

Considerations in Use of the EPA's ToxCast Data for Environmental Toxicology WP062

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ToxCast Phase I & II Summary: What Have We Learned?

ACEA: red

Attagene: orange

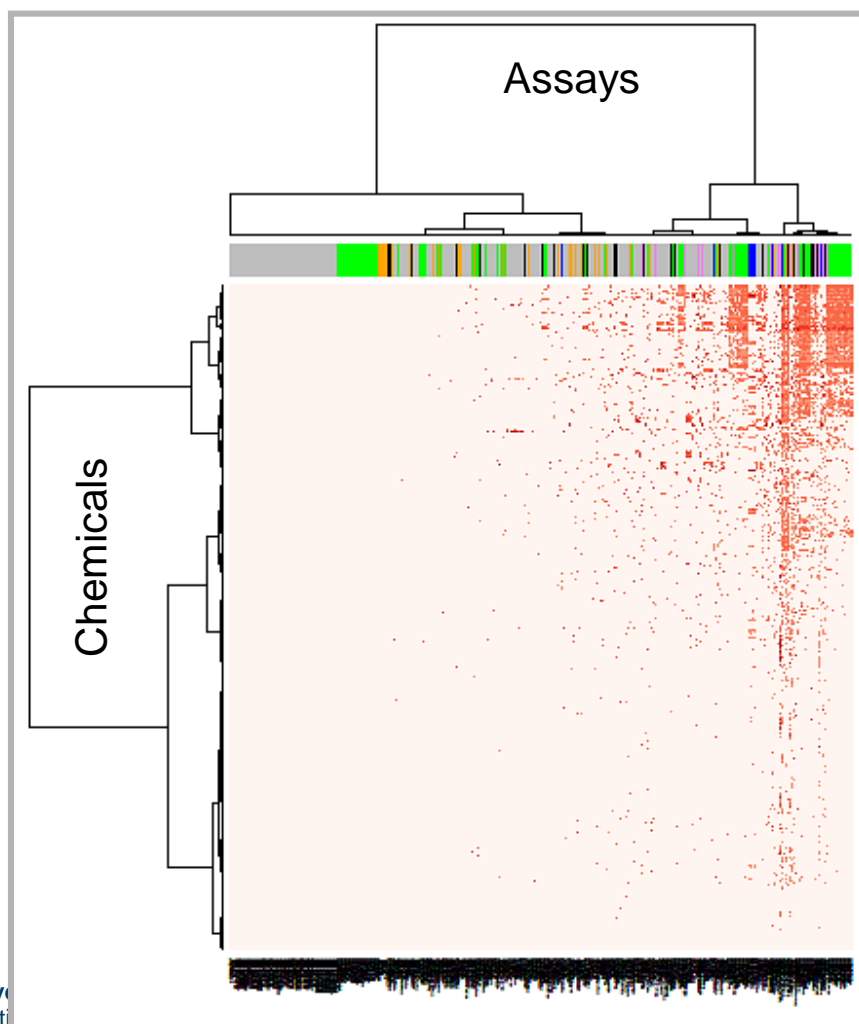
Apredica: black

BioSeek: green

Novascreen: gray

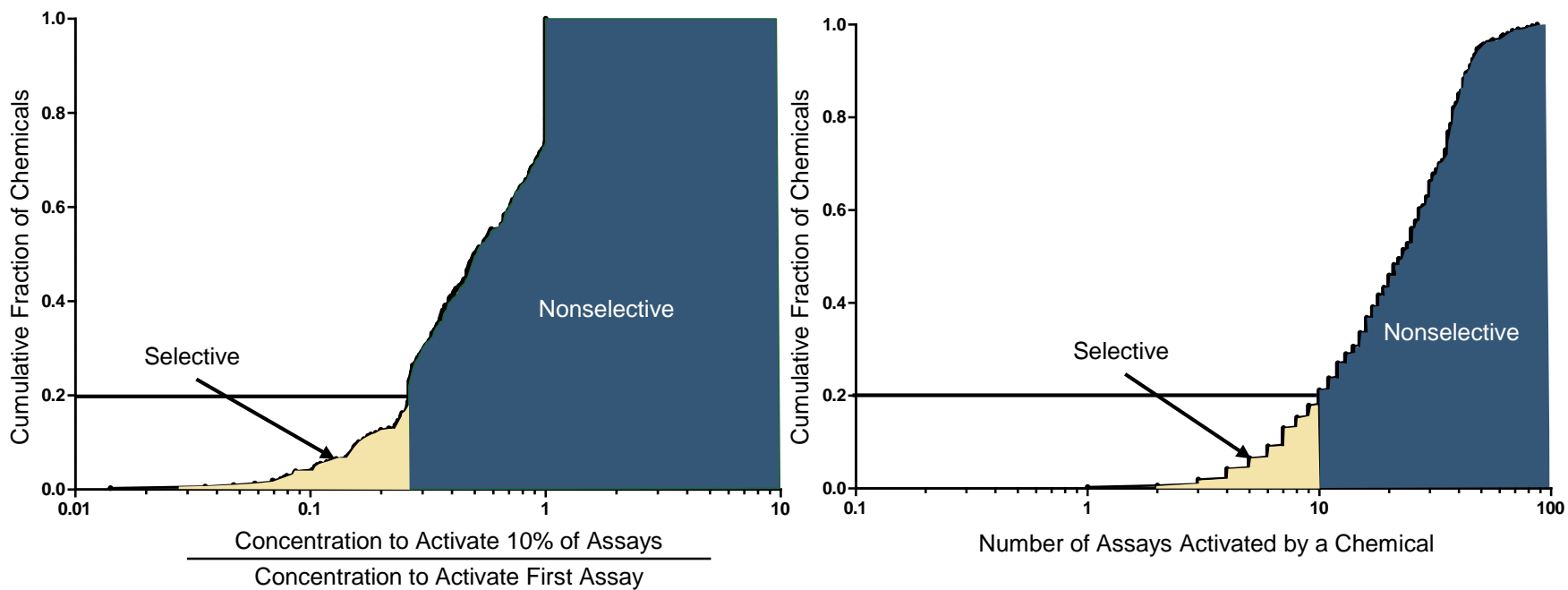
Tox21: violet

OT: blue

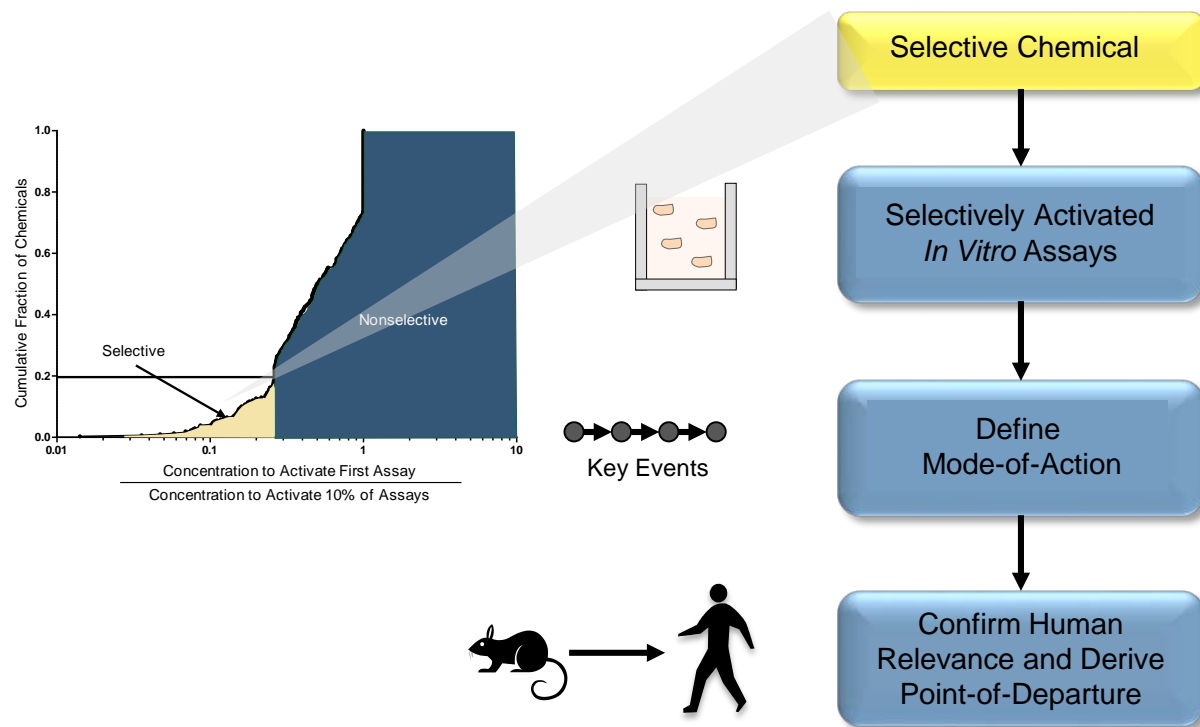


1000 chemicals
821 assay endpoints
85% human

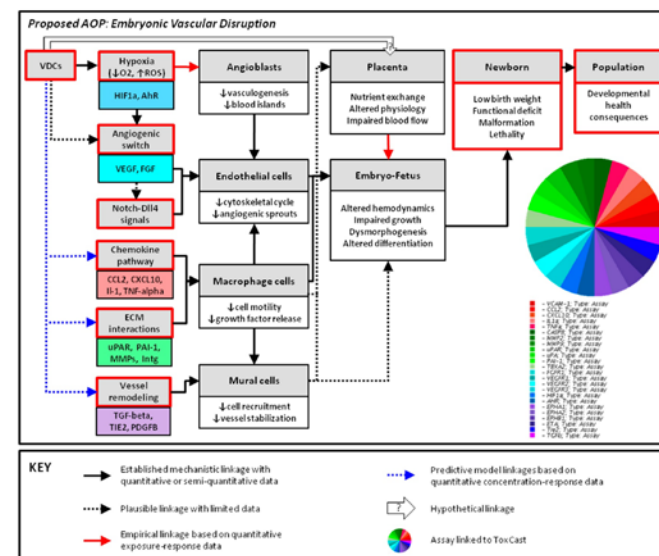
What Did High-Throughput Screening Tell Us?



In Vitro Assay Selectivity as a Starting Point for Chemical Mechanisms Of Action/Adverse Outcome Pathways



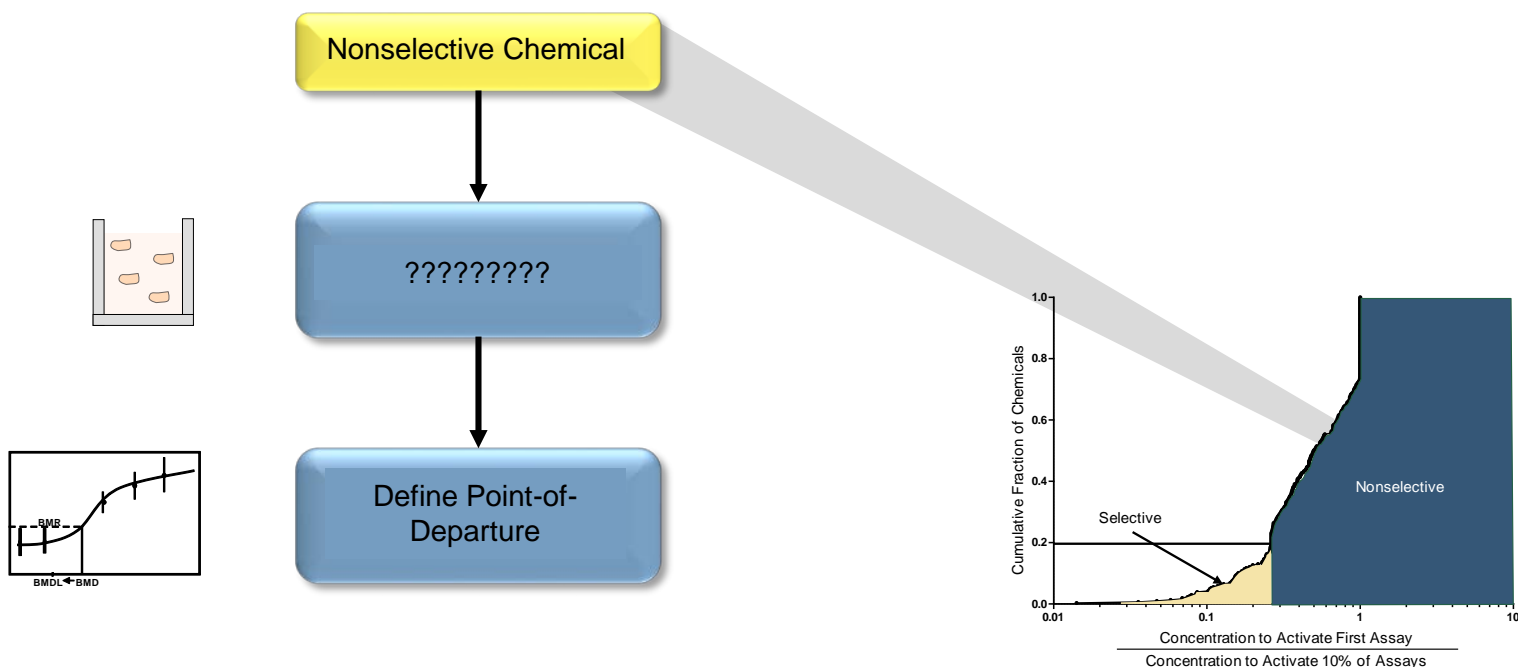
Use of HTS Results in an Adverse Outcome Pathway (AOP)



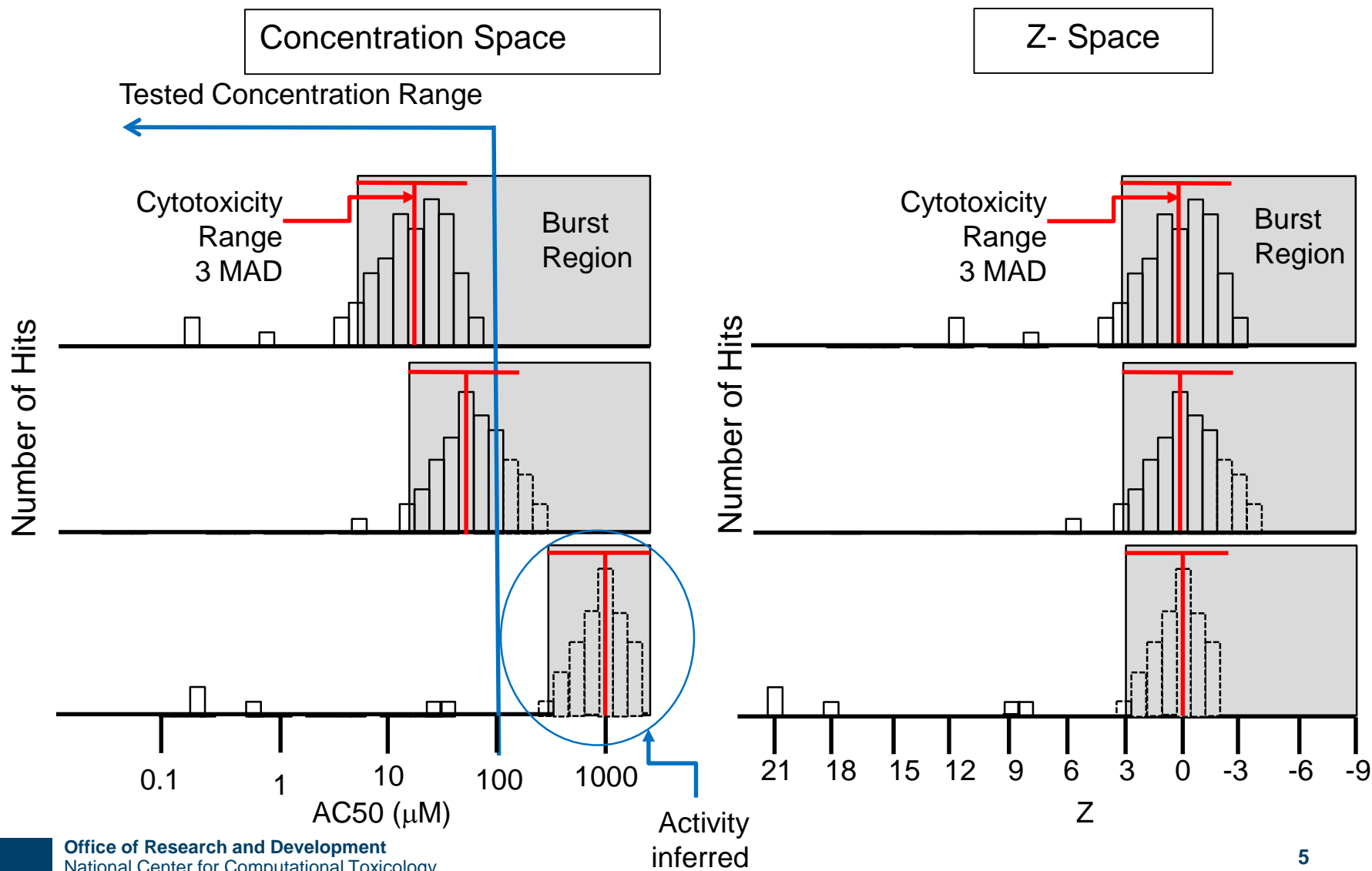
Knudsen and Kleinstreuer. Birth Def Res C. 2012

Need for species/taxa specific AOP

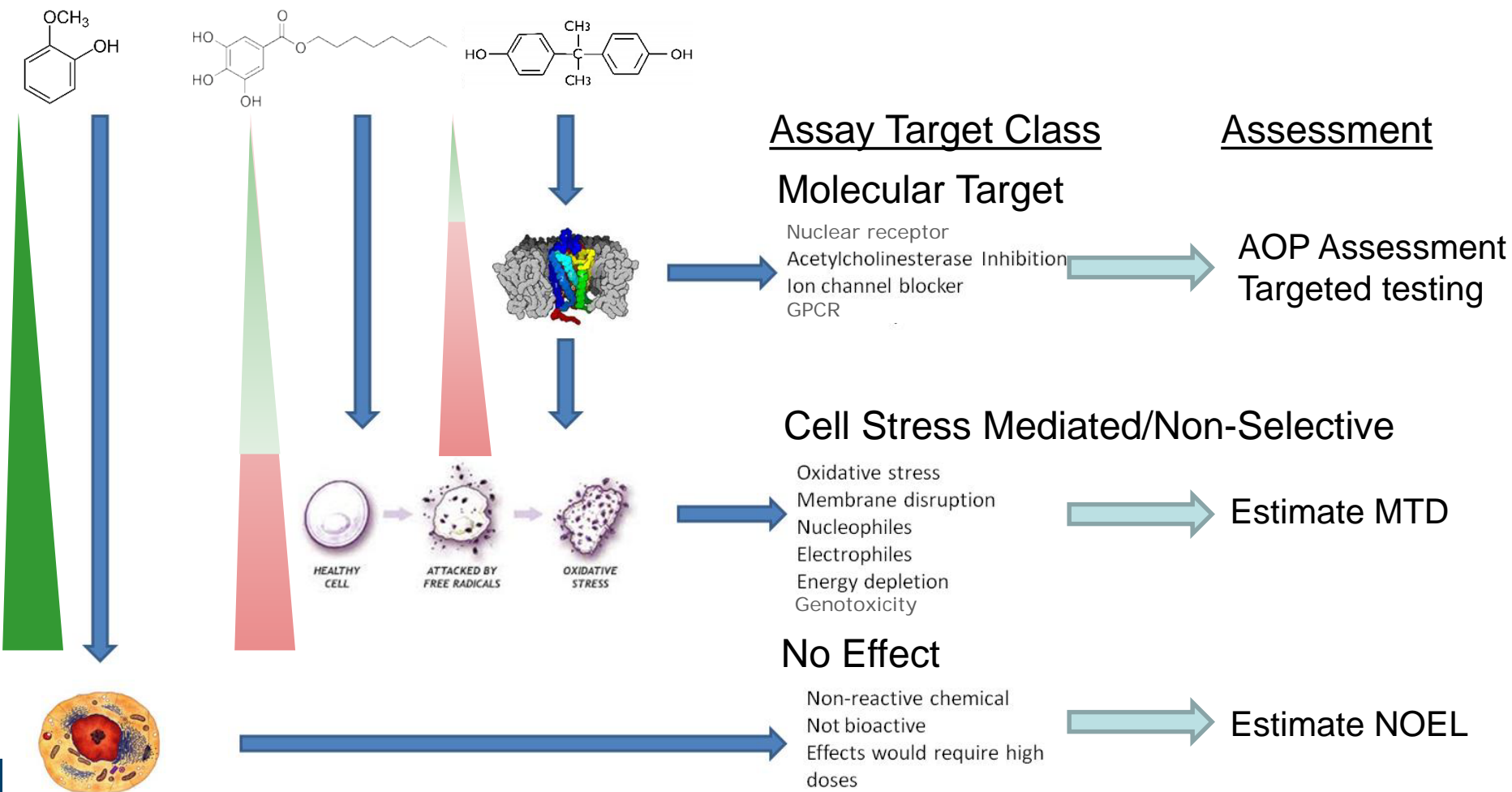
What Have We Learned About the Non-Selective Chemicals?



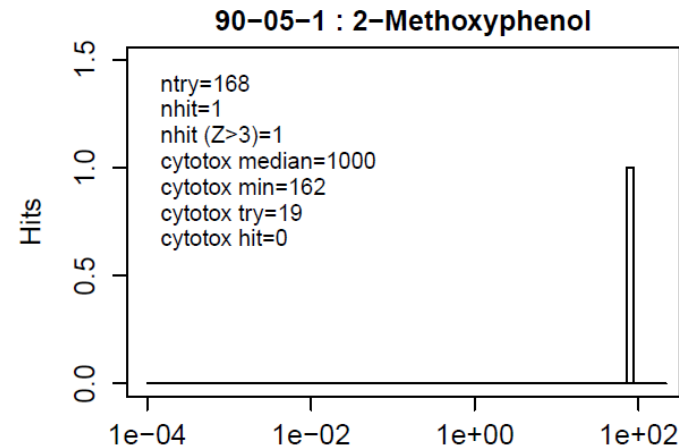
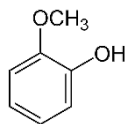
Most chemicals display a “burst” of activity near cytotoxicity concentration



Significance of *In Vitro* Effects



Significance of *In Vitro* Effects



Assay Target Class

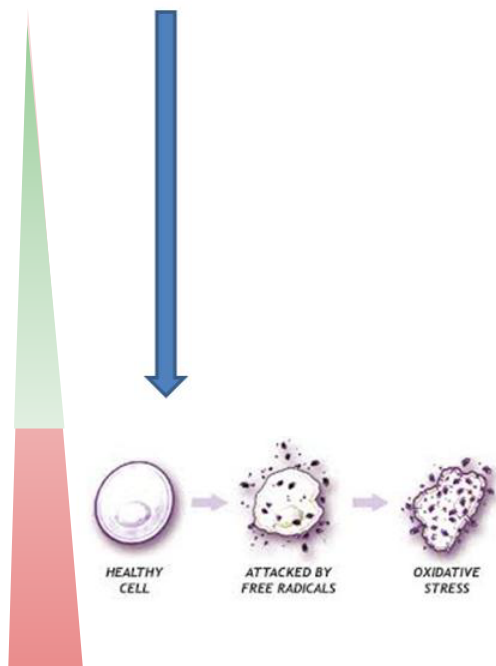
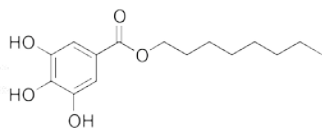
Assessment

No Effect

Non-reactive chemical
Not bioactive
Effects would require high
doses

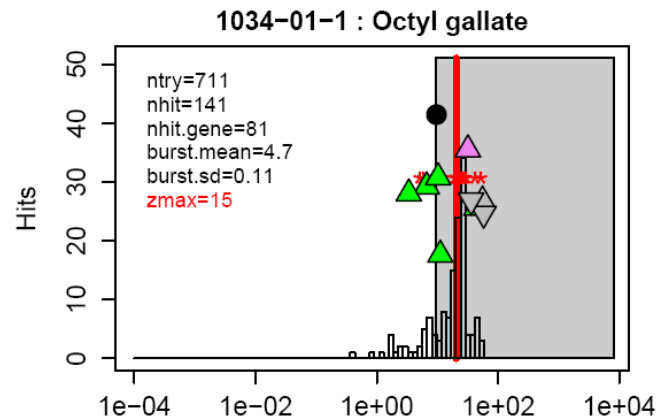
Estimate NOEL

Significance of *In Vitro* Effects

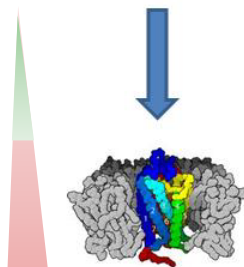
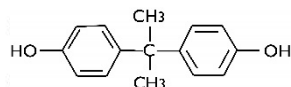


Oxidative stress
Membrane disruption
Nucleophiles
Electrophiles
Energy depletion
Genotoxicity

Estimate MTD



Significance of *In Vitro* Effects



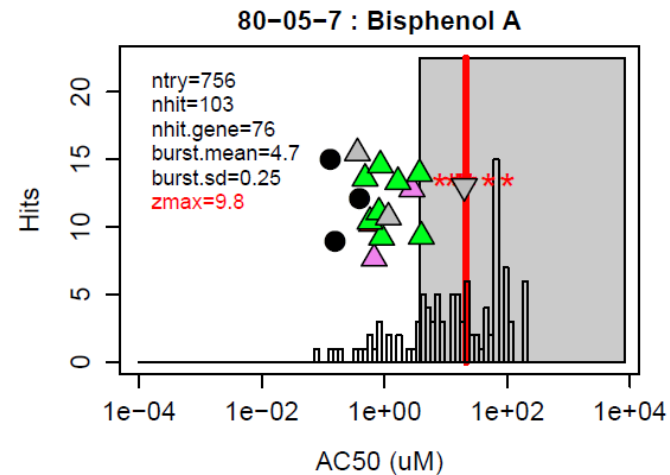
Assay Target Class

Molecular Target

Nuclear receptor
Acetylcholinesterase Inhibition
Ion channel blocker
GPCR



AOP Assessment
Targeted testing



Extrapolation of Nonselective Chemical Effects

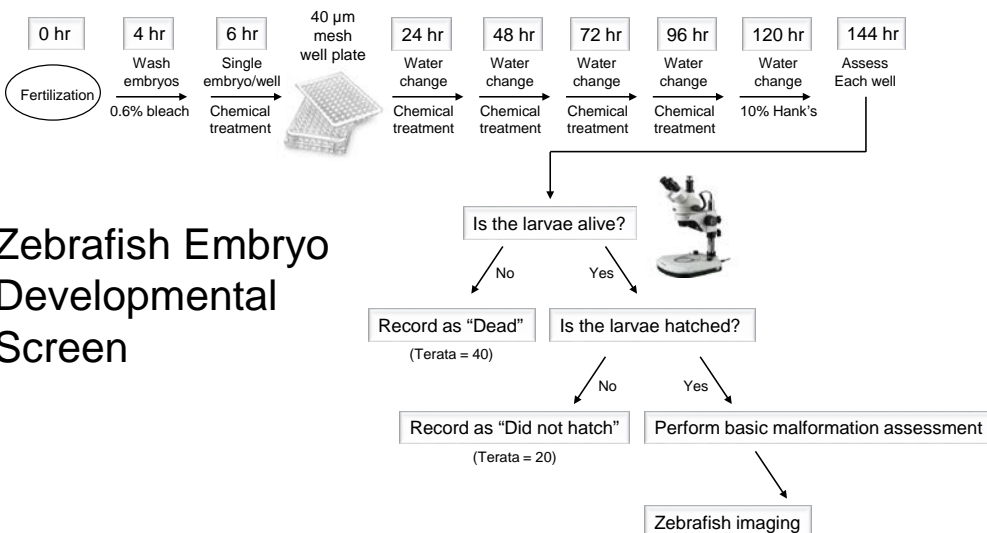
Zfish Mortality (% lethal)

	QLogPow Range		
Cytotoxicity	< 2	2-4	> 4
Low	5.0% (188)	18.3% (181)	17.7% (73)
Moderate	30.0% (26)	29.6% (35)	40.7% (38)
High	52.0% (38)	73.3% (78)	59.5% (126)

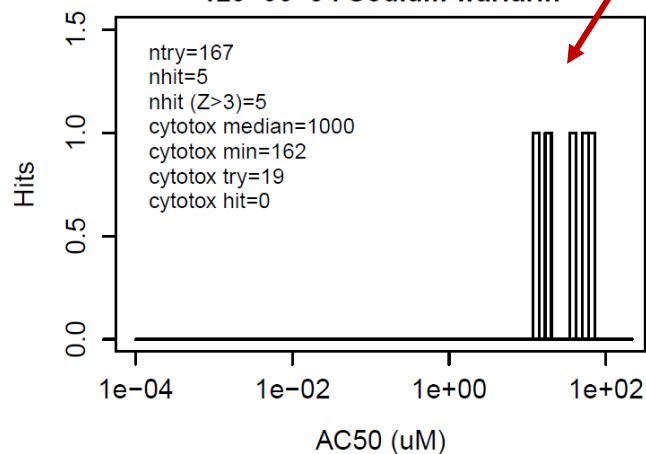
Zfish Mortality (Median -logM LEC)

	QLogPow Range		
Cytotoxicity	< 2	2-4	> 4
Low	4.1	4.5	4.6
Moderate	4.6	4.6	4.6
High	4.6	4.9	5.0

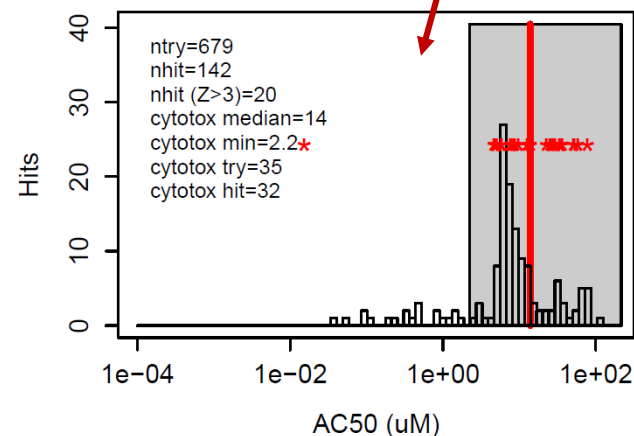
Zebrafish Embryo Developmental Screen



129-06-6 : Sodium warfarin

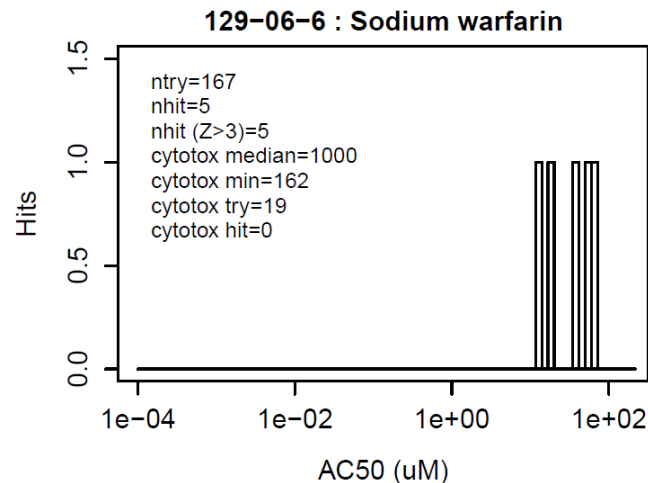


10540-29-1 : Tamoxifen

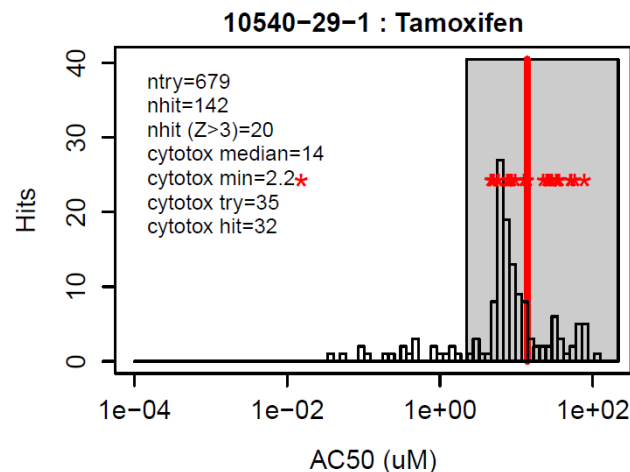


Zebrafish Embryo Developmental Screen

- Warfarin: targets Vitamin K epoxide reductase leading to anticoagulation in mammals
 - No enzyme target in ToxCast
 - Coagulation not important to *in vitro* cell health
 - Known zebrafish toxicant: hemorrhages in brain, skeletal deformities and triggered ectopic calcifications



- Tamoxifen: anti-estrogen at low conc and cytotoxic at μ M conc
 - No anti-estrogenic phenotype seen in zfish
 - Selective estrogenic activity in biochemical and mammalian cellular assays
 - Cytotoxic in mammalian and zfish



Considerations for Species Extrapolations

High-Throughput Screening Analysis

No Effect

- Low priority
- Need for improved assay coverage to increase confidence in negatives

Cell Stress Mediated/Non-Selective

- Expect similar MOA across species
 - multiple molecular targets
 - specificity averaged out
 - conserved sensitive residues (e.g. protein disulfides, membrane lipids)
- Focus on exposure (BCF)
- Biotransformation differences may be critical

Molecular Target

- AOP Focus
- Species/Target Similarity (SeqAPASS)
- False negative potential due to focus on human targets in screening



NCCT 2014