NexGen

Human Health Risk Assessment Approaches for Chemicals with Limited Data

Dr. David Dix

U.S. EPA, Office of Research and Development

EPA United States Environmental Protection Agency

NIEHS National Institute of Environmental Health Sciences

NCGC NIH Chemical Genomics Center

CDC Centers for Disease Control and Prevention

Office of Environmental Health Hazard Assessment, State of California

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Advancing the Next Generation (NexGen) of Risk Assessment: Public Dialogue Conference
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Office of Research and Development

The views expressed in this presentation are those of the author and do not necessarily reflect the views or policies of the U.S. EPA

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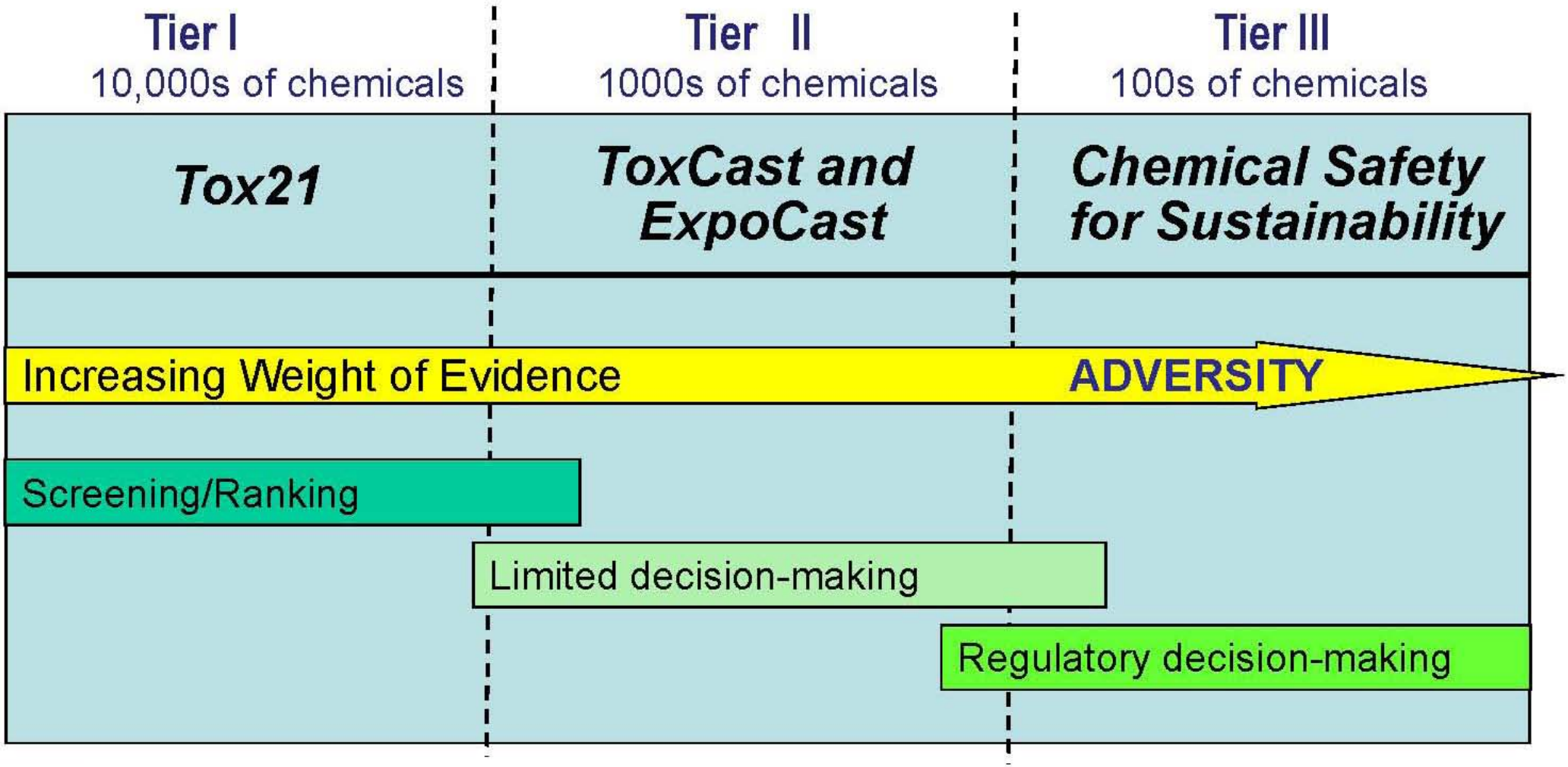
NexGen Risk Assessments: Screening to Biological Pathway Altering Doses and Ultimately Adversity



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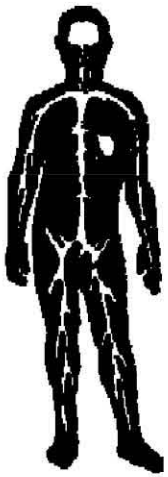
CompTox and Chemical Safety for Sustainability: Supporting High Throughput Risk Assessments

- Understand chemical toxicity at a molecular level
- Understand using as few animal as possible
- Build predictive models
- Initially screen and prioritization, eventually provide quantitative points-of-departure
- Assess many chemicals – deal with the data gaps

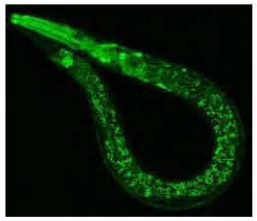
High-Throughput Screening Assays

batch testing of chemicals for pharmacological/toxicological endpoints using automated liquid handling, detectors, and data acquisition

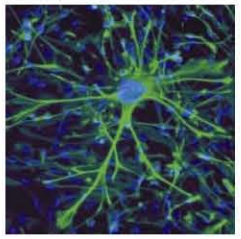


10s-100s/yr

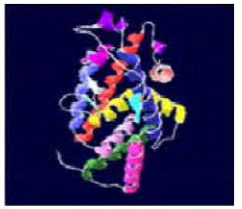
Gene-expression

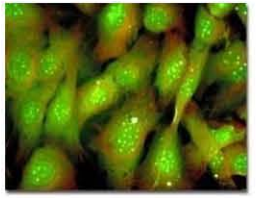


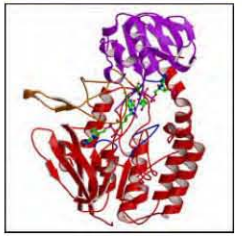
10s-100s/day



1000s/day







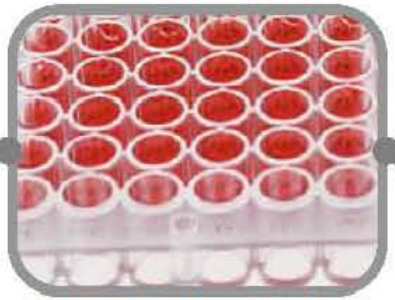
10,000s-100,000s/day

Human Relevance/
Cost/Complexity

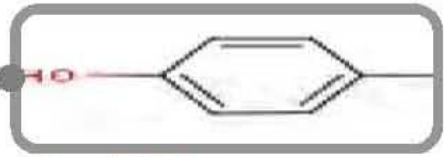
Throughput/
Simplicity

High Throughput Screening 101

HTS Robotic Platform

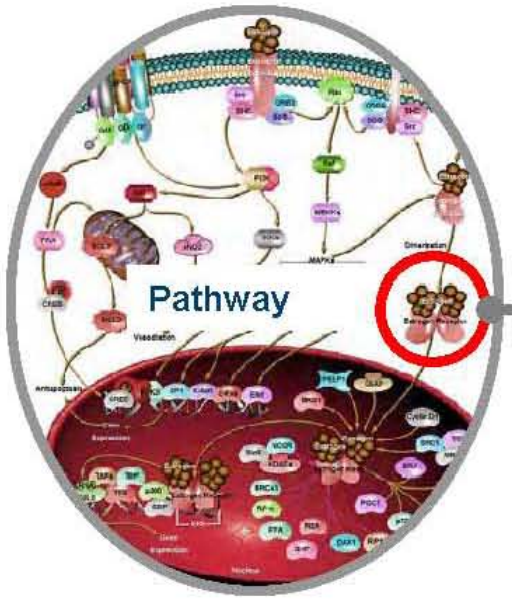


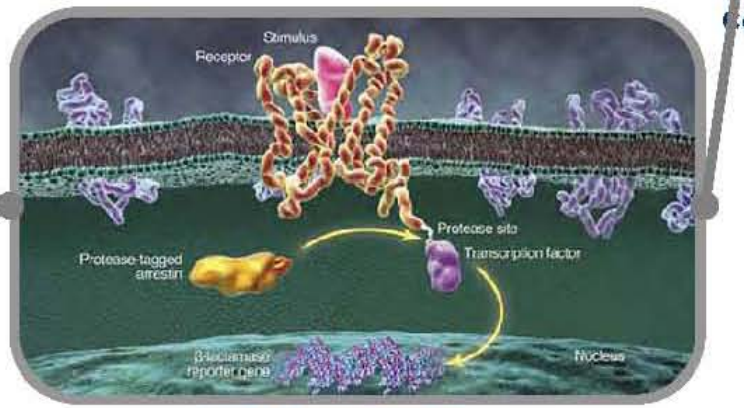
96-, 384-, 1536 Well Plates



Chemical Exposure

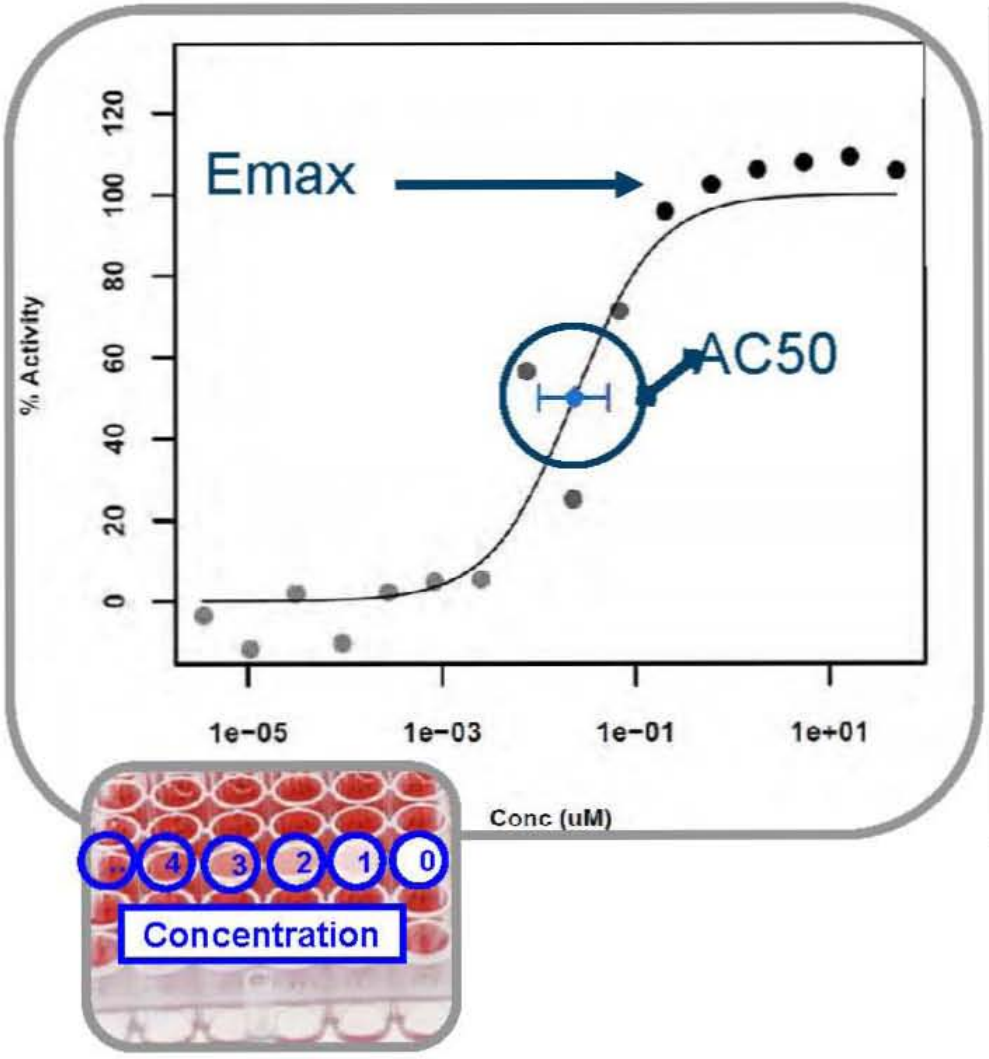
Cell Population

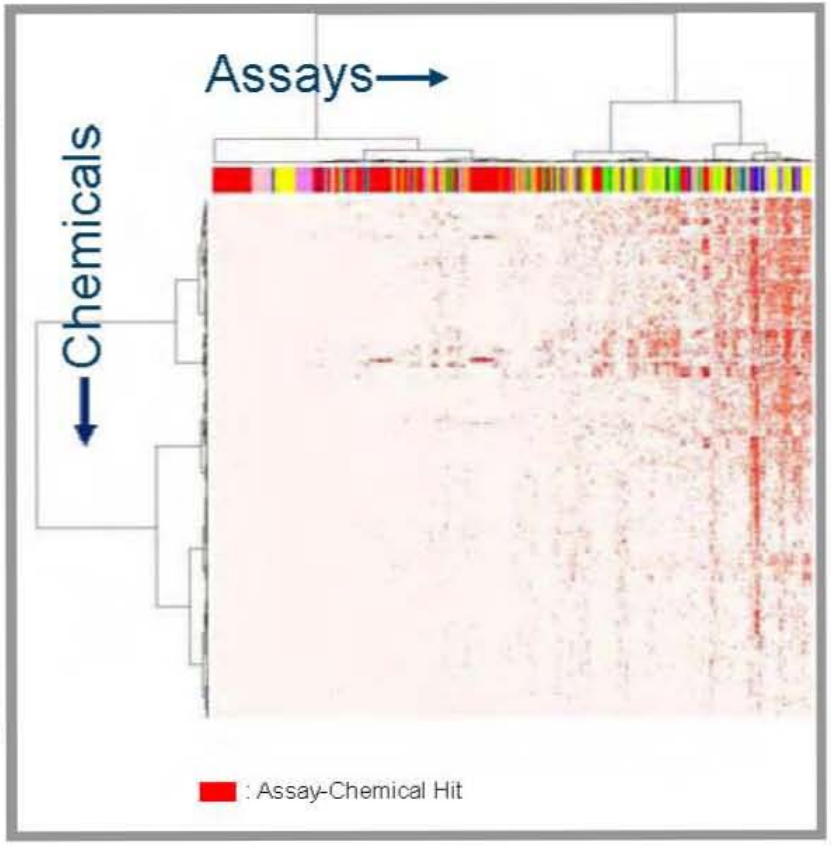




Assay Target Biology
(e.g., Estrogen Receptor)

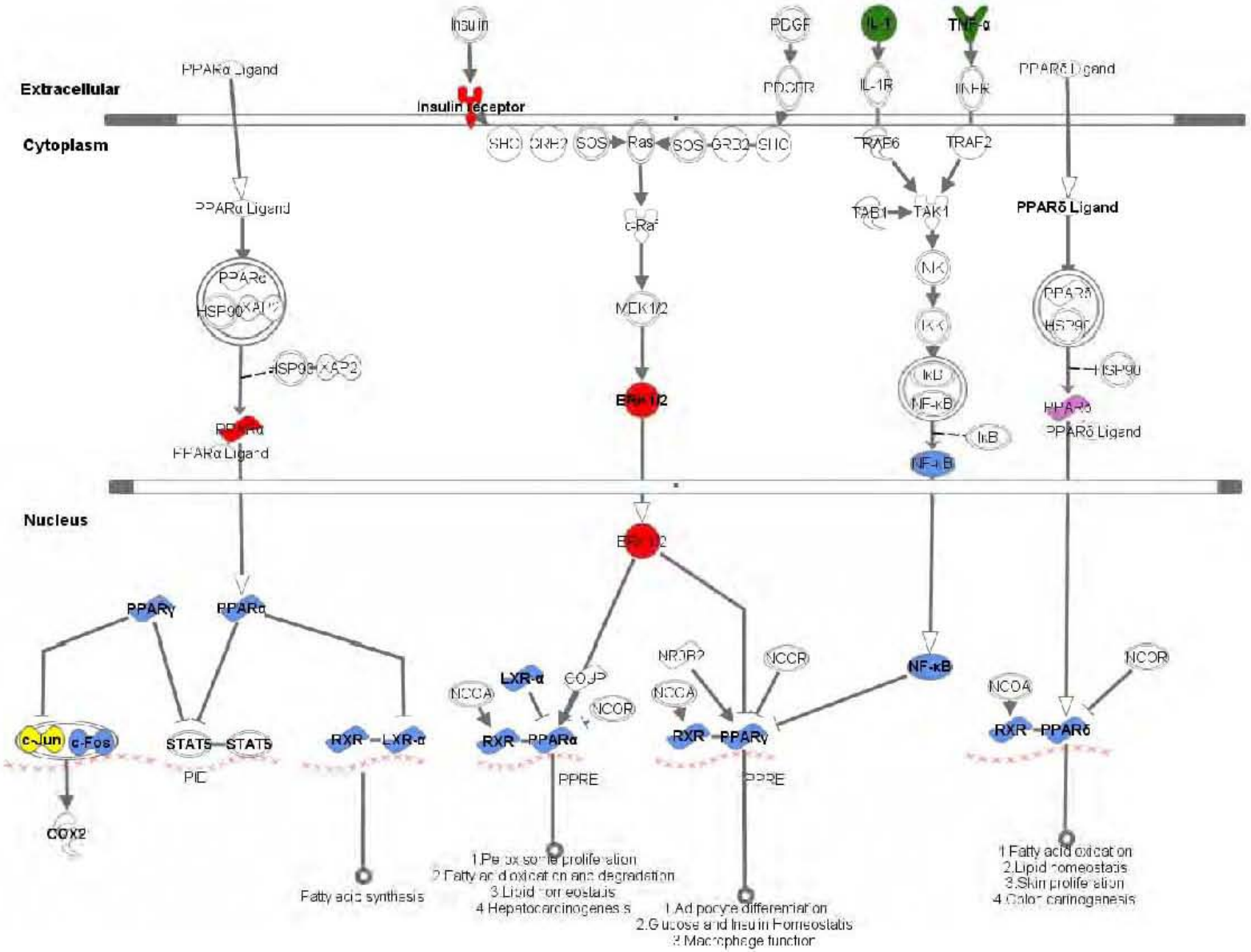
Tox21/ToxCast Data Analysis



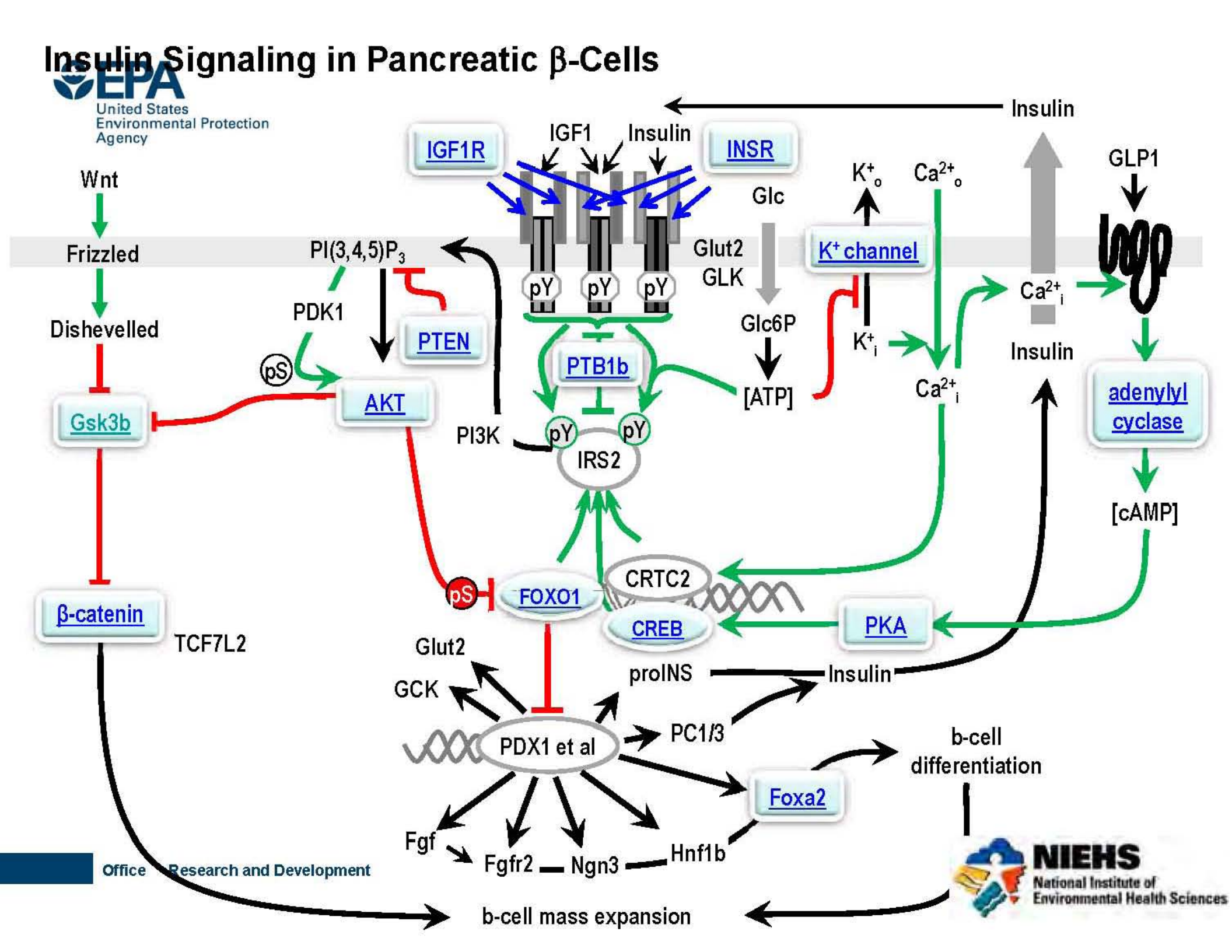


ToxCast: Multiple Targets per Pathway

- **Biologically Multiplexed Activity Profiling (BioMAP)**
- **Multiplex Transcription Reporter Assay**
- **Cell-based HTS Assays**
- **Cell-free HTS Assays**
- **High Content Cell Imaging Assays**



Insulin Signaling in Pancreatic β-Cells



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Sample Output From Signaling Hyperlinks to ToxCastDB

GSK3b

You are here: EPA Home » National Center for Computational Toxicology » ToxCastDB » Assay

ACToR | ToxRefDB | ToxCastDB | ExpoCastDB | DSSToxDB

Home | Basic Info | Data Collection List | Chemical List | Genes Associated with Assays | Help

Assay: Novascreen Human GSK3b

Assay Id:	914
Source	Novascreen
Source Name AID	NVS_ENZ_hGSK3b
Name	Novascreen Human GSK3b
Description	Human GSK3b Fluorescein-peptide
Number of Substances	320
Number of Components	1
Species	Homo sapiens

Parameters

Parameter	Value
CATALOG NUMBER	203-0425
ASSAY CATEGORY	Enzyme Inhibition
ASSAY CATEGORY	In vitro (Biochemical)
ASSAY TARGET	GSK3b
ASSAY TARGET FAMILY	Kinase
ASSAY TARGET SOURCE	Recombinant
ASSAY GENE ID	2932
ASSAY GENE NAME	GSK3B
ASSAY TECHNOLOGY	Fluorescence -EMO
ASSAY REFERENCE COMPOUND	Staurosporine
ASSAY NOTE	KINASE
ASSAY SUBSTRATE NAME	CMGC group
ASSAY ATP CONCENTRATION (M)	NCCT_v2
ASSAY LIGAND NAME	1.5 E-06
ASSAY LIGAND CONCENTRATION (M)	1.20E-05
ASSAY BMAX	Fluorescein-peptide + ATP --> Fluorescein-phosphopeptide + ADP

Data

Name	CASRN	NVS_ENZ_hGSK3b (uM)
Mancozeb	8018-01-7	0.27
Maneb	12427-38-2	0.32
Metiram-zinc	9006-42-2	16.0

number of "actives" = 3

CREB

Assay: Attagene Factorial cis CRE

Assay Id:	16
Source	Attagene
Source Name AID	ATG_CRE_CIS
Name	Attagene Factorial cis CRE
Description	Factorial reporter gene assay
Number of Substances	320
Number of Components	1
Species	Homo sapiens

Parameters

Parameter	Value
ASSAY URL	Link Out EXIT Disclaimer
ASSAY CATEGORY	In vitro (Cellular)
ASSAY TARGET	cAMP Response Element
ASSAY TARGET FAMILY	Transcription Factor
ASSAY TARGET SOURCE	Cell line
ASSAY TARGET SOURCE TYPE	HepG2
ASSAY GENE ID	10488
ASSAY GENE NAME	CREB3
ASSAY TECHNOLOGY	Reporter gene assay
ASSAY MODE	DNA sequencer
ASSAY REFERENCE COMPOUND	Forskolin cAMP
ASSAY NOTE	"Multiplexed reporter gene assay; cAMP, cGMP, NO receptor, GPCR pathways"

Data

Name	CASRN	ATG_CRE_CIS (uM)
Alachlor	15972-60-8	3.4
Anilazine	101-05-3	59.0
Azinphos-methyl	86-50-0	27.0
Azoxystrobin	131860-33-8	46.0
Bendiocarb	22781-23-3	51.0
Bisphenol A	80-05-7	30.0
Bromoxynil	1689-84-5	40.0
Chlorpropham	101-21-3	31.0
Cyazofamid	120116-88-3	10.0
Cyprodinil	121552-61-2	23.0
Dazomet	533-74-4	49.0
Allethrin (d-cis,trans)	584-79-2	46.0
Dichlobran	99-30-9	43.0

partial list:
number of total "actives" = 52

NIEHS National Institute of Environmental Health Sciences

http://actor.epa.gov/actor/faces/ToxMiner/Home.jsp

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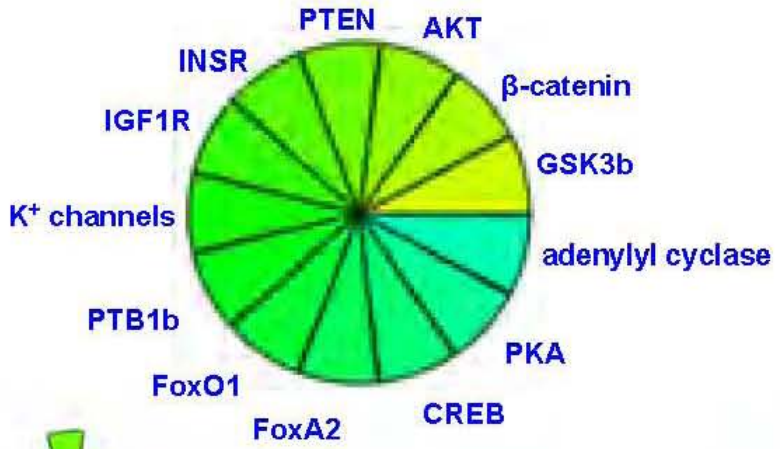
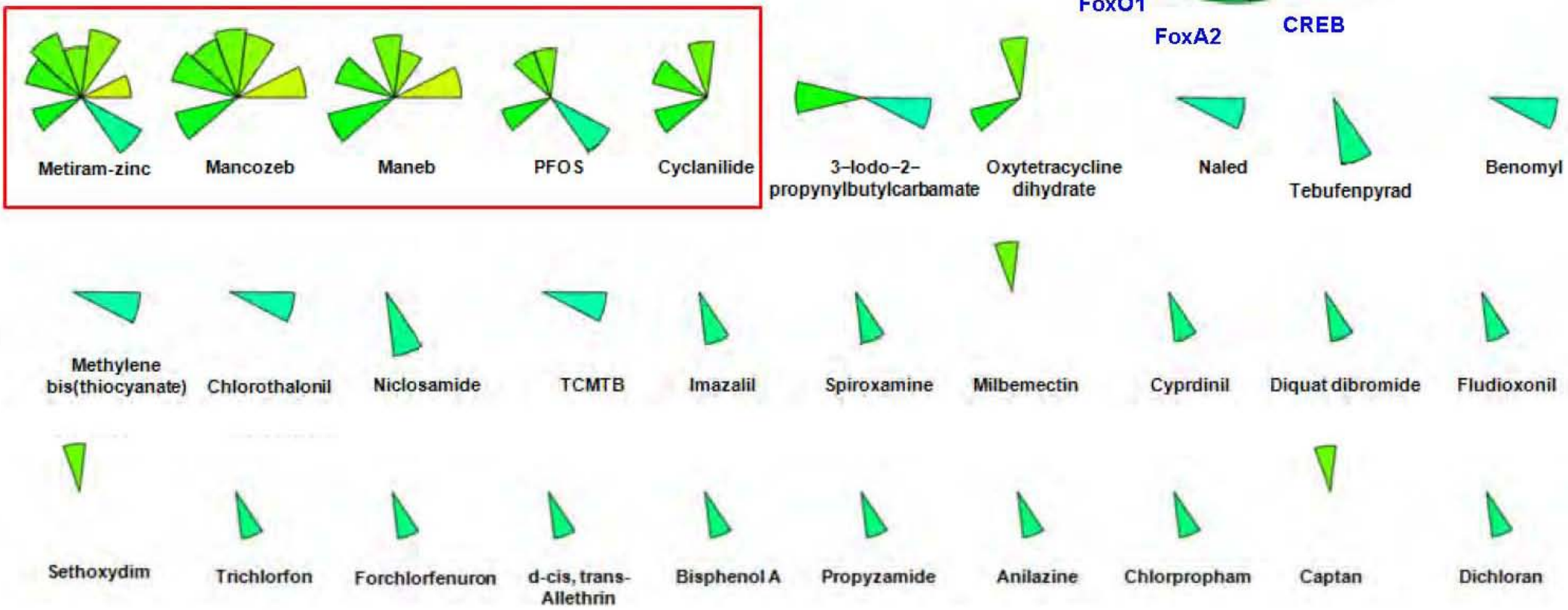
ToxPi™ for Insulin Signaling in Pancreatic β Cells- Top 30 from 309 Chemicals in ToxCast Phase I





http://epa.gov/ncct/ToxPi/

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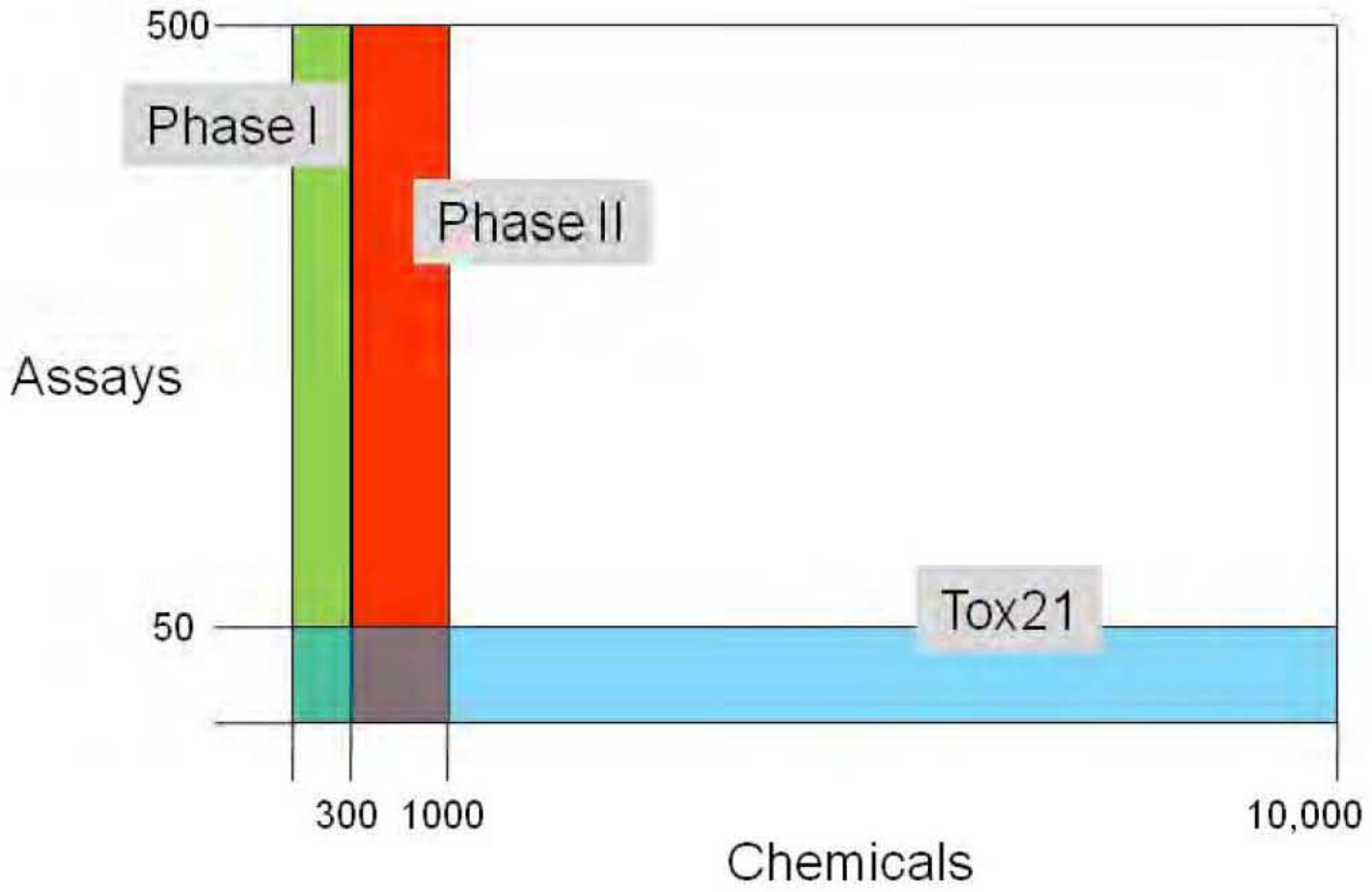
ToxCast and Tox21 Assays and Chemicals



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What is High-Throughput Risk Assessment?

- Where does risk assessment come in?
  - Estimate upper dose that is still protective
  - RfD, BMD, POD
- Where does high-throughput come in?
  - Focus on molecular pathways and targets whose perturbation can lead to adversity
  - Screen hundreds to thousands of chemicals in *in vitro* assays for those targets
  - Get oral dose using H-T pharmacokinetic modeling
- Incorporate population variability and uncertainty

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Why do we need High Throughput Risk Assessment (HTRA)?

- Thousands of chemicals with no or little animal data
- Need starting points for setting health-protective exposure levels
- These starting points can be used to prioritize and target further testing

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HTRA Basic Outline

1. Define molecular pathways linked to adverse outcomes
2. Measure activity *in vitro* in concentration-response (PD)
3. Estimate external dose to internal concentration scaling (PK)
4. Estimate dose at which pathway is perturbed *in vivo*
5. Estimate population variability and uncertainty in PK and PD
6. Estimate lower end of dose range for perturbation of pathway

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HTRA Publication

Chemical Research in Toxicology, in press, 2011

Estimating Toxicity-Related Biological Pathway Altering Doses for High-Throughput Chemical Risk Assessment

Richard S. Judson, Robert J. Kavlock, R. Woodrow Setzer, Elaine A. Cohen Hubal, Matthew T. Martin, Thomas B. Knudsen, Keith A. Houck, Russell S. Thomas, Barbara A. Wetmore, David J. Dix

National Center for Computational Toxicology, Office of Research and Development, U.S. Environmental Protection Agency

The Hamner Institutes for Health Sciences, Research Triangle Park, North Carolina

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What Pathways to Use?

- Start with known targets (genes, proteins) and pathways
- Define levels of evidence and prioritize for analysis
  - **Class 1** - the link between *in vitro* activity and adversity is clear (e.g. cholinesterase activity). There is a single target which, if significantly perturbed, can lead directly to undesirable phenotypic change.
  - **Class 2** - here is an association between perturbations of a pathway and some disease outcome, but the details and causal linkage is not clear (e.g. PPAR pathway perturbations and potential linkage with human disease).
  - **Class 3** - no clear linkage between *in vitro* activity and adverse *in vivo* outcomes is currently known.

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Measuring the Pathways

- ToxCast and Tox21 are using hundreds of assays on thousands of chemicals
- Need to determine concentration at which pathway is "altered"
- Many ways to do this
  - Simple – take minimum AC50 (AC20, etc.) of any assay mapping to the pathway
  - Harder – develop a systems-level model of the pathway and build a probabilistic concentration-response profile
- Add in estimates of population variability and uncertainty

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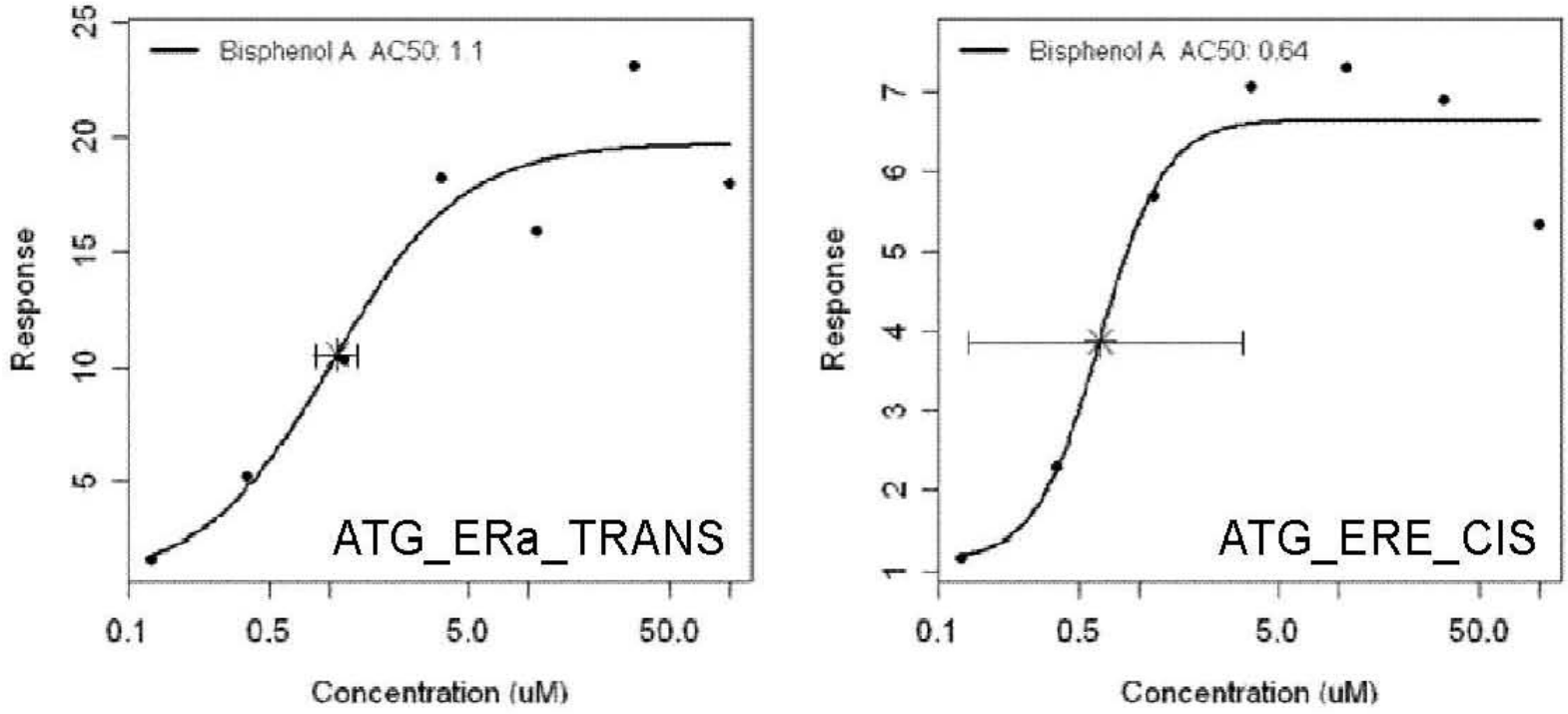
Example: Concentration-Response Curves for Bisphenol A



Sample curves for BPA in two of six ToxCast ER assays

Note that full concentration-response profiles can be measured, at arbitrary spacing and to arbitrarily low concentrations (at moderate cost for a given chemical)

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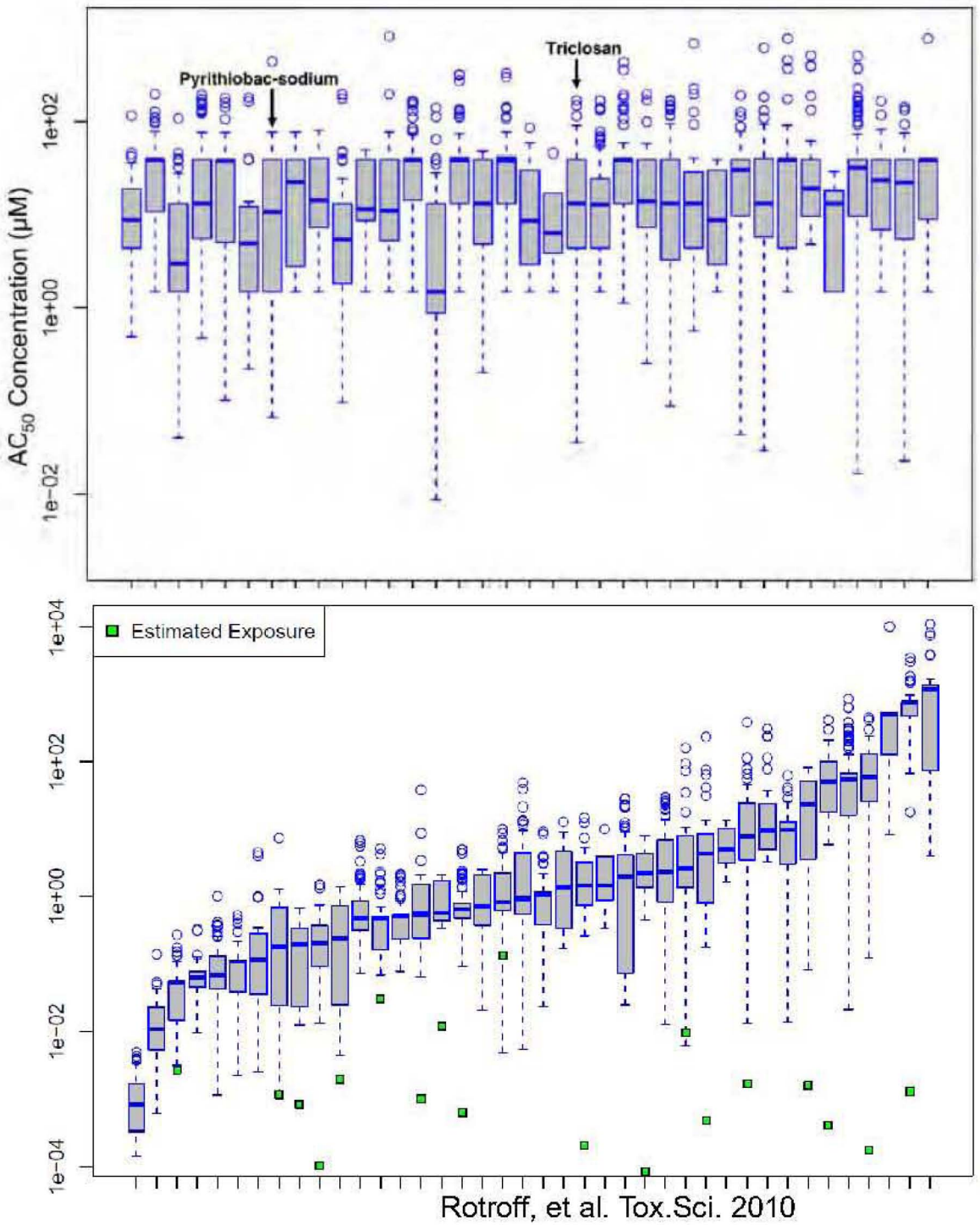
The Significance of Reverse Toxicokinetics: Adding Kinetics is Critical to Understanding Dynamics



Rotroff, et al. Tox.Sci. 2010

Reverse Toxicokinetics (rTK): *in vitro concentration to in vivo dose*

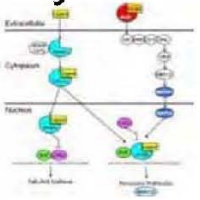
Pharmacodynamics

Adverse Effect

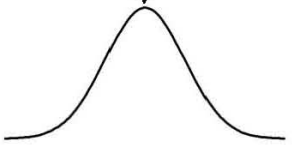
MOA

Key Events

Toxicity Pathway

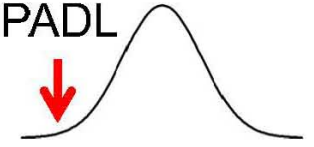


HTS Assays



Biological Pathway Activating Concentration (BPAC) Probability Distribution

BPADL



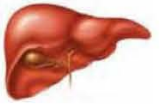
Probability Distribution for Dose that Activates Biological Pathway

Pharmacokinetics

Dose-to-Concentration Scaling Function (C_{ss}/DR) Probability Distribution

PK Model

Populations



Intrinsic Clearance

Plasma Protein Binding

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Uncertainty and Variability

- RTK modeling explicitly incorporates human population variability in PK (SimCyp)
- Other uncertainty and variability …
  - PK uncertainty due to model and data uncertainty
  - PD variability due to intrinsic variability in enzymes, receptors, pathways
  - PD uncertainty due to details of assay performance, etc.
- Need to develop approach to move away from using defaults for HTRA
  - Follow similar path to what is being developed for standard RA

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Example: Bisphenol A Estrogenicity In Vitro vs. In Vivo Reproductive Toxicity

- Rat reproduction tests resulted in a No Effect Level of 50 mg/kg/day
- Adjusted for uncertainty and variability, the no effect dose is 0.5 mg/kg/day
- HTRA lower limit BPADL99 is 0.16 mg/kg/day, derived from six ToxCast estrogen receptor assays

Example: Bisphenol A Estrogenicity In Vitro vs. In Vivo Reproductive Toxicity

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Pharmacodynamics

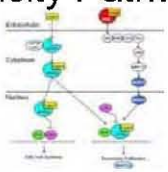
Estrogenicity

MOA

Adverse Effect

Key Events

Toxicity Pathway



HTS Assays

Lowest AC50 from 6 ToxCast ER assays

BPAC = 0.64 µM

Biological Pathway Activating Concentration (BPAC) Probability Distribution

NEL/100 = 0.5 mg/kg/day

Female ReproTox

$BPADL_{99}$

Probability Distribution for Dose that Activates Biological Pathway

Estrogenicity

$BPADL_{99}$ = 0.16 mg/kg/day

Pharmacokinetics

Dose-to-Concentration Scaling Function (C_{ss}/DR) Probability Distribution

$BPAD_{99}$ = 0.44 mg/kg/day

PK Model

Populations

Intrinsic Clearance

Plasma Protein Binding

Exposure = 0.00008 mg/kg/day

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HTRA Summary

1. Select Toxicity-related pathways
2. Develop assays to probe them
3. Estimate concentration at which pathway is “altered” (PD)
4. Estimate concentration-to-dose scaling (PK)
5. Estimate PK and PD uncertainty and variability
6. Combine to get BPAD distribution and safe tail

- Many (better) variants can be developed for each step (1-6)
- Use for analysis and prioritization of data poor chemicals

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HTRA Summary (2)

- Pathway perturbation = MOA Key Event evidence Necessary for MOA
  - Sets lowest dose at which chemical acts through MOA
  - Do not need to do low-dose extrapolation – just measure it

NexGen Risk Assessment Data Requirements Being Met by CompTox and CSS Research

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Tier I 10,000s of chemicals	Tier II 1000s of chemicals	Tier III 100s of chemicals
Ultra-High Throughput Screening (uHTS) for Bioactivity Profiling- Tox21	***HTS, Metabolism, Dosimetry, Exposure, Modes of Action and Systems Integration- ToxCast and ExpoCast***	***High Content, Lower Throughput, Animal or Human Studies- Chemical Safety for Sustainability***
• In vitro assays (dozens) • In silico assays ✓QSAR ✓Molecular Docking • ToxPi (Toxicological Prioritization index) data integration ✓Pathway, toxicity or disease associations	• In vitro & in silico assays (hundreds) • Metabolism and dosimetry • Exposure estimates • Alternative species, systems modeling , virtual tissue simulations • High Throughput Risk Assessments (HTRA) based on modes of action	• Molecular epidemiology & clinical studies • Exposure modeling and biomarkers of exposure • Molecular biology & targeted animal testing • Pathway and disease outcomes • Knowledge-Base integration and visualization

Increasing Weight of Evidence → **Adversity**

Screening/Ranking

Limited decision-making

Regulatory decision-making

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Acknowledgements

- Participants in the Day 3/Tier 2 portion of Nov2010 NexGen Workshop: Stan Barone (EPA), Derek Knight (ECHA), Karen Leach (Pfizer), Chris Portier (ATSDR), Mike Devito (NTP), Alex Tropsha UNC), Richard Judson (EPA), David Reif (EPA), Rusty Thomas (Hamner), Jason Lambert and Ila Cote (EPA)
- Tox21 teams at EPA, NIEHS/NTP, NHGRI/NCGC and FDA: led by Bob Kavlock, Ray Tice, Chris Austin and David Jacobson-Kram
- ToxCast/ExpoCast EPA teams: led by Keith Houck and Elaine Cohen Hubal
- Newly forming Chemical Safety for Sustainability teams at EPA