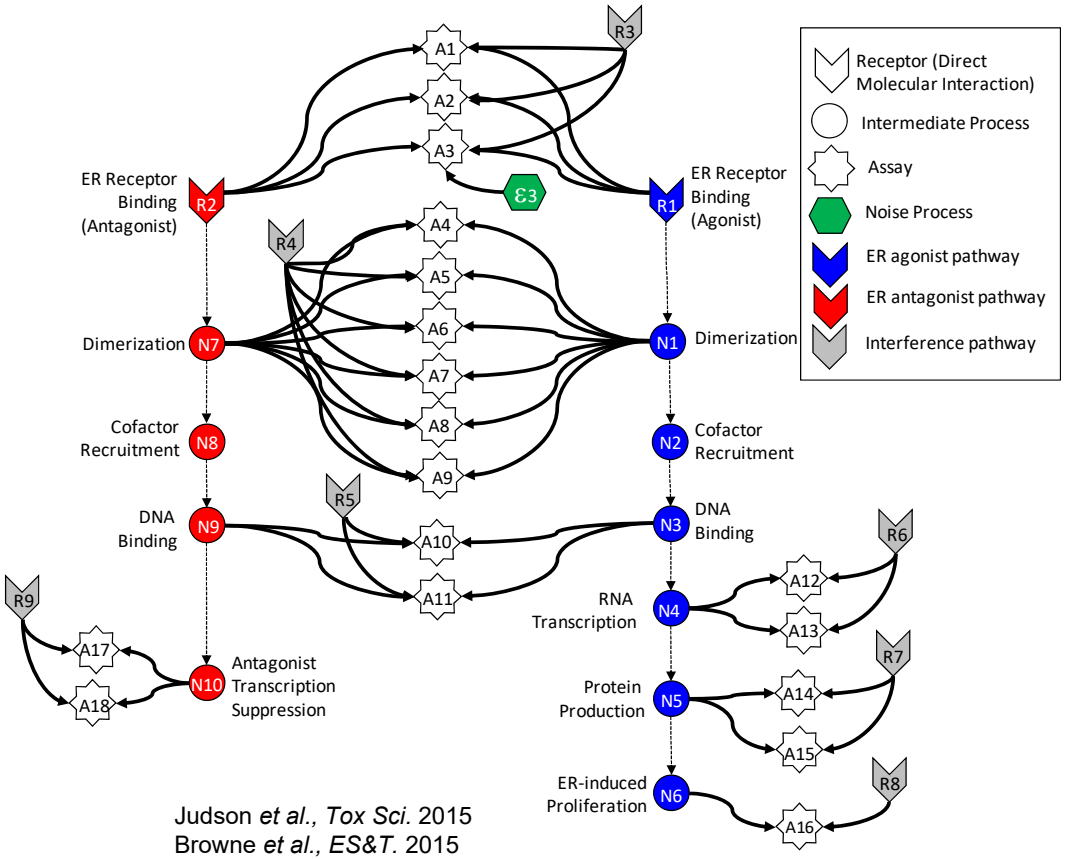


The Estrogen Receptor Model

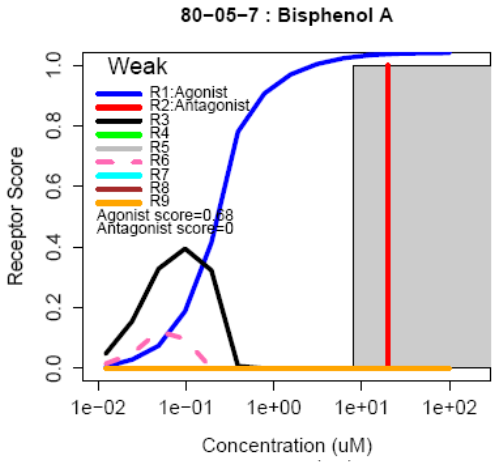
- Public solicitation for diverse high-throughput assays to cover broad range of bioactivity/toxicity endpoints
- Many estrogen receptor assays included
  - Binding
  - Nuclear localization
  - Transactivation
  - Cell proliferation
- No single assay perfect for a variety of reasons
- Decided to develop computational model utilizing all data

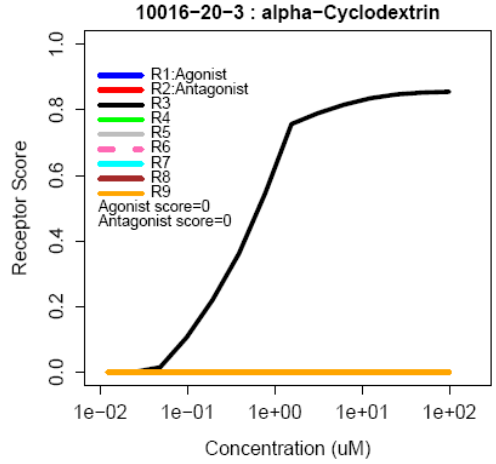
Targeted Pathways

18 *In Vitro* Assays Measure ER-Related Activity

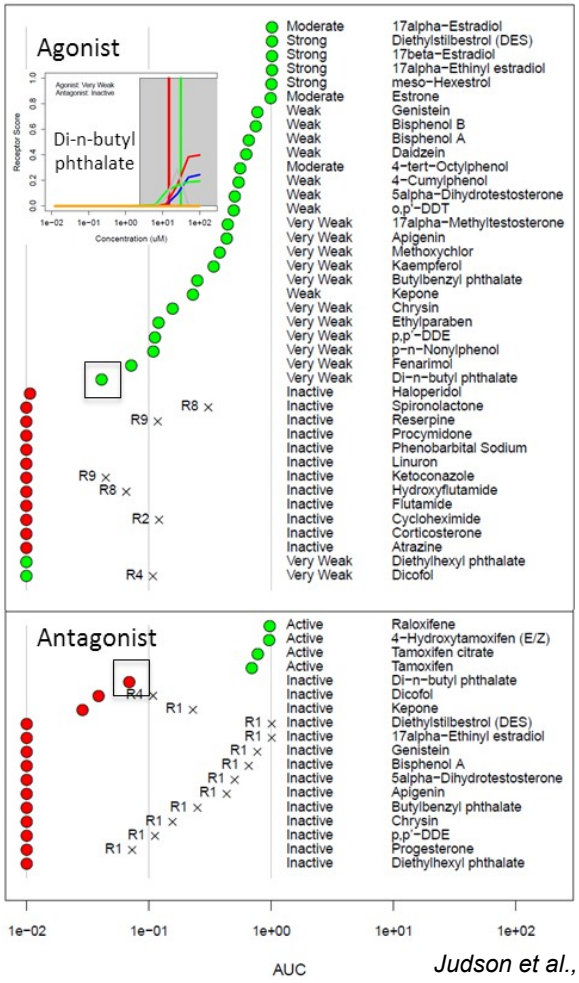


Judson *et al., Tox Sci.* 2015
Browne *et al., ES&T.* 2015
Kleinstreuer *et al., EHP* 2016

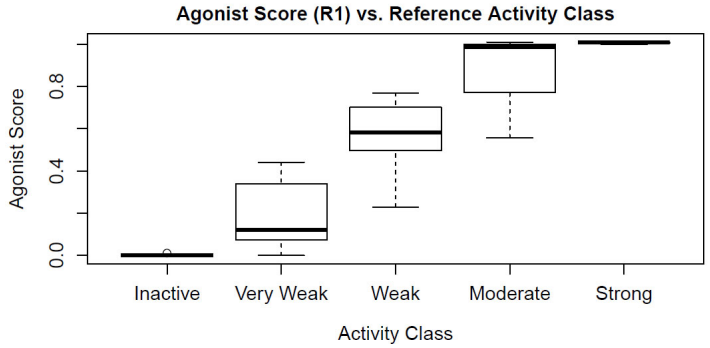


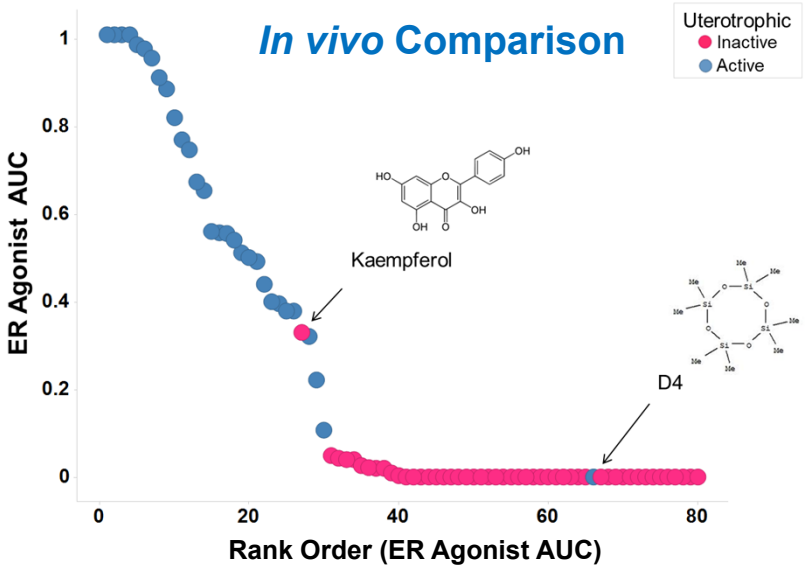


ER Model Performance



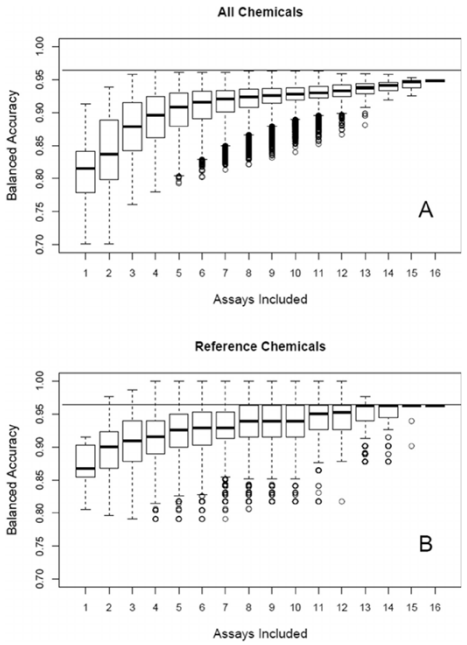
Judson et al., Tox Sci 2015

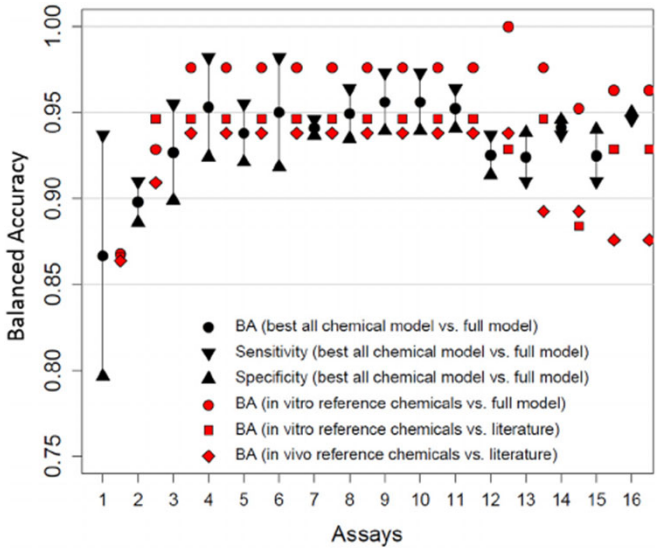




Browne et al., Environ. Sci. Technol., **2015**

ER Minimal Model

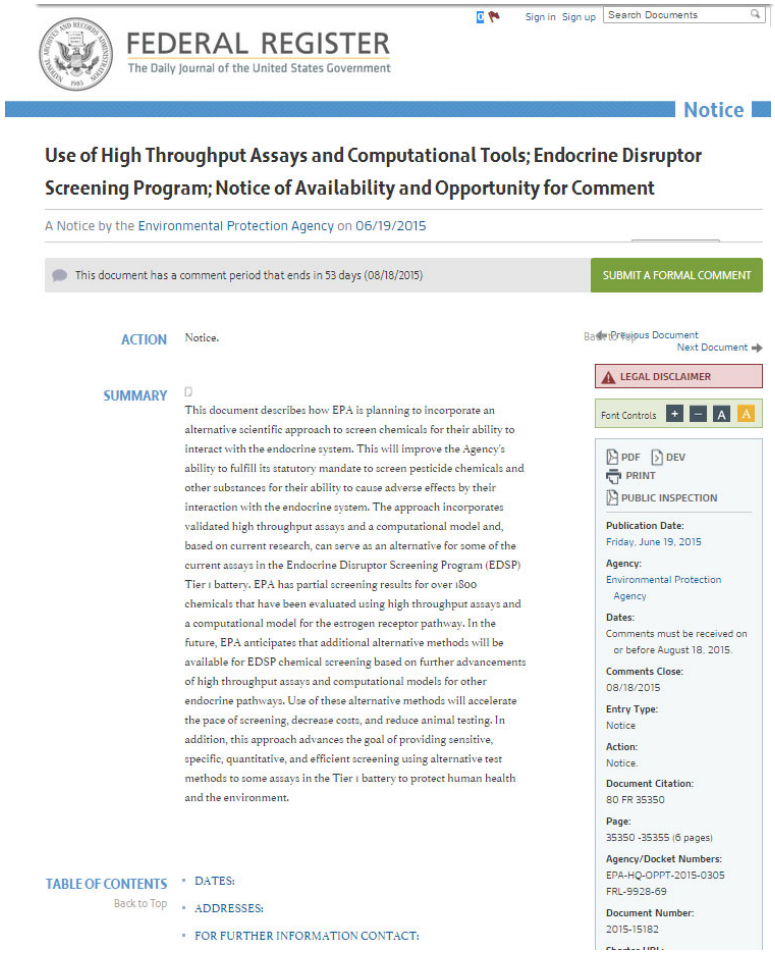




Combinations of four assays provide good balanced accuracy

R.S. Judson et al. / Regulatory Toxicology and Pharmacology 91 (2017) 39e49

Regulatory Applications: EDSP



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Notice

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

A Notice by the Environmental Protection Agency on 06/19/2015

This document has a comment period that ends in 53 days (08/18/2015) SUBMIT A FORMAL COMMENT

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 efficient screening using alternative test methods to some assays in the Tier 1 battery to protect human health and the environment.

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Page: 35350 -35355 (6 pages)
Agency/Docket Numbers: EPA-HQ-OPPT-2015-0305 FRL-9928-69
Document Number: 2015-15182

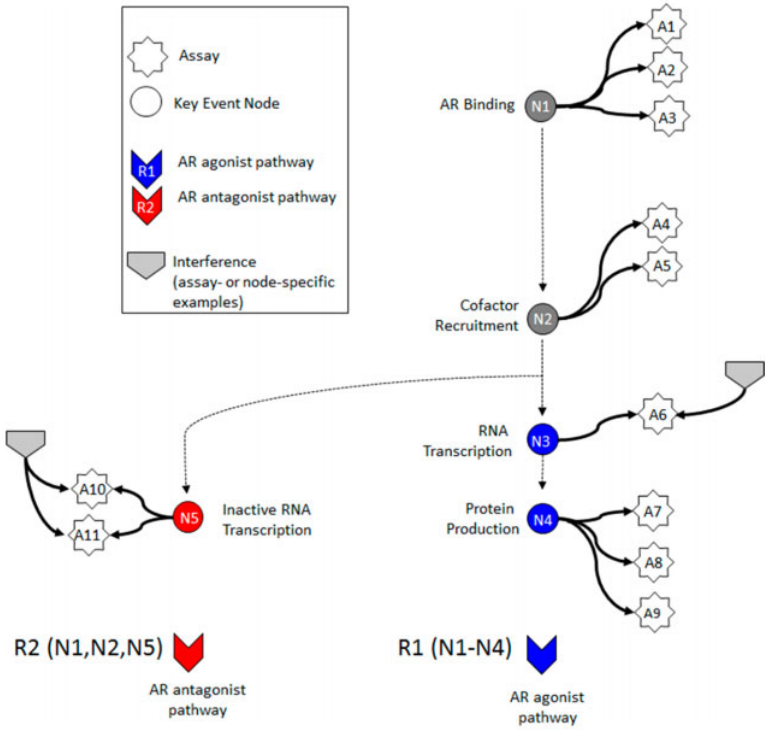
"The approach incorporates validated high-throughput assays and a computational model and, based on current research, can serve as an alternative for some of the current assays in the Endocrine Disruptor Screening Program (EDSP) Tier 1 battery."

Androgen Receptor Screening

- Utilize existing ToxCast/Tox21 assays to develop AR model
- Cytotoxic chemicals confounded antagonist cell-based assays
- Run additional confirmation assay for antagonists
  - Higher agonist concentration
  - Competitive antagonists show right-shift in potency

ToxCast/Tox21 Assays

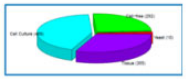
ID	node	assay name	source	gene[a]	species	type	associated pathways[b]
A1	N1	NVS_NR_hAR	Novascreen	AR	*Homo sapiens*	receptor binding	R1; R2; R3
A2	N1	NVS_NR_cAR	Novascreen	AR	P. troglodytes	receptor binding	R1; R2; R3
A3	N1	NVS_NR_rAR	Novascreen	AR	*Rattus norvegicus*	receptor binding	R1; R2; R3
A4	N2	OT_AR_ARSRC1_0480	Odyssey Thera	AR; SRC	*Homo sapiens*	coregulator recruitment	R1; R2; R4
A5	N2	OT_AR_ARSRC1_0960	Odyssey Thera	AR; SRC	*Homo sapiens*	coregulator recruitment	R1; R2; R4
A6	N3	ATG_AR_TRANS	Attagene	AR	*Homo sapiens*	RNA reporter gene	R1; R5
A7	N4	OT_AR_ARELUC_AG_1440	Odyssey Thera	AR; ARE	*Homo sapiens*	reporter gene	R1; R6
A8	N4	Tox21_AR_BLA_Agonist_ratio	NCATS/ NCGC	AR	*Homo sapiens*	reporter gene	R1; R6
A9	N4	Tox21_AR_LUC_MDAKB2_Agonist	NCATS/ NCGC	AR	*Homo sapiens*	reporter gene	R1; R6
A10	N5	Tox21_AR_BLA_Antagonist_ratio	NCATS/ NCGC	AR	*Homo sapiens*	reporter gene	R2; R7
A11	N5	Tox21_AR_LUC_MDAKB2_Antagonist	NCATS/ NCGC	AR	*Homo sapiens*	reporter gene	R2; R7
A11[c]	N5	Tox21_AR_LUC_MDAKB2_Antagonist-confirmation	NCATS/ NCGC	AR	*Homo sapiens*	reporter gene	NA



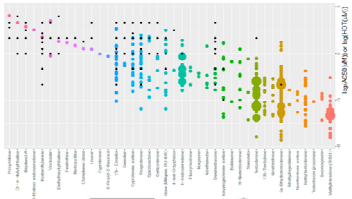
Antagonist Mode

Evaluation of AR Model

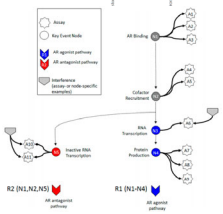
Reference Data Literature Review



Reference Chemical Classifications

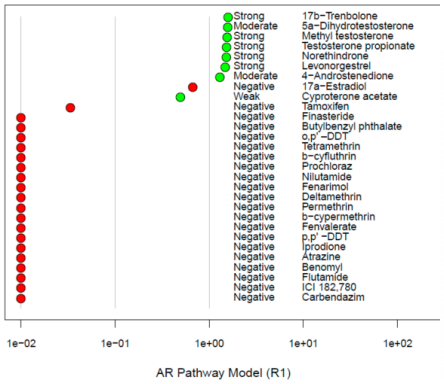


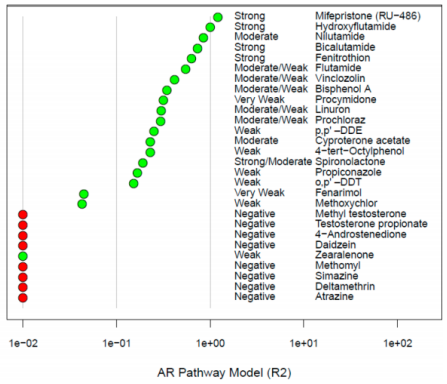
Model Performance Evaluation



Agonist

Antagonist





Validated Model for Chemical Screening

Summary

- Model has high sensitivity
- Antagonist mode specificity improved by considering antagonist assay with high agonist concentration
- Weakly active chemicals most difficult to detect
- Broad screening suggested cytotoxic compounds not all excluded

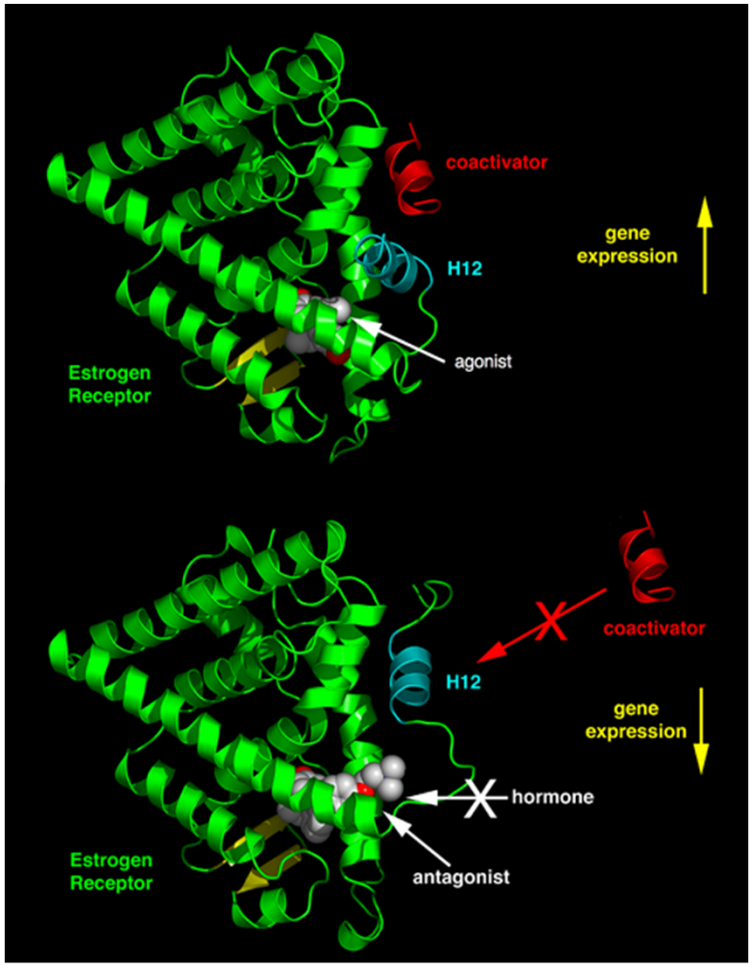
Chemical Research in Toxicology

Development and Validation of a Computational Model for Androgen Receptor Activity

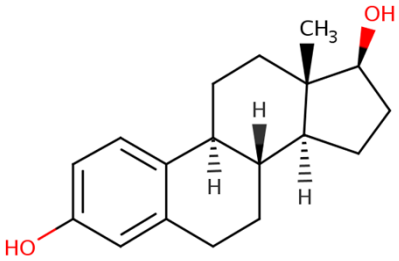
Nicole C. Kleinstreuer,*,† Patricia Ceger,‡ Eric D. Watt,§ Matthew Martin,§ Keith Houck,§ Patience Browne,∥ Russell S. Thomas,§ Warren M. Casey,† David J. Dix,⊥ David Allen,‡ Srilatha Sakamuru,# Menghang Xia,# Ruili Huang,# and Richard Judson§

Chem Res Toxicol. 30:946-964, 2017.

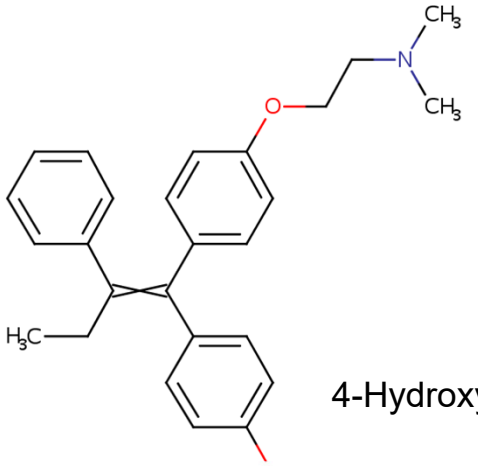
Agonists versus Antagonist



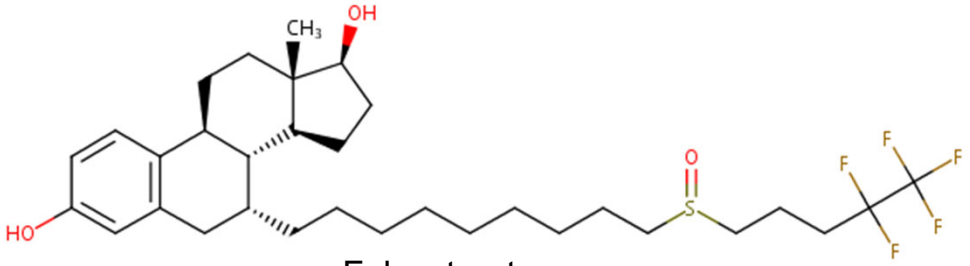
Selective receptor modulators behave conditionally as agonists and/or antagonists



17β-Estradiol



4-Hydroxytamoxifen



Fulvestrant

Brzozowski et al., Nature. ***389****: 753–8, 1997).*

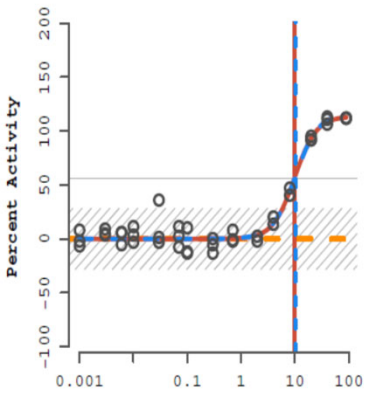
Tox21 AR Screening Results
~ 8,000 chemicals

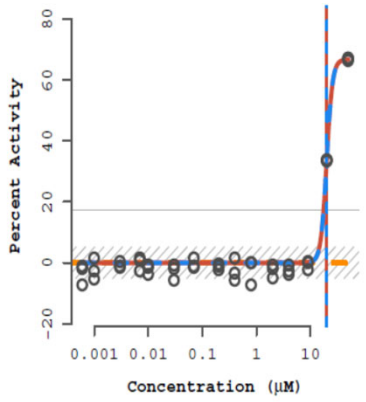
- Only 102 chemicals positive using strictest criteria
- Expanding criteria allows for ranking of chemicals based on strength of evidence
- Chemicals that are confounded by cytotoxicity are not eliminated but evidence is weaker
- Potency not currently considered but is another important factor

Hydroxyflutamide
Bis(tributyltin)oxide
Dipyrithione
Ziram
NTP Mix21 AR2 2-EQP
17alpha-Ethinylestradiol
Bis(1-piperidinylthioxomethyl)hexasulfide
Triphenyltin acetate
Tributyltin benzoate
Nilutamide
Triethyltin bromide
Equilin
17alpha-Estradiol
(Acryloyloxy)(tributyl)stannane
Triphenyltin fluoride
Ethylestrenol
Copper dimethyldithiocarbamate
Vinclozolin

Challenges with assessing NR antagonism *in vitro*

- Measuring loss of signal-confounded by cytotoxicity
- To address:
  - Two different assay platforms
  - Use bootstrapping techniques to determine effect of cytotoxicity
  - Two concentrations of agonist R1881
  - MARCoNI assay for corepressor/activator recruitment






```
ASSAY:   AEID1816 (TOX21_AR_LUC_MDAKB2_Antagonist2)

NAME:    Benzethonium chloride
CHID:    23810   CASRN: 121-54-0
SPID(S): Tox21_202488
M4ID:    4754104

HILL MODEL (in red):
      tp      ga      gw
val:  114     0.996   2.14
sd:   2.71    0.0262  0.208

GAIN-LOSS MODEL (in blue):
      tp      ga      gw      la      lw
val:  117     1.01    2.04    2.13    8.85
sd:   NaN     NaN     NaN     NaN     NaN

      CNST      HILL      GNLS
AIC:  441.3     291.95    295.54
PROB: 0         0.86      0.14
RMSE: 50.36     8.11      8.1

MAX MEAN: 112      MAX MED: 112      BMAD: 9.44
ASSAY:   AEID1817 (TOX21_AR_LUC_MDAKB2_Antagonist2_viab:

NAME:    Benzethonium chloride
CHID:    23810   CASRN: 121-54-0
SPID(S): Tox21_110403_1
M4ID:    10861077

HILL MODEL (in red):
      tp      ga      gw
val:  66.8    1.3     8
sd:   1.03    0.00424 4.68

GAIN-LOSS MODEL (in blue):
      tp      ga      gw      la      lw
val:  66.8    1.3     8       3.49    6.26
sd:   NA      NA      NA      NA      NA

      CNST      HILL      GNLS
AIC:  345.27    225.7     229.7
PROB: 0         0.88      0.12
RMSE: 19.49     2.82      2.82

MAX_MEAN: 66.7      MAX_MED: 67      BMAD: 1.74

COFF: 17.4   HIT-CALL: 1   FITC: 41   ACTP: 1

FLAGS:
```

Antagonist Reference Chemical Results

Antagonist Screening

- LUC: R1881 = 0.5 nM
- LUC_counterscreen: R1881 = 10 nM

Criteria	Sensitivity	Specificity	Balanced Accuracy
Active in BLA	0.9	0.88	0.89
Active in LUC_counterscreen	0.8	1	0.9
Active in LUC	0.9	0.88	0.89
Active in at least 2 assays	0.9	1	0.95
Active in all three assays	0.7	1	0.85
Active in all three assays, LUC vs. LUCcs difference, not confounded by cytotoxicity	0.5	1	0.75
Active in LUC, LUC vs. LUCcs difference, not confounded by cytotoxicity	0.7	1	0.85

Chemical	Designation	Assay Hitcalls	LUC vs LUC_counterscreen	LUC vs LUC_viability
Procymidone	Very Weak Antagonist	BLA, LUC, LUCcs	Yes	Yes
Fenarimol	Very Weak Antagonist	BLA, LUC, LUCcs	Yes	Yes
4-(1,1,3,3-Tetramethylbutyl)phenol	Weak Antagonist	LUC	Yes	Yes
o,p'-DDT	Weak Antagonist	BLA, LUC	Yes	Yes
p,p'-DDE	Weak Antagonist	LUC	Yes	Yes
Propiconazole	Weak Antagonist	BLA, LUC, LUCcs	Yes	No
Zearalenone	Weak Antagonist	BLA, LUC, LUCcs	No	No
Methoxychlor	Weak Antagonist	BLA, LUC, LUC2	No	No
Linuron	Moderate/Weak Antagonist	BLA, LUC	Yes	No
Vinclozolin	Moderate/Weak Antagonist	BLA, LUC, LUCcs	Yes	Yes
Flutamide	Moderate/Weak Antagonist	BLA, LUC, LUCcs	Yes	Yes
Bisphenol A	Moderate/Weak Antagonist	BLA, LUC, LUCcs	Yes	Yes
Prochloraz	Moderate/Weak Antagonist	BLA, LUC, LUCcs	Yes	Yes
Cyproterone acetate	Moderate Antagonist	BLA, LUC	Yes	Yes
Nilutamide	Moderate Antagonist	BLA, LUC, LUCcs	Yes	Yes
Spironolactone	Strong/Moderate Antagonist	BLA, LUC	No	Yes
Mifepristone	Strong/Moderate Antagonist	BLA, LUC, LUCcs	No	Yes
Fenitrothion	Strong Antagonist	BLA, LUC, LUCcs	Yes	Yes
Hydroxyflutamide	Strong Antagonist	BLA, LUC, LUCcs	Yes	Yes
Bicalutamide	Strong Antagonist	BLA, LUC, LUCcs	Yes	Yes
17-Methyltestosterone	Negative Antagonist	NA	NA	NA
4-Androstene-3,17-dione	Negative Antagonist	NA	NA	No
Atrazine	Negative Antagonist	NA	NA	NA
Daidzein	Negative Antagonist	BLA	NA	NA
Deltamethrin	Negative Antagonist	NA	NA	NA
Methomyl	Negative Antagonist	LUCcs	NA	No
Simazine	Negative Antagonist	NA	NA	NA

MARCoNI assay

Microarray **A**ssay for **R**eal-time **C**oregulator-**N**uclear receptor **I**nteraction

- Cell-free assay measuring co-regulator recruitment to AR-LBD
  - 154 co-regulators
  - 3 concentrations (1, 10, 100 uM)
  - log fold-change of binding compared to DMSO
- Tested 318 suspected AR antagonists
- Reduced variables (co-regulators) to 28 most affected
- Goal: to see if patterns of coregulatory recruitment can distinguish between true antagonists and false antagonists (cytotoxicity/artifacts)

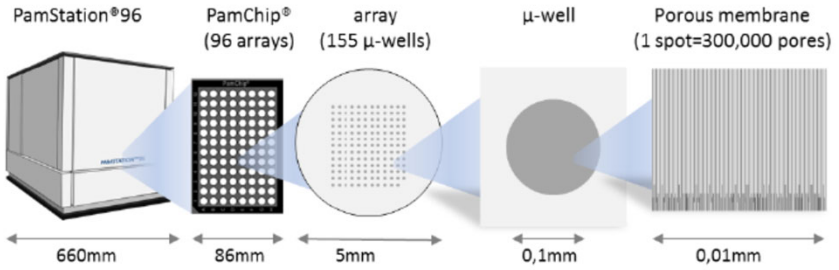


Figure 1: Outline of PamStation®96 instrument and PamChip®96 microarray, each array consisting of 155 µ-wells, (The PamChip®96 is therefore a 14880 µ-well plate)

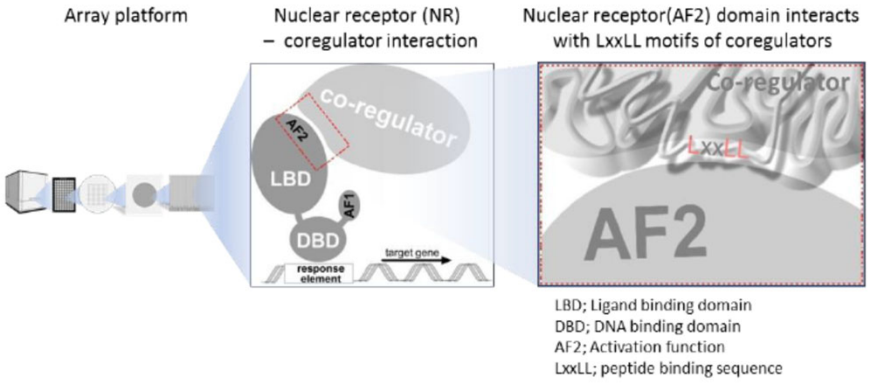
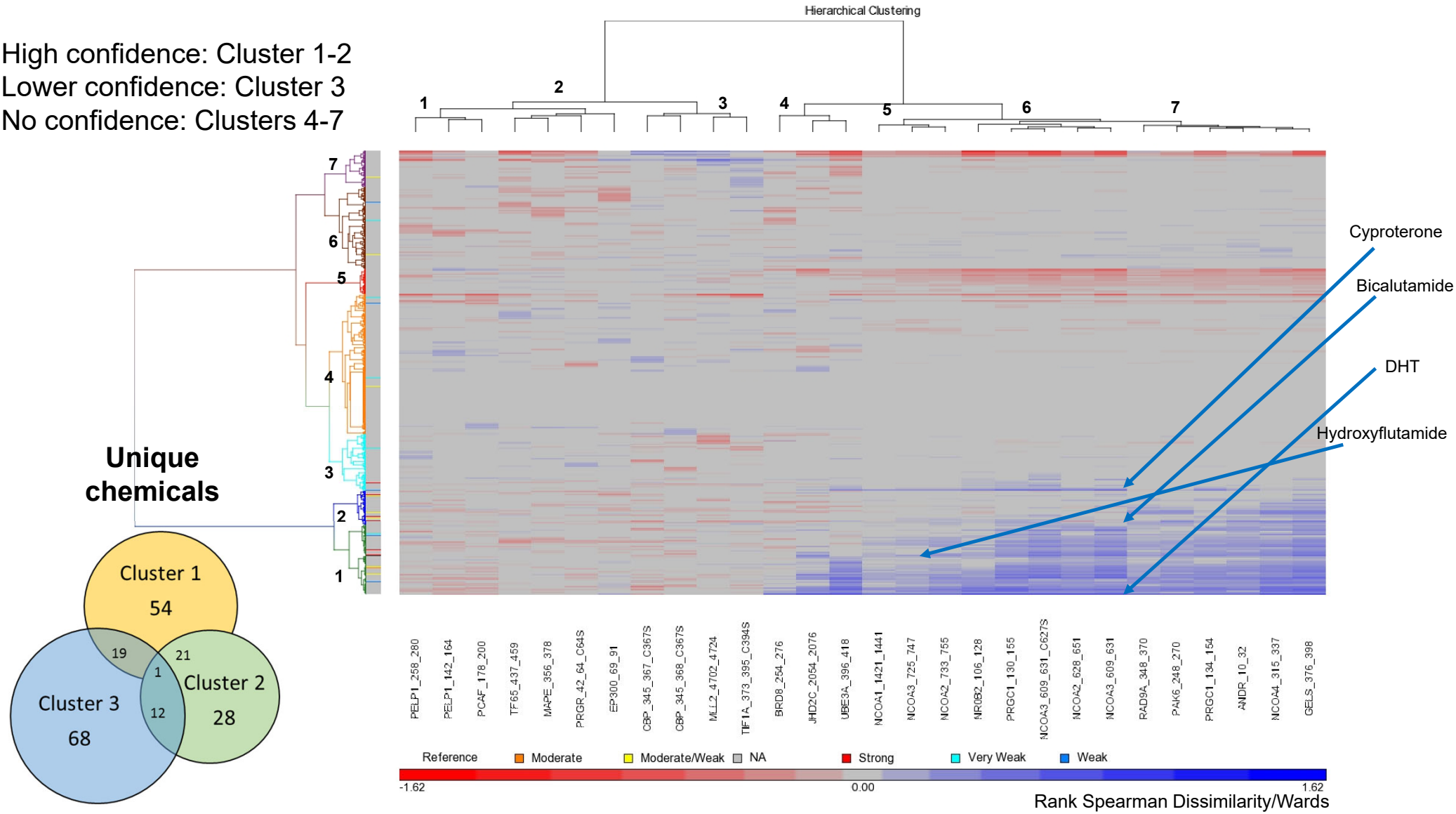


Figure 2: Outline of the nuclear receptor – coregulator interactions in the µ-wells

Image: pamgene.com

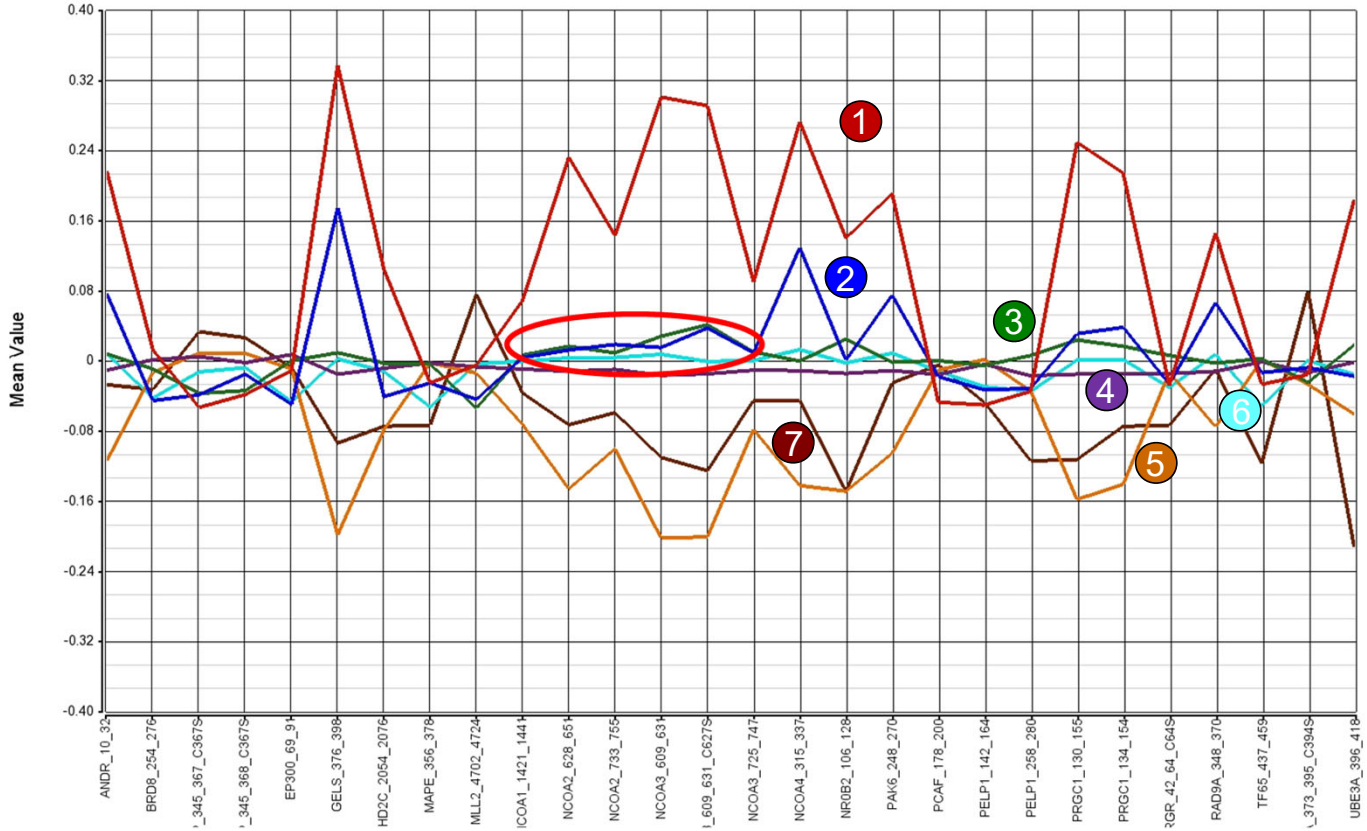
High confidence: Cluster 1-2
Lower confidence: Cluster 3
No confidence: Clusters 4-7



Unique chemicals

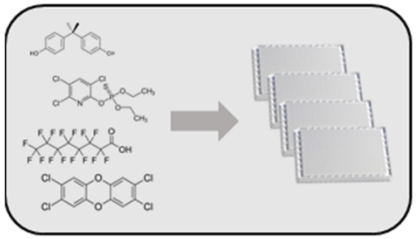
Co-regulator Recruitment Patterns

- Mean value of cluster plotted per coregulator
- Loss of binding seen in cluster 2&3 versus 1 (red oval)
- These represent SRC coactivators that have histone acetyl transferase activity
- Selective receptor modulators; likely would influence biological response



Thyroid Hormone Receptor Modulators: Tox21 qHTS Campaign

Primary Screen



Tox21 Library 10K
15 concentrations

qHTS:
3X Agonist
3X Antagonist
3X Viability

Agonists
Antagonists
Viability

tcpl curve fitting

Hit Characterization

35 total → 28 total

2395 total → 2375 total

699
$AC50^{Via}/AC50^{TR}$ >10

manual curve review

Assay	# Tested
gh3-tre-agonist-followup	84
TRa_CoA	84
TRb_CoA	84
TRb-bla-agonist	84
RXR-bla-agonist	84

Assay	# Tested
gh3-tre-antagonist-highAgonist	408
gh3-tre-antagonist_followup	120
RXR-bla-antagonist	120
RXR-bla-antagonist	120
TRb-bla-antagonist	120
TRb-bla-antagonist_viability	120
TR_GR_GFP_nuctrans	263

Characterization assays

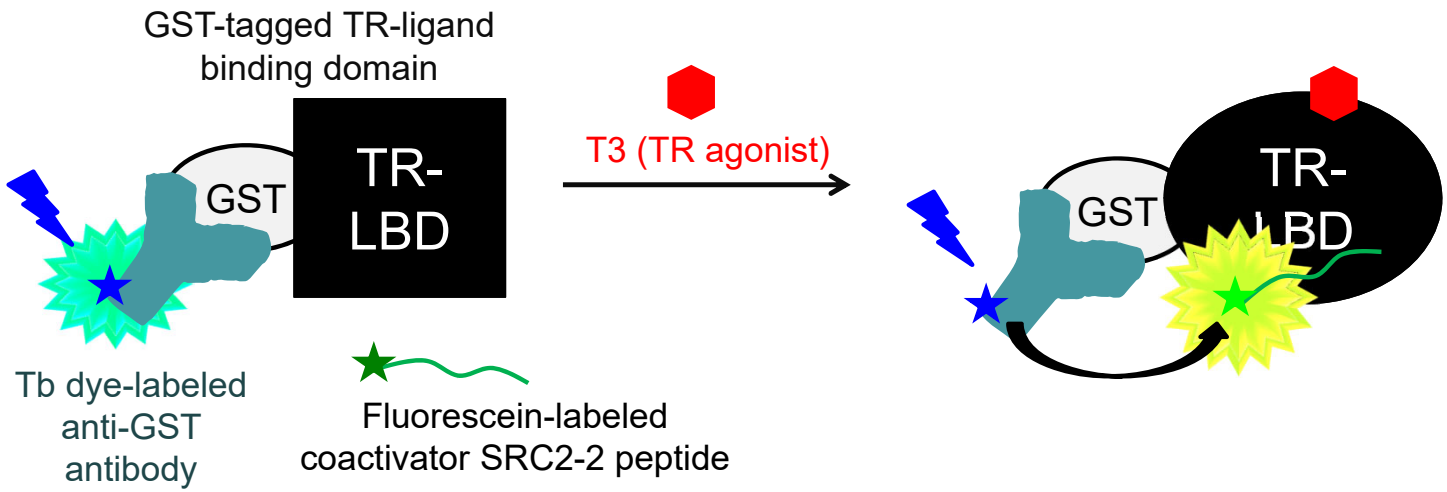
ASSAYS
Rat pituitary GH3 cell line*
expressing endogenous TRα
and TRβ, with TRE regulating
luciferase expression
Cell viability

ASSAYS
GH3
GAL4-TRβ (human)
GAL4-RXRα (human)
TRα/TRβ coactivator recruitment
TR nuclear translocation

* *Developed by Albertinka Murk, Wageningen University, the Netherlands*

TR Modulator Hit Characterization: TR Coactivator Assay (Invitrogen):

- Direct-acting modulators should regulate coactivator recruitment
- Test in both agonist (recruitment) and antagonist (disassociation) format

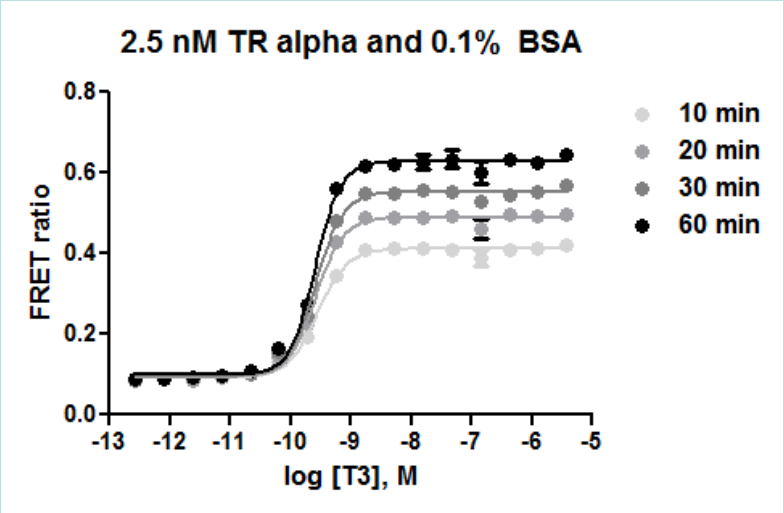


FRET = $I_{em, Fl}/I_{em, Tb}$

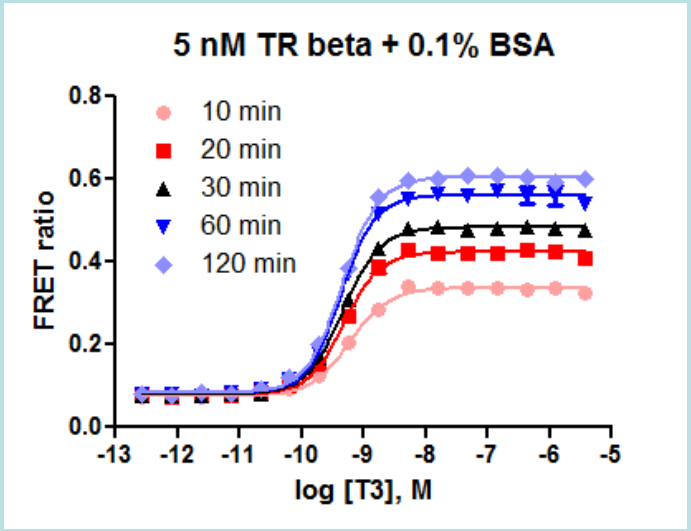
Low FRET

High FRET

Optimization of TR Coactivator Assay



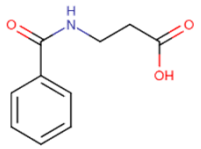
2.5 nM TRα-LBD	S/B	CV%	EC50 (M)
10 min	5.0	3.6	2.786E-10
20 min	5.9	3.3	2.605E-10
30 min	6.7	4.2	2.629E-10
60 min	7.0	4.6	2.571E-10



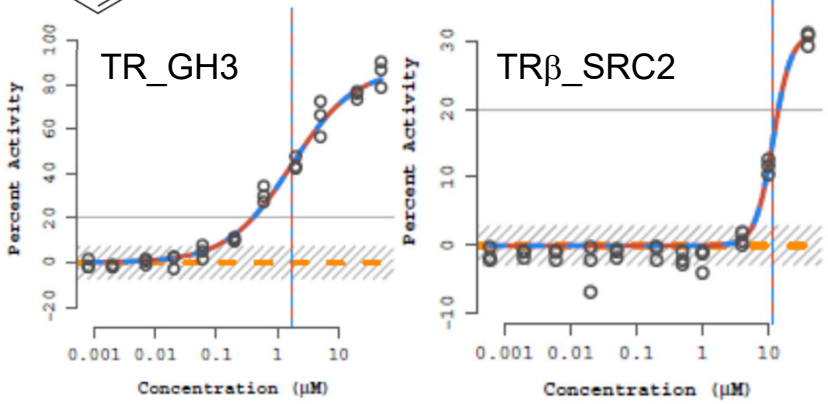
TRβ-LBD	S/B	CV%	EC50 (M)
10 min	4.4	3.9	5.911E-10
20 min	5.3	4.7	4.786E-10
30 min	6.1	4.6	4.661E-10
60 min	6.8	4.8	4.380E-10
120 min	7.4	5.7	4.507E-10

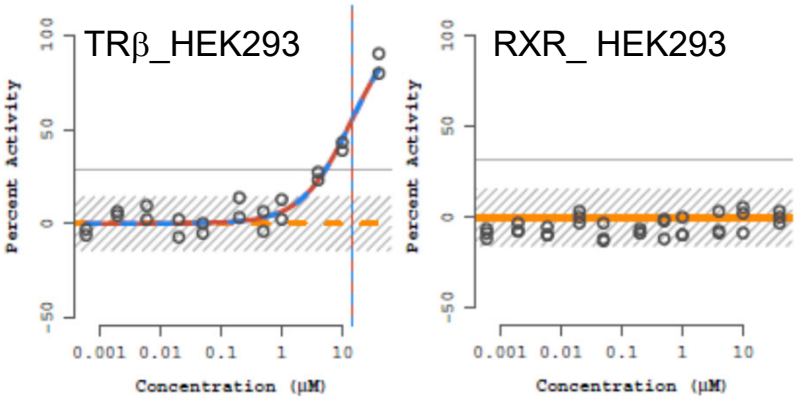
Example Agonists

Direct TR Agonist

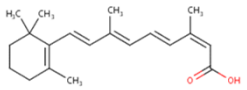


Betamipron

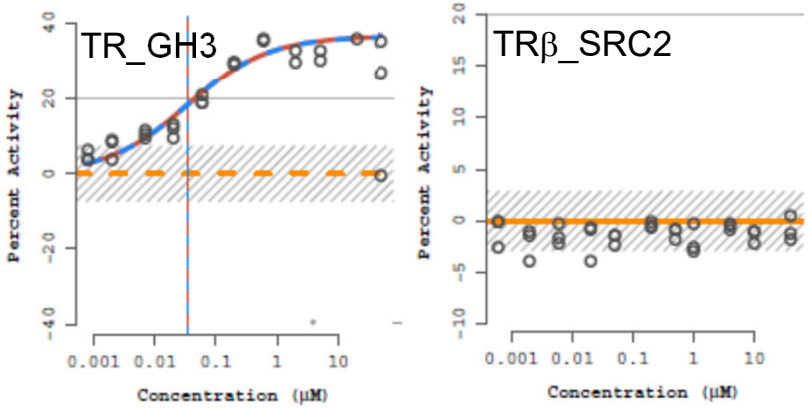


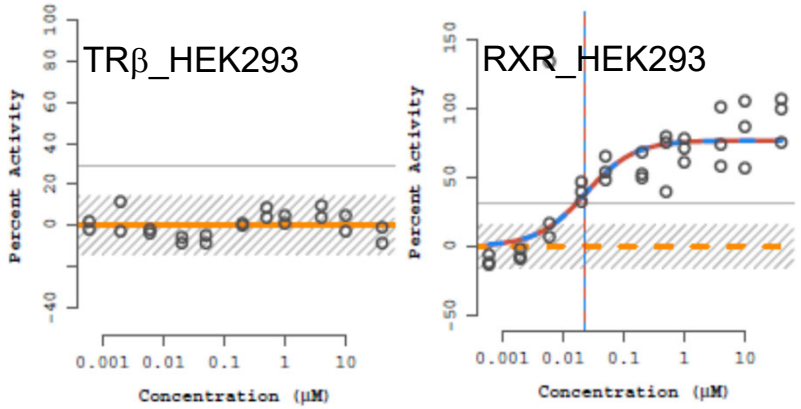


Indirect TR Agonist/RXR Agonist



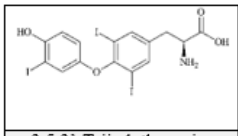
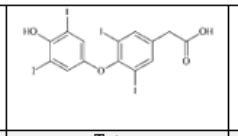
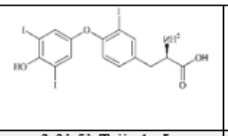
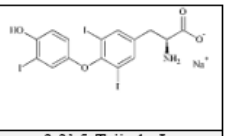
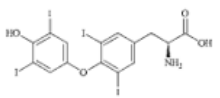
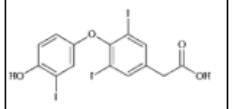
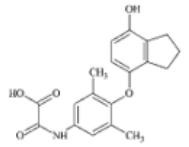
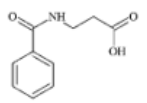
13-cis retinoic acid



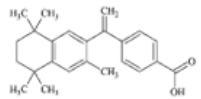
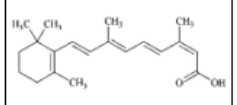
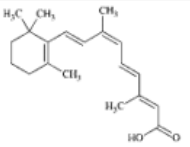
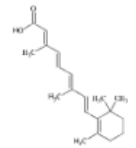
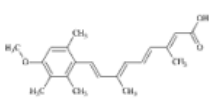
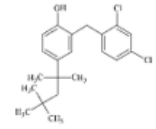
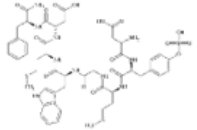


TR Agonists

Direct

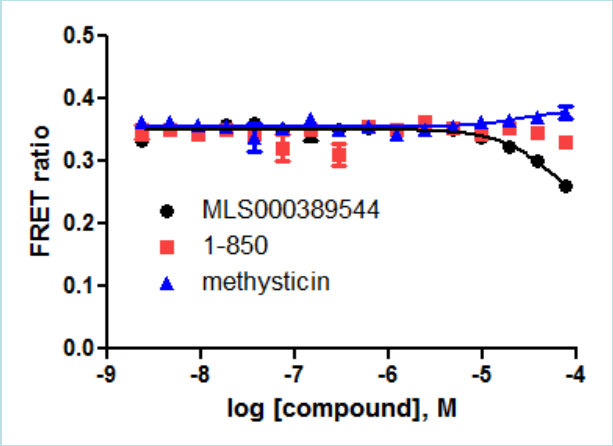
			
3,5,3'-Triiodothyronine 6893-02-3	Tetrac 67-30-1	3,3',5'-Triiodo-L-thyronine 5817-39-0	3,3',5-Triiodo-L-thyronine 55-06-1
			
Levothyroxine 51-48-9	Tiratricol 51-24-1	CP-634384 290352-28-2	Betamipron 3440-28-6

Indirect

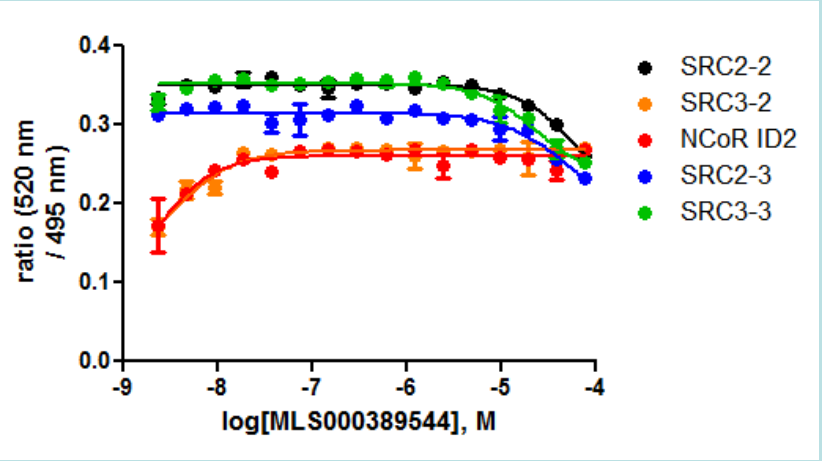
			
Bexarotene 153559-49-0	13-cis Retinoic acid 4759-48-2	9-cis Retinoic acid 5300-03-8	All-trans-retinoic acid 302-79-4
			
Acitretin 55079-83-9	Clofoctol 37693-01-9	Sincalide 25126-32-3	

TR-FRET TRβ Coactivator Assay, Antagonist Mode

Three known TR antagonists tested with the SRC2-2 peptide:



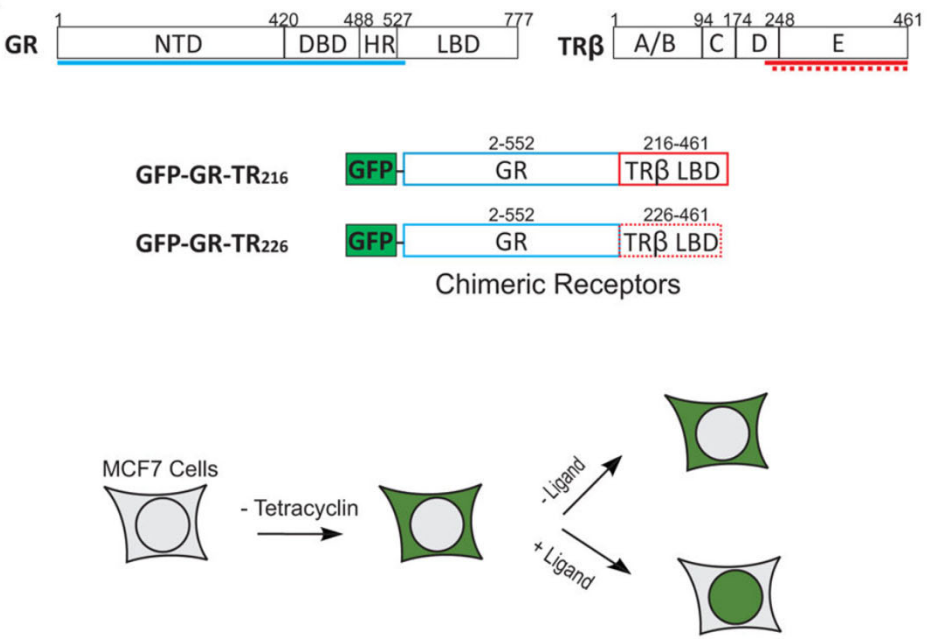
MLS000389544 tested on various coactivator peptides:

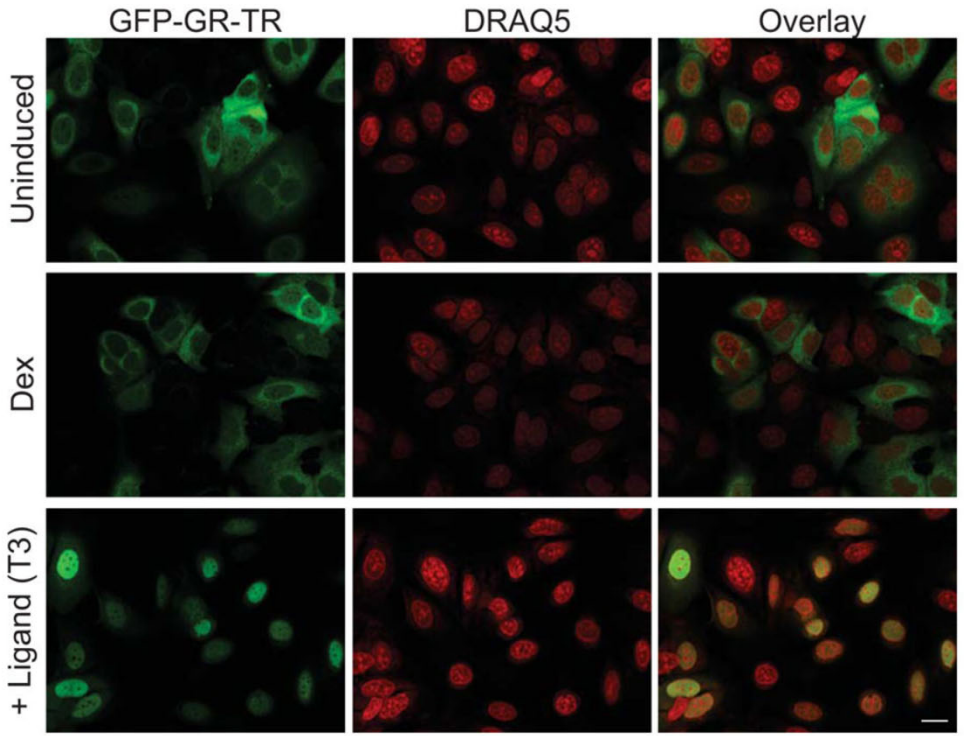


No understanding of why this assay failed.

Reference for MLS000389544: J Biomol Screen. 2011 Jul;16(6):618-27.

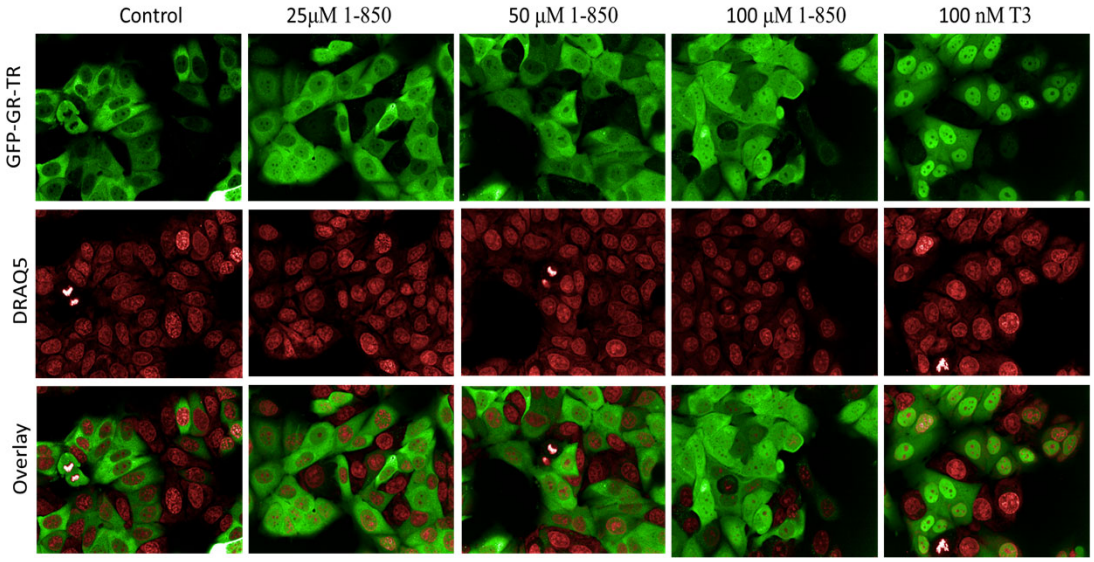
Development of a TR Nuclear Localization Assay

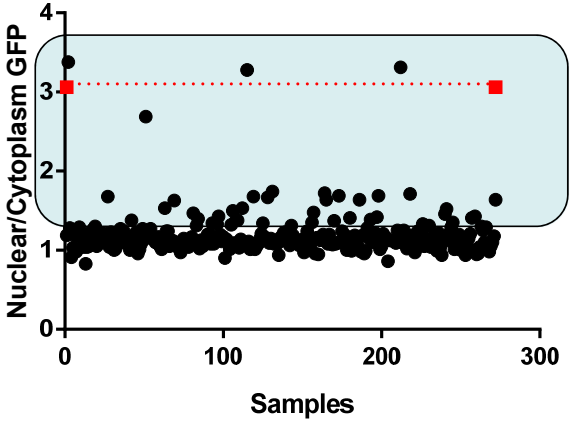




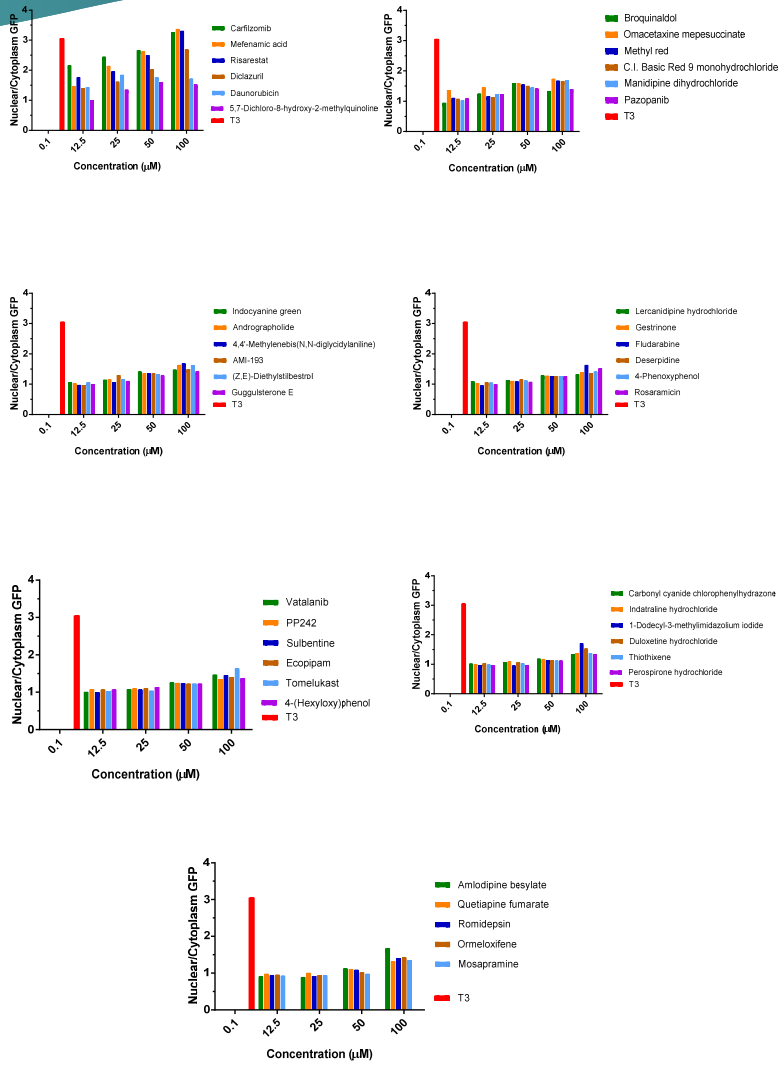
Stavreva et al., Toxicology 368–369: 69-79, 2016.

TR Nuclear Translocation Assay



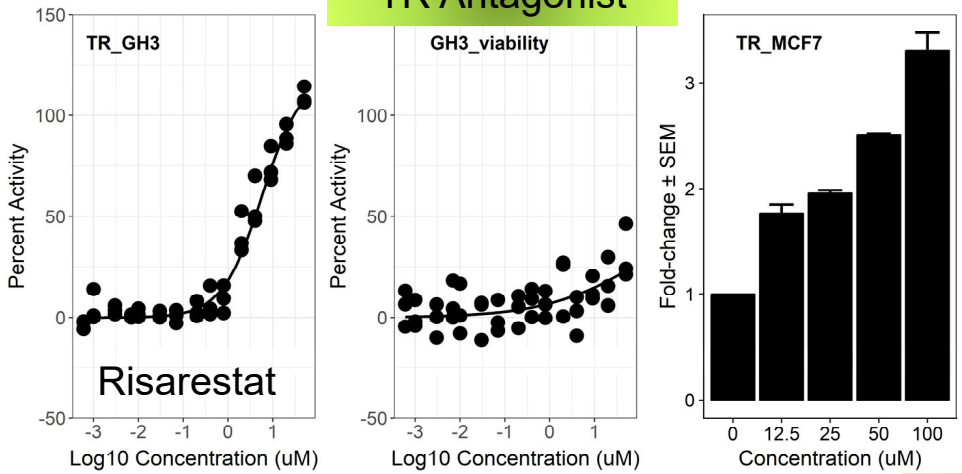


Screened 300 chemicals

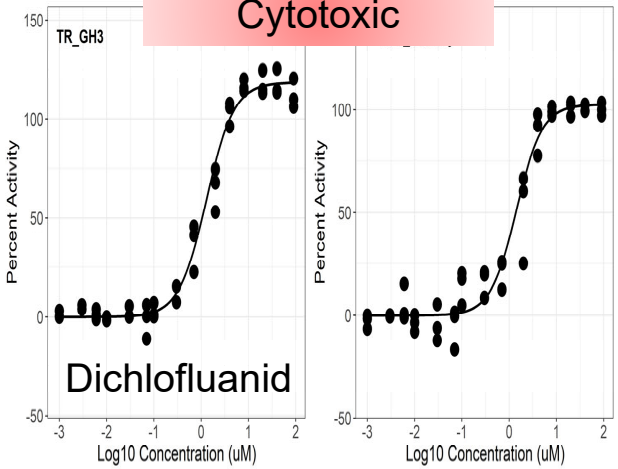


Antagonist Characterization Examples

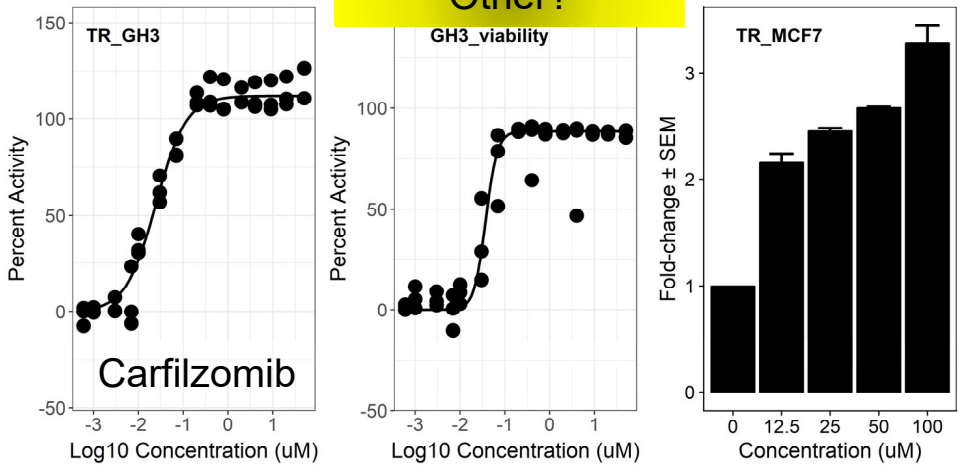
TR Antagonist



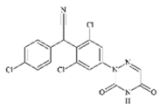
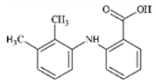
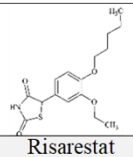
Cytotoxic



Other?



TR Antagonist Candidates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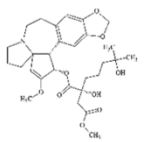	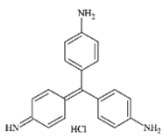	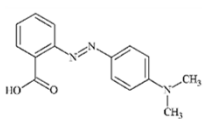	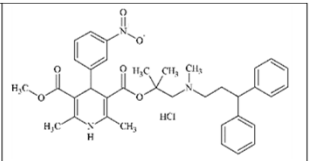
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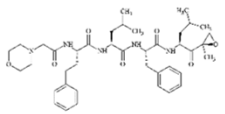	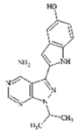	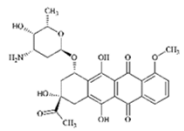	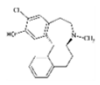
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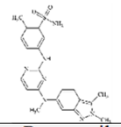
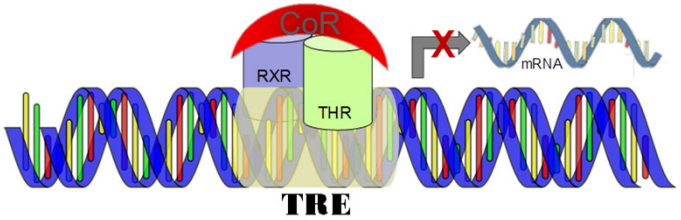
			
Pazopanib 444731-52-6			

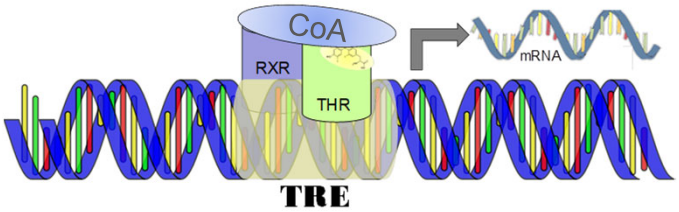
Modes of TR Modulation

Ligand-Independent Repression
Antagonism

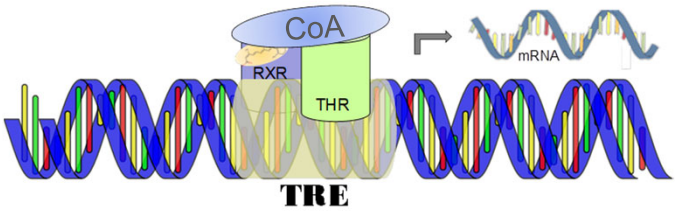


apo receptor

Agonism

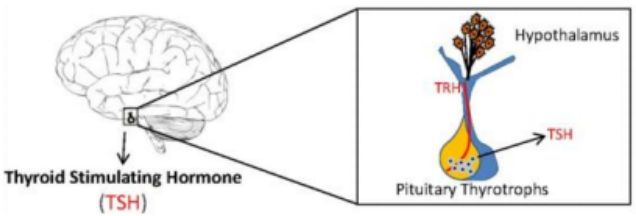


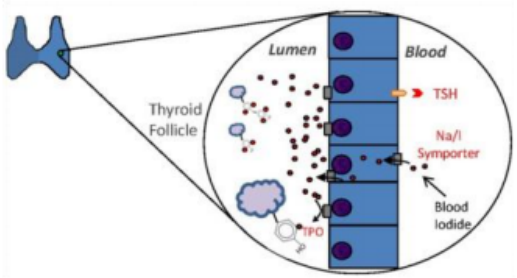
Agonism by Permissive
Heterodimer

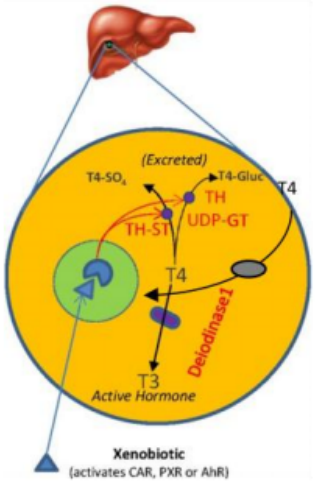


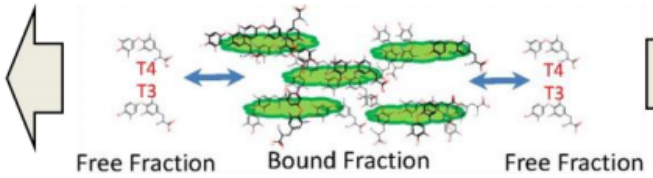
Physiological/Toxicological
Relevance?

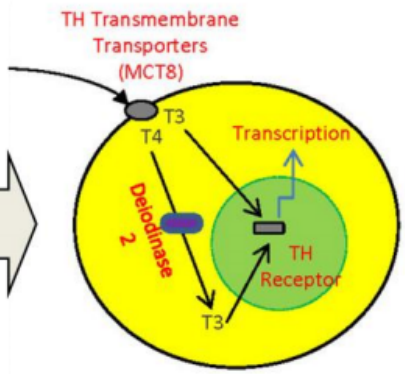
Thyroid Axis Targets









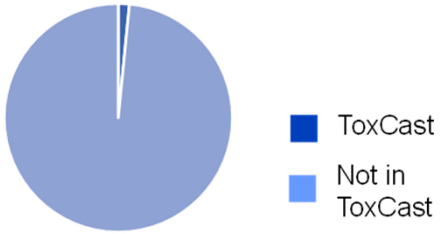


Molecular Target	Screening Assay Status	
	Existing	In Development
TRH Receptor	X (ToxCast)	
TSH Receptor	X	
Sodium-Iodide Symporter (NIS)	X	
Pendrin		
Dual Oxidase (DUOX)		
Thyroperoxidase (TPO)	X	
TH Serum Transport Proteins	X	
TH Membrane Transporters		
Iodothyronine Deiodinase Type I	X	
Iodothyronine Deiodinase Type II	X	
Iodothyronine Deiodinase Type III	X	
Iodotyrosine Deiodinase		X
Nuclear Receptors	X (ToxCast)	
Sulfation and Glucuronidation		
Alanine Side Chain Activation		
TH Receptor Binding		
TH Transcription (Agonist/Antagonist)	X (ToxCast)	

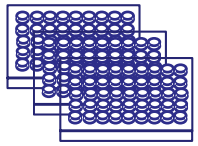
Some Existing Limitations in High-Throughput and *In Vitro* Test Systems

Human Focus

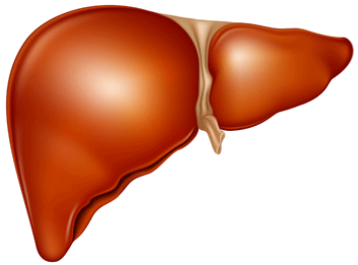
Biological Coverage (Gene Basis)



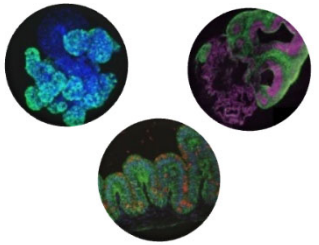
Chemical Coverage and Specific Chemical Types (e.g., VOCs)



Metabolic Competence

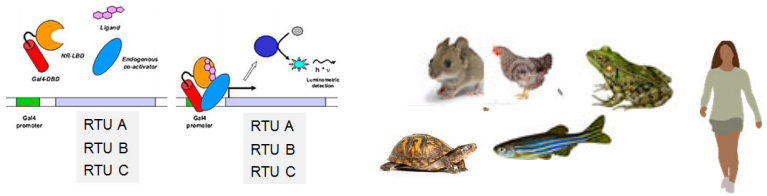


Organ and Tissue Responses

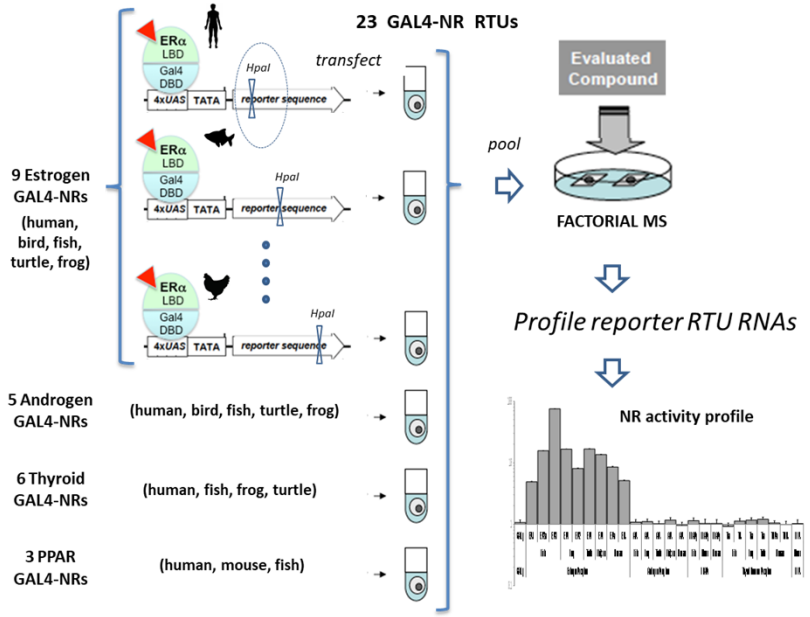


Assessing Cross-Species Differences in Response

Multispecies Attagene *Trans* Reporter Assay



Highly multiplexed reporter gene assay

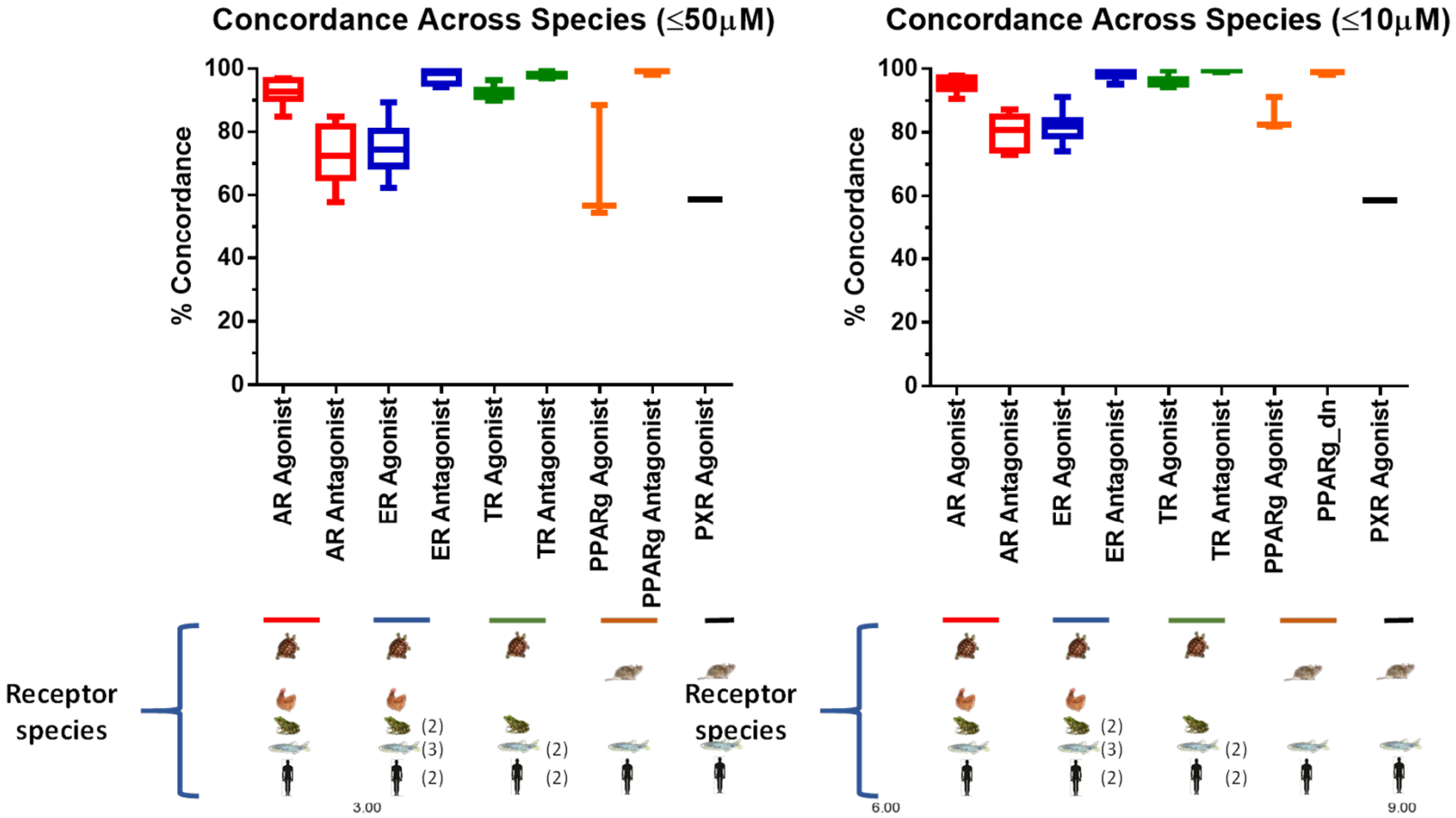


Houck et al., unpublished

NR family	NR	Class	Species	Sequence ID
Estrogen	ER1	Fish	*Danio rerio*	BC162466
	ER2a		*Danio rerio*	BC044349
	ER2b		*Danio rerio*	BC086848
	ER1	Amphibian	*Xenopus laevis*	NM_001089617
	ER2		*Xenopus laevis*	NM_001130954
	ER1	Reptilian	*Chrysemys picta*	NM_001282246
	ER1	Avian	*Gallus gallus*	NM_205183
	ERa	Mammalian	*Homo Sapiens*	NM_000125
	ERb		*Homo Sapiens*	NM_001437
Androgen	AR	Fish	*Danio rerio*	NM_001083123
	AR	Amphibian	*Xenopus laevis*	NM_001090884
	AR	Reptilian	*Chrysemys picta*	XM_005279527
	AR	Avian	*Gallus gallus*	NM_001040090
	AR	Mammalian	*Homo Sapiens*	NM_000044
Thyroid	TRa	Fish	*Danio rerio*	BC096778
	TRb		*Danio rerio*	BC163114
	TRa	Amphibian	*Xenopus laevis*	NM_001088126
	TRa	Reptilian	*Chrysemys picta*	XM_005294120
	THRa	Mammalian	*Homo Sapiens*	NM_199334
	THRb		*Homo Sapiens*	NM_000461
PPAR	PPARg	Fish	*Danio rerio*	NM_131467
	PPARg	Mammalian	*Mus musculus*	NM_001127330
	PPARg		*Homo Sapiens*	BC006811

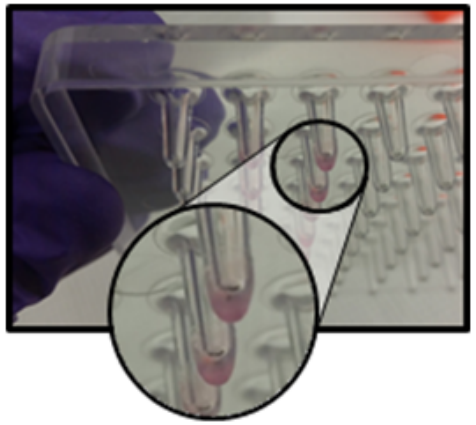
- Host cell: human HepG2
- Agonist mode for all receptors
- Antagonist for ER and AR

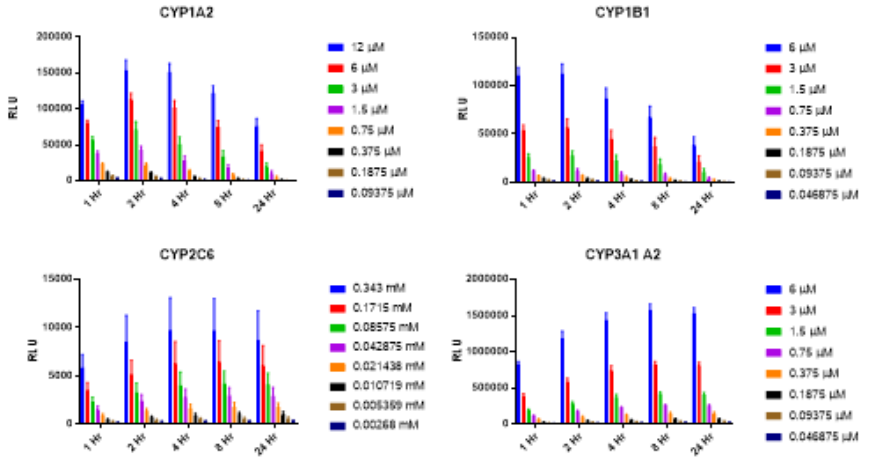
Cross-Species Differences in Nuclear Receptor Responses

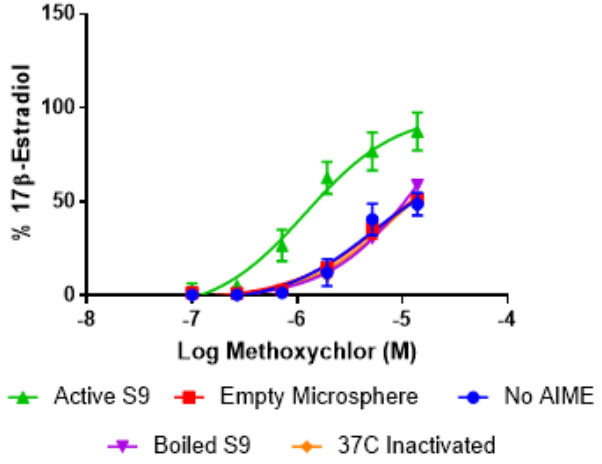


- 180 Chemicals tested in concentration-response
- Chemicals selected for NR activity

Assays Retrofit for Xenobiotic Metabolism: Extracellular



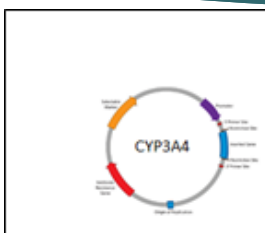


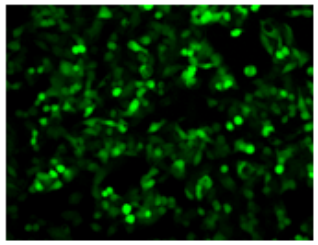


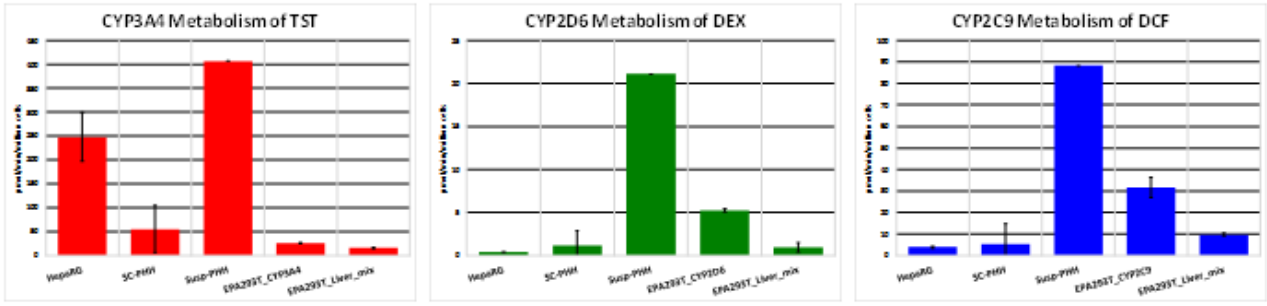
96-Well	EC50 (µM)	EC50 Potency Shift
Active S9	0.71	
Heat Inactivated S9	6.8	9.6
No AIME	4.98	7.0
384-Well	**EC50 (µM)**	**EC50 Potency Shift**
Active S9	1.2	
Heat Inactivated S9	15.9	13.2
No AIME	5.1	4.2

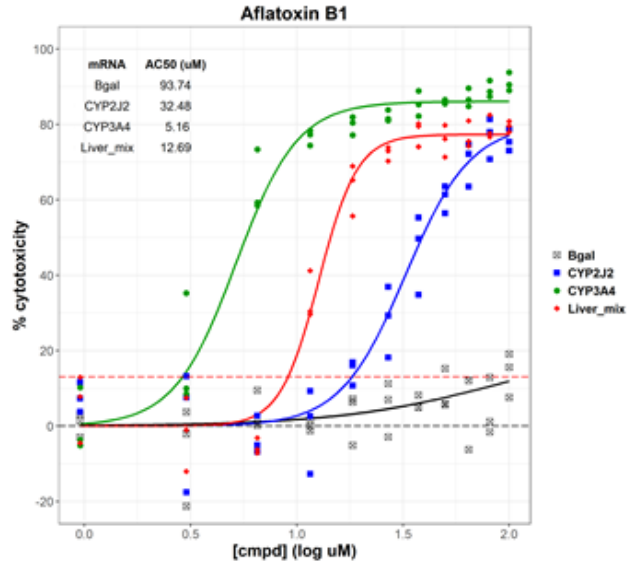
DeGroot, Simmons, and Deisenroth, Unpublished

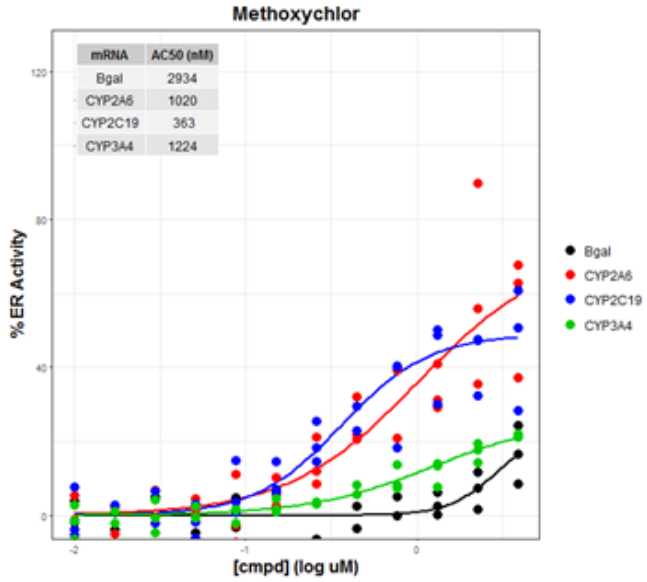
Assays Retrofit for Xenobiotic Metabolism: Intracellular







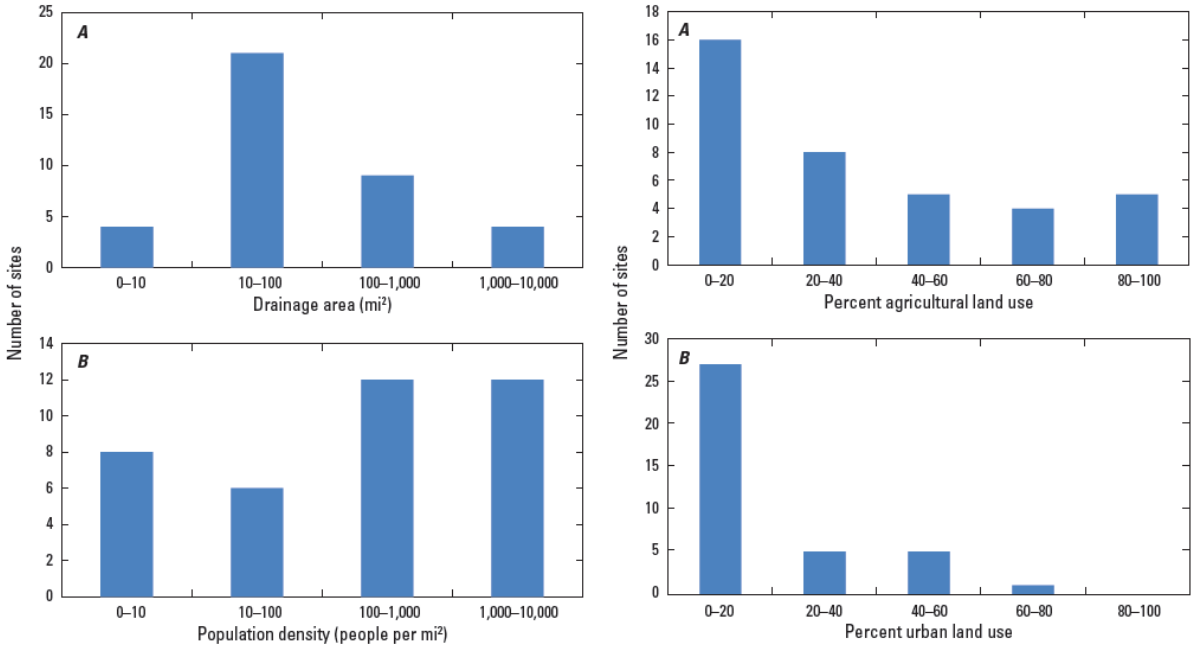


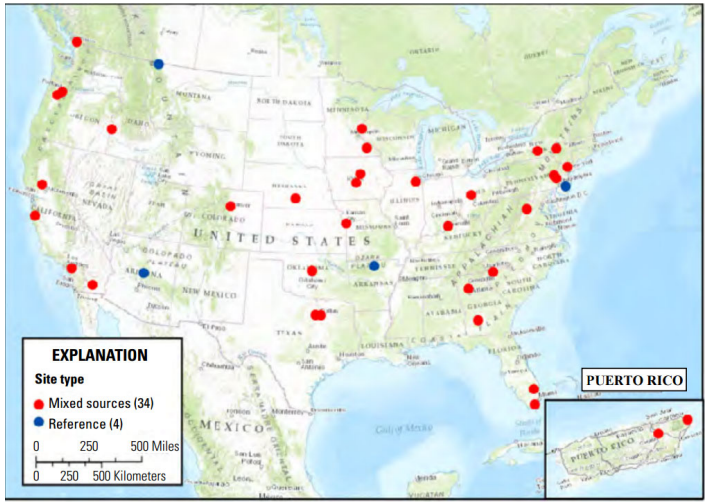


Simmons et al., J Pharmacol Tox Methods, 2018

Environmental Monitoring Application: Nationwide Streams Surveillance

- 38 total sites (4 reference sites) across US and PR
- Water samples collected 2012-2014
- Locations varied by watershed drainage





Brett Blackwell/ORD/EPA

Bioassay Analysis Workflow

Ambient Water Sample

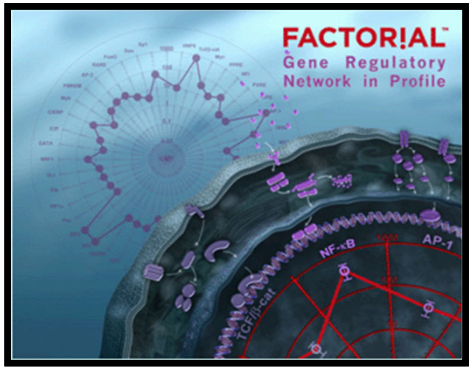
Filtered

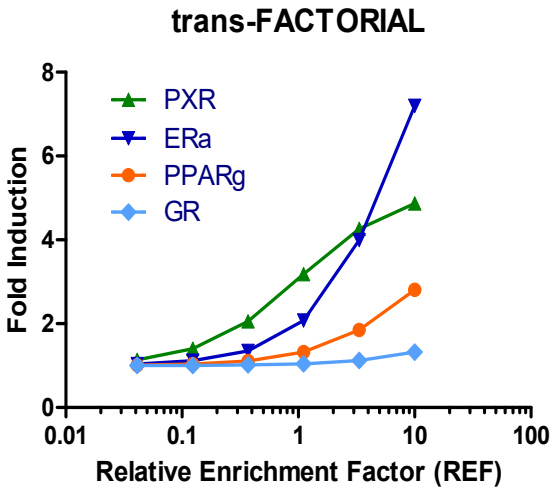
Extracted 200mg HLB

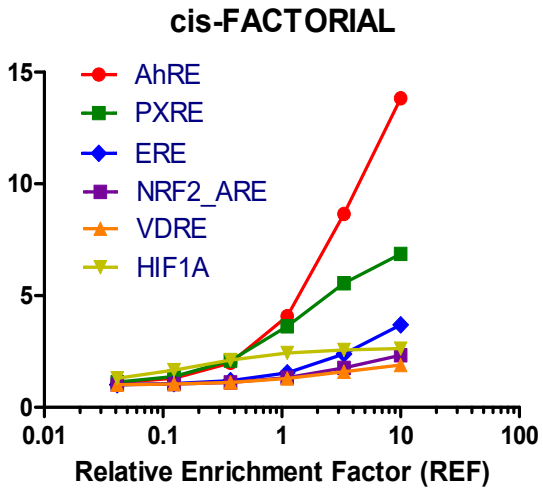
"Unknown" Chemical Mixture

DMSO

1000x conc.







Extract Analysis

- 6-point curve; 3-fold dilution
- 24h exposure

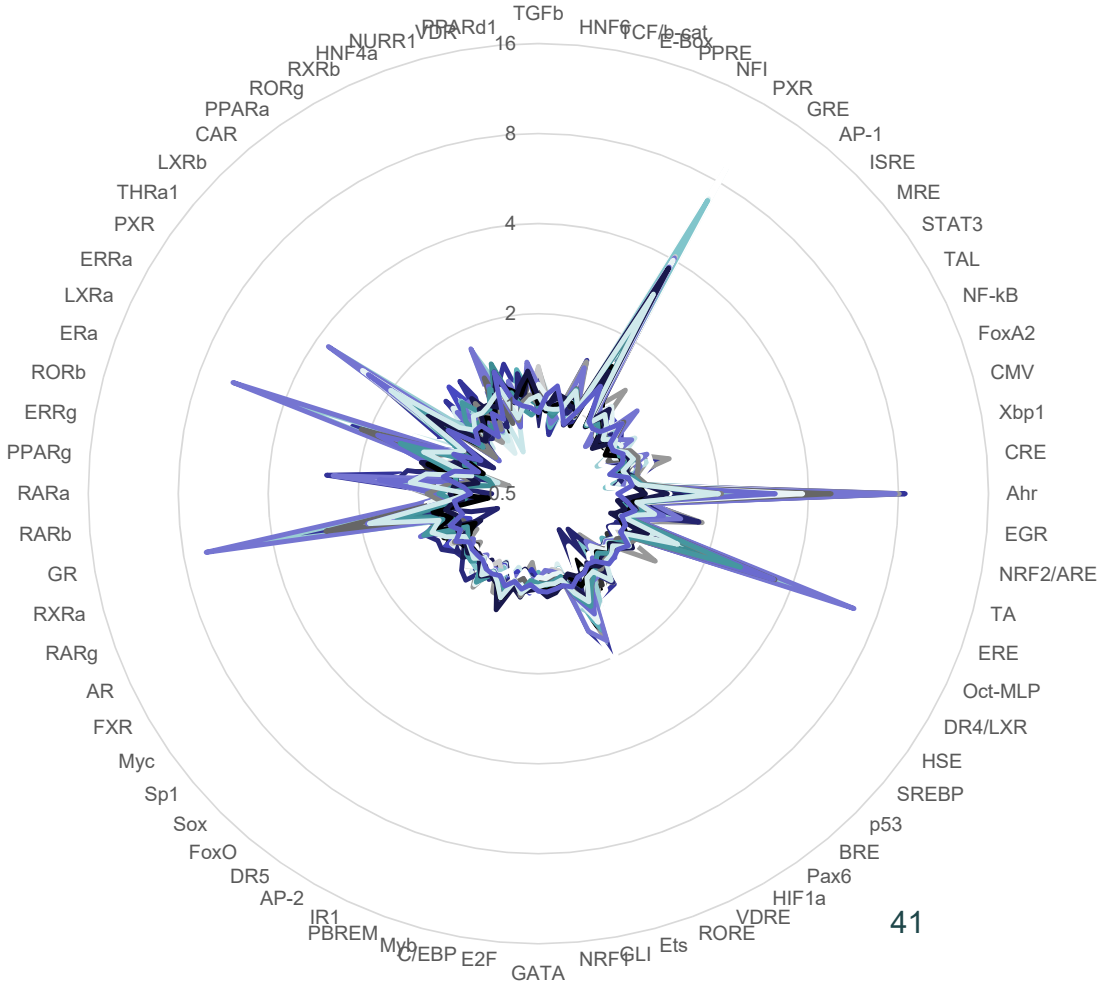
- Area Under Curve (AUC)
  - Response relative to extract blank

Bioassay Results

- 26/70 endpoints AUC >1.25-fold (borderline active)
- 11/70 endpoints AUC >1.5-fold (active)

<u>Active Endpoints</u>

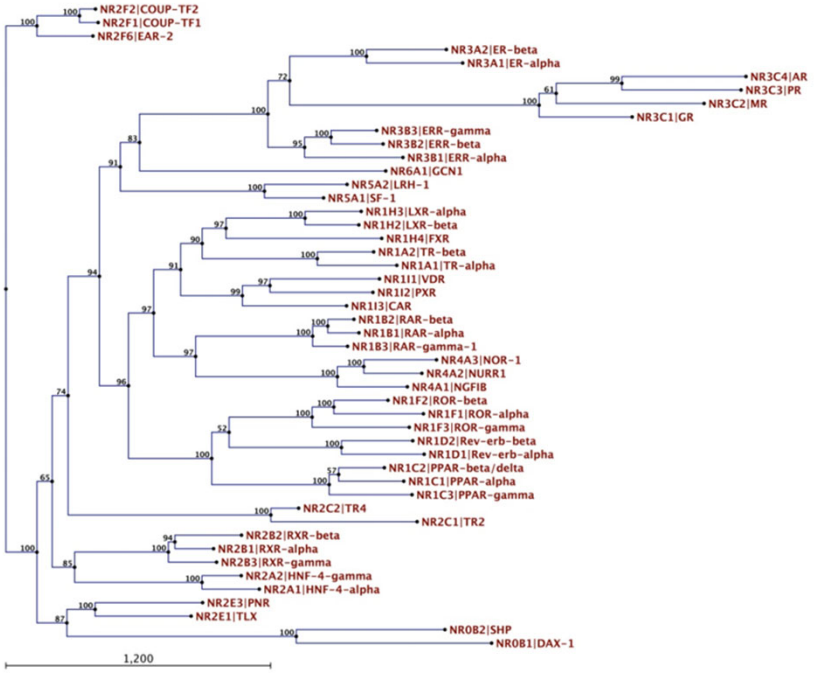
- PXRE, PXR, AhRE – 30-36 sites
- ERE – 17 sites
- ERα, PPARγ – 10 sites
- GR, VDRE, NRF2 – 6-8 sites
- RORE, RXRβ – 2 sites



Concluding Remarks

- In vitro/alternative and computational approaches are valuable for chemical prioritization, especially where we understand the targets and toxicity pathways
- Nuclear receptors are an inherently important target of environmental chemicals

- Endocrine disruption is one important mode of action mediated by NR's but there are many more receptors with varied, important physiology
- Using high-throughput approaches will require systematically addressing key technical and data analysis challenges
  - assay interferences
  - selective receptor modulators
  - cytotoxicity



Thank You for Your Attention!

- ICCVAM
  - Nichole Kleinstreuer
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  - Anthony Schroeder
  - Gerald Ankley
  - Brett R Blackwell

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  - Matt Martin
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  - Eric Watt
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