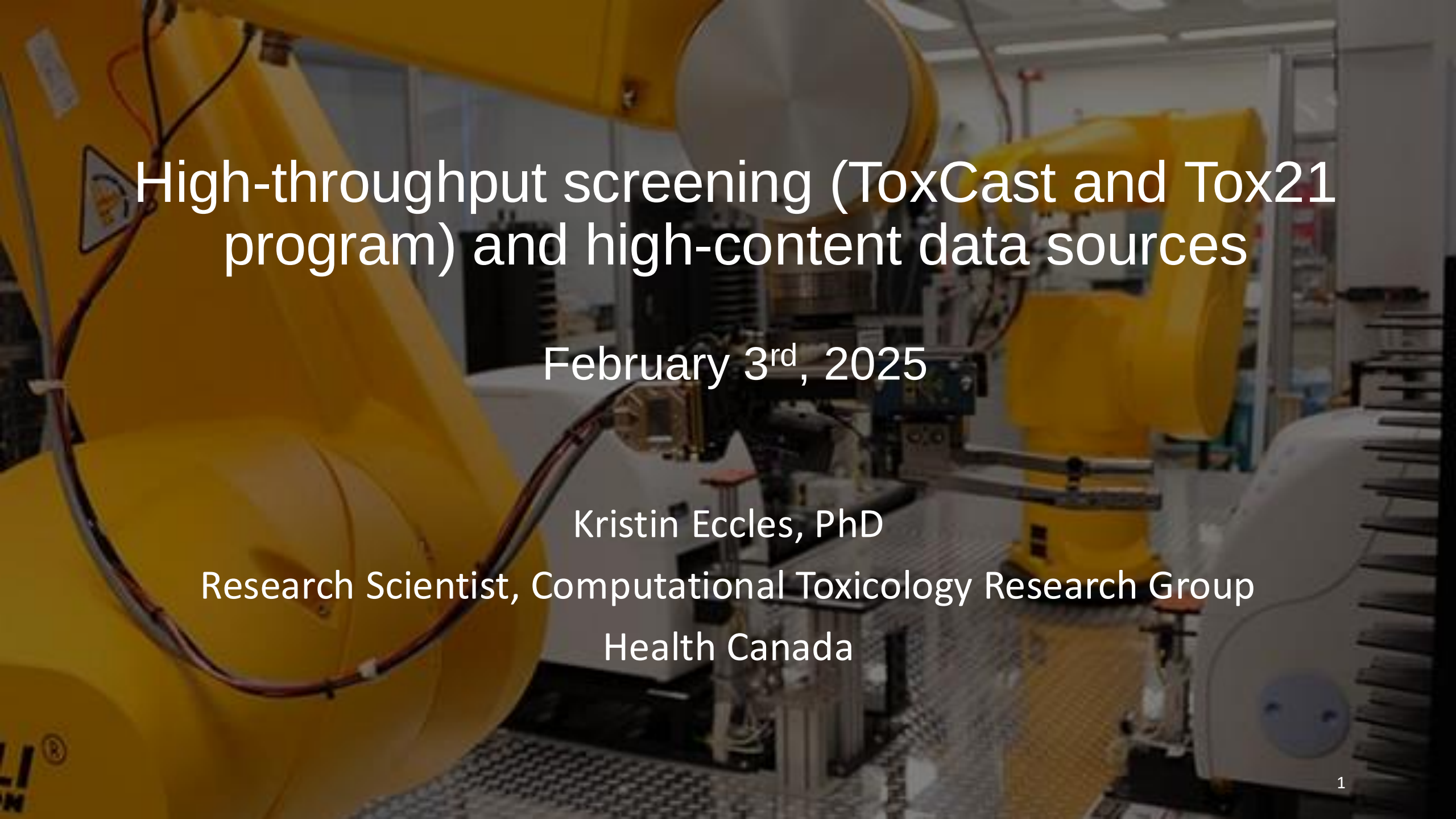

High-throughput screening (ToxCast and Tox21 program) and high-content data sources

February 3rd, 2025

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Health Canada

Outline

1. The Problem
2. What is High- throughput screening and why do we need it
3. The ToxCast/Tox21 Program
    1. Chemical Selection
    2. HTS Analysis/ Assays
    3. Data Processing
        1. Model Fitting, PODs, and Flags
    4. Data Release/ Data Exploration
        1. EPA: CompTox Dashboard
        2. NTP: Integrated Chemical Environment
    5. Data Interpretation
4. Wednesday: HTS Dose-Response Modeling in R

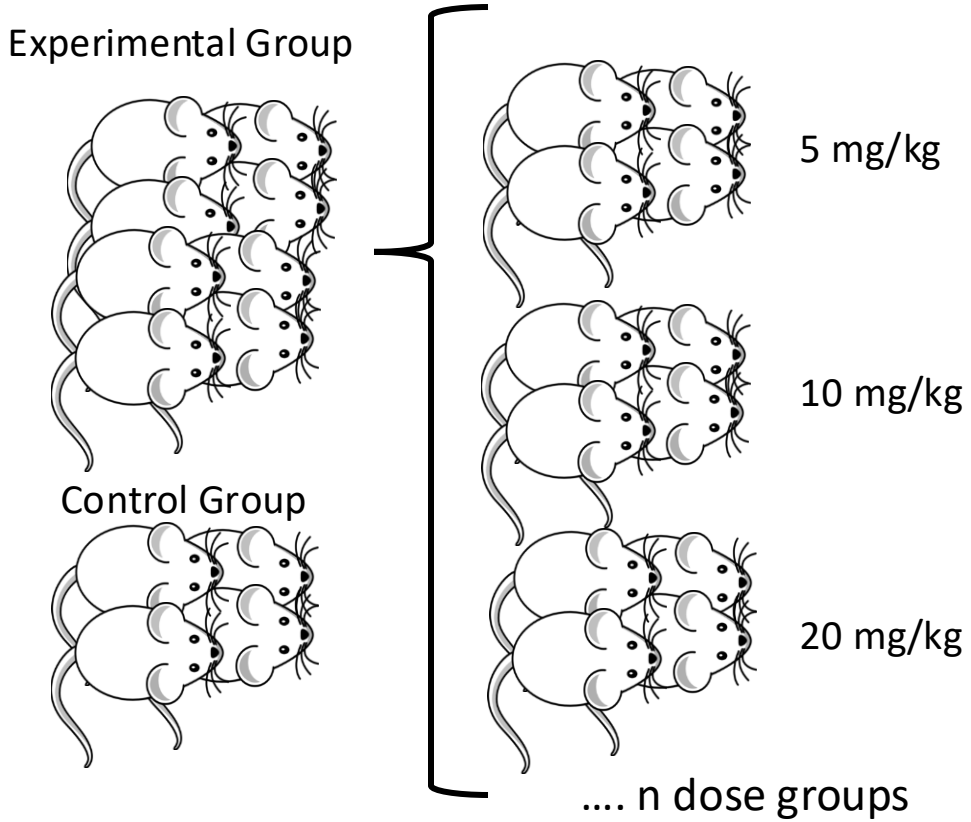
The Problem

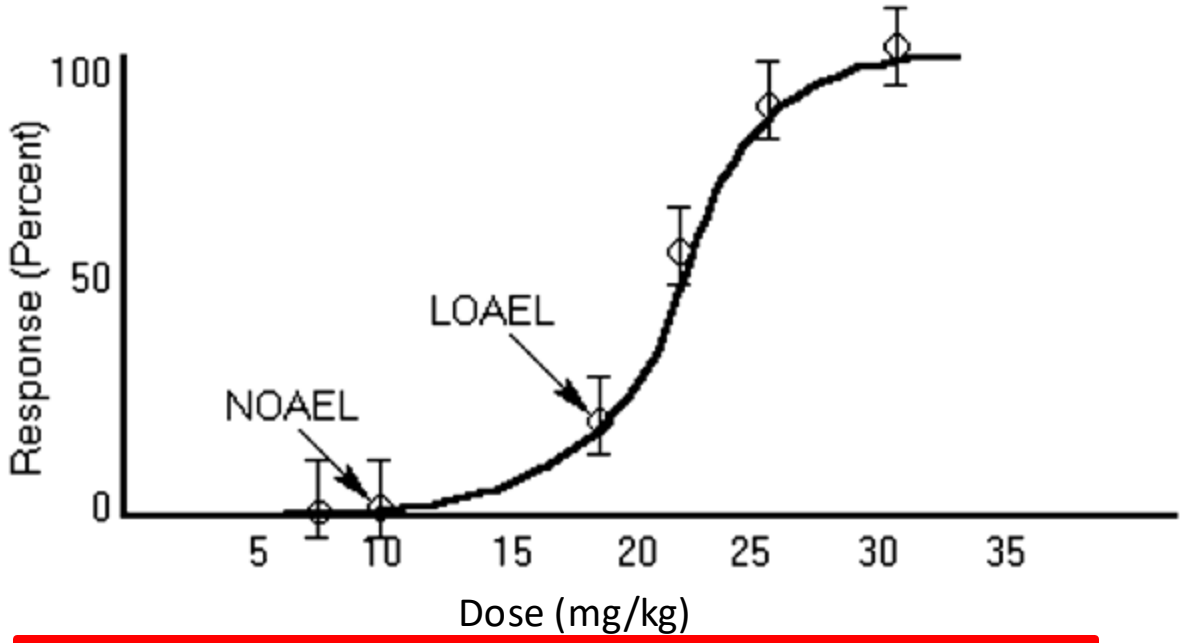
- The “too many chemicals” problem
- A lot of toxicity data (potency, target tissues, etc.) on few chemicals
- Many data poor chemicals
  - Measured in serum, environmental media, in commerce
  - Lack of toxicity information

The risk assessment process

Traditional methods for hazard characterization

in vivo



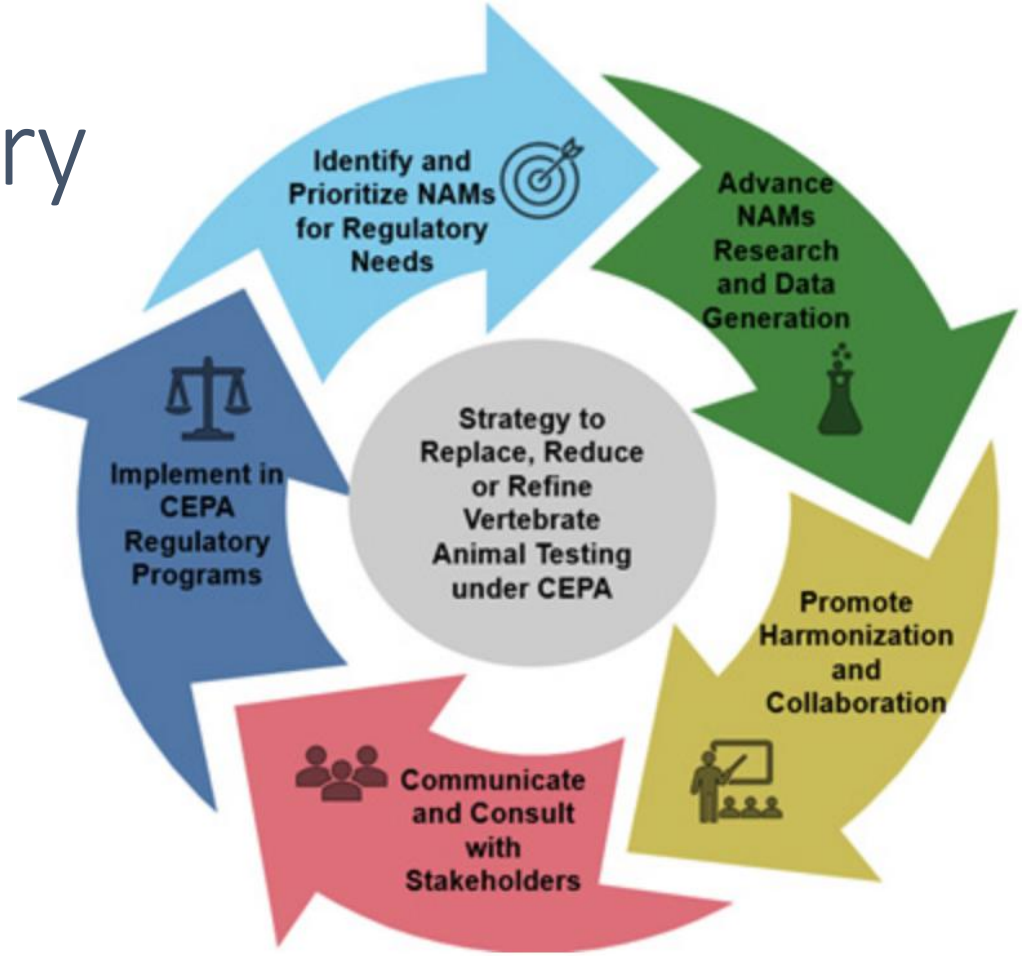


The problem:

- Expensive and time consuming
- Animal welfare issues to consider
- Focus on apical endpoints
- Biological relevance to humans

Toxicology for the 21st century

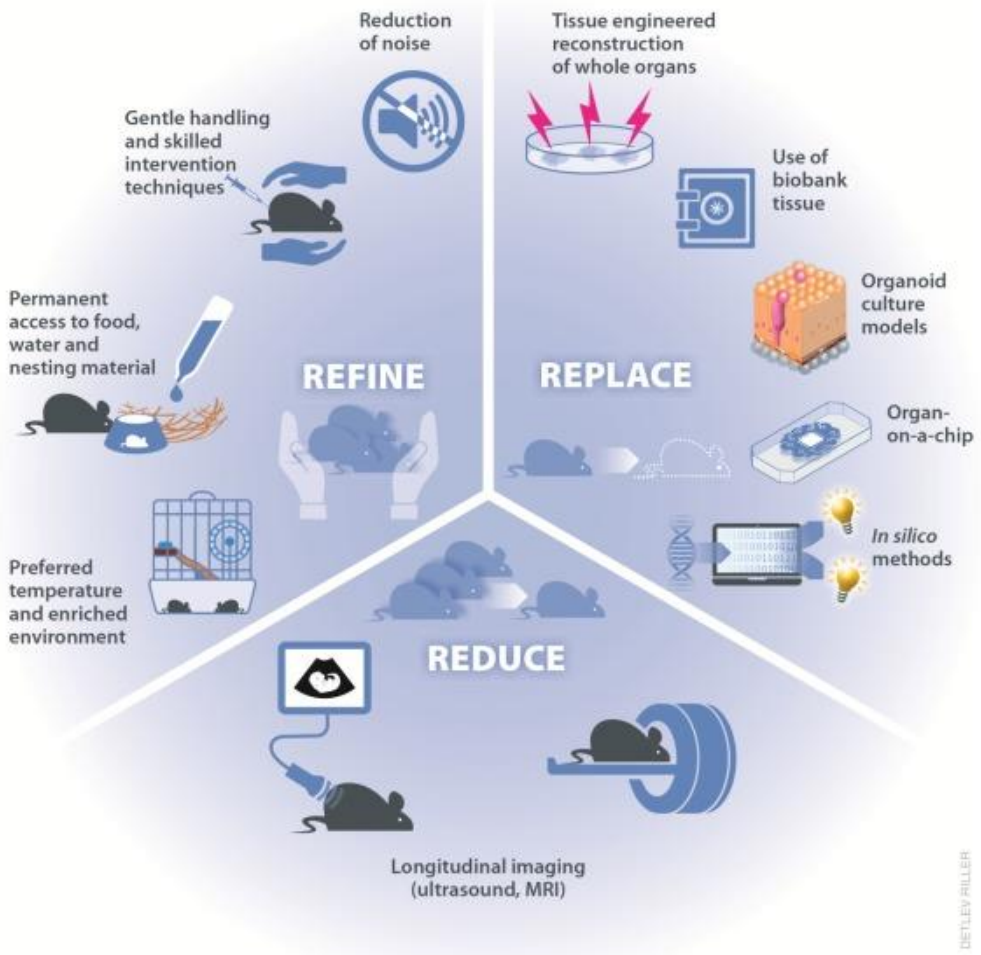
- New Approach Methods: broadly defined as any technology, methodology, or approach that can be used to replace, reduce or refine animals used for toxicity testing and allow for more rapid or effective prioritization and/or assessment of chemicals.
  - Computational models (e.g., physiological based toxicokinetic models)
  - *in chemico* (cell free), *in vitro* (cell based) assays, or 'omics' methods
  - Whole Organism (e.g., zebrafish embryo)



Joint Environment and Climate Change Canda and Health Canada [Draft] Strategy to Replace, Reduce or Refine Vertebrate Animal Testing

The 3Rs

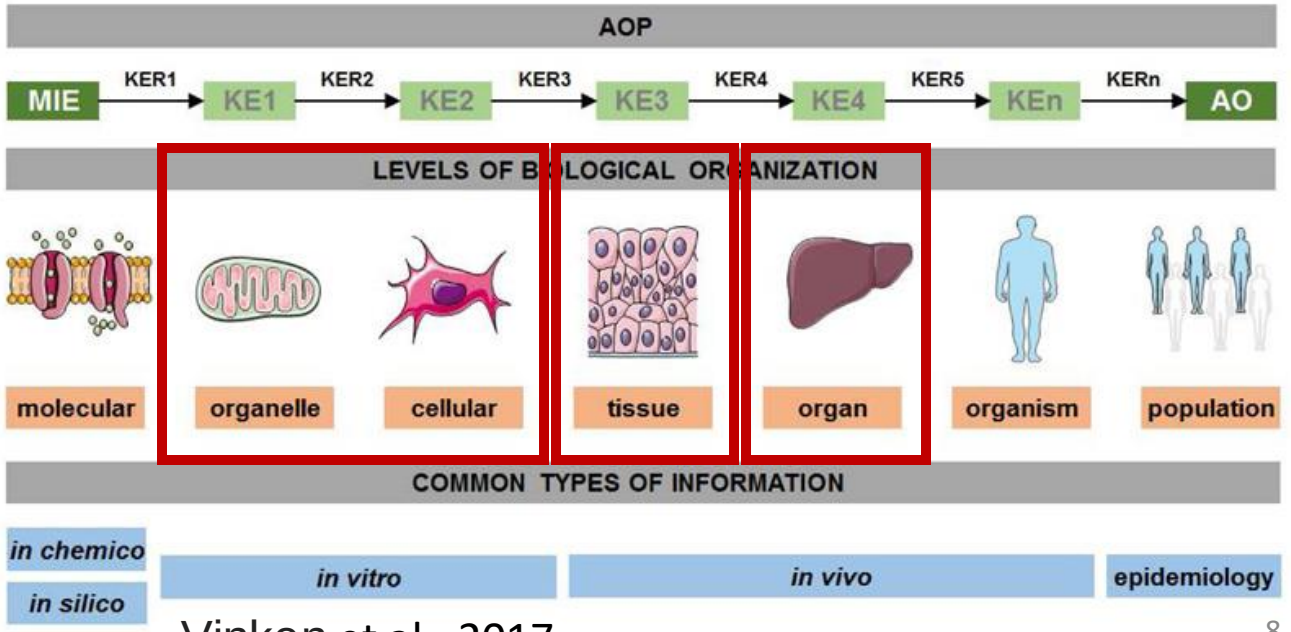
- Replace: Avoiding or replacing the use of animals in areas where they otherwise would have been used.
- Reduce: Minimising the number of animals used consistent with scientific aims.
- Refine:Minimising the pain, suffering, distress or lasting harm that research animals might experience.



Arck, 2019 *Journal of Reproductive Immunology*

What is high- throughput screening?

- High throughput screening (HTS) is the use of automated equipment to rapidly test thousands to millions of samples for biological activity at varying levels of biological organisation
- *in vitro* approach rapidly provides screening level data on chemical hazard

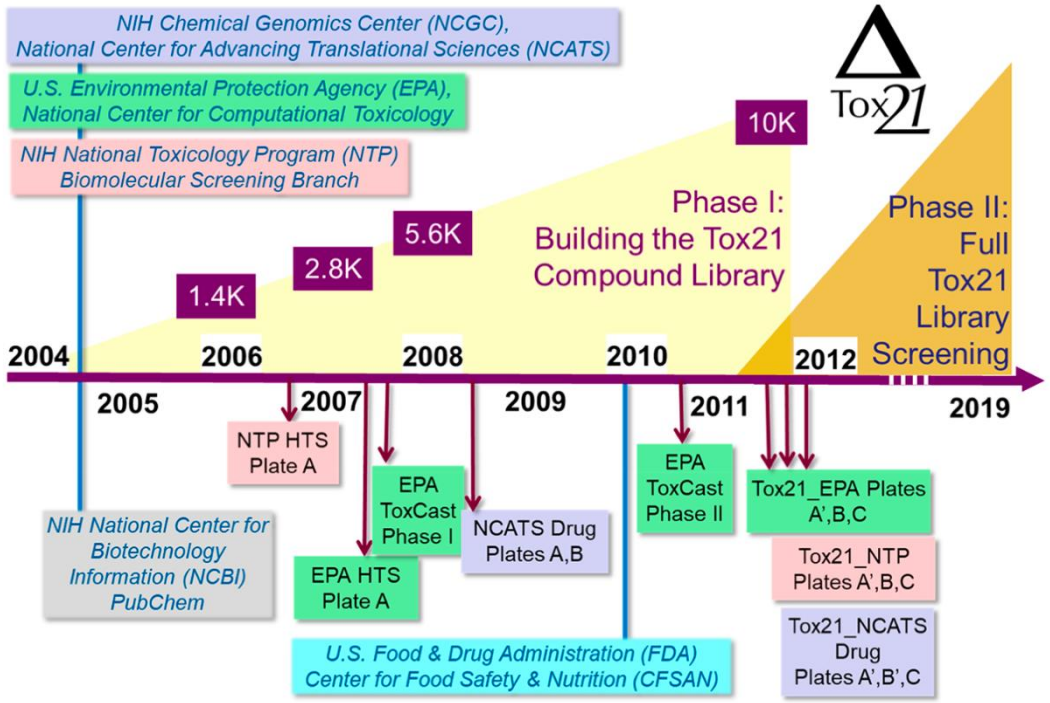


Vinken et al., 2017

Tox21 Program

- USA Federal collaboration among EPA, NIH, including National Center for Advancing Translational Sciences (NCATS) and the National Toxicology Program (NTP) at the National Institute of Environmental Health Sciences (NIEHS), and the Food and Drug Administration (FDA).
- Tox21 aims to:
  - Prioritize substances for further in-depth toxicological evaluation
  - Identify mechanisms of action for further investigation (e.g., toxicity-associated and disease-associated pathways)
  - Develop models that better predict how substances will affect biological responses (predictive toxicology)
  - Employ testing methods using human cells (*in vitro* approaches)
  - Reduce time, effort, and costs associated with testing
  - Contribute to the reduction, refinement, and replacement of animals used in toxicity testing

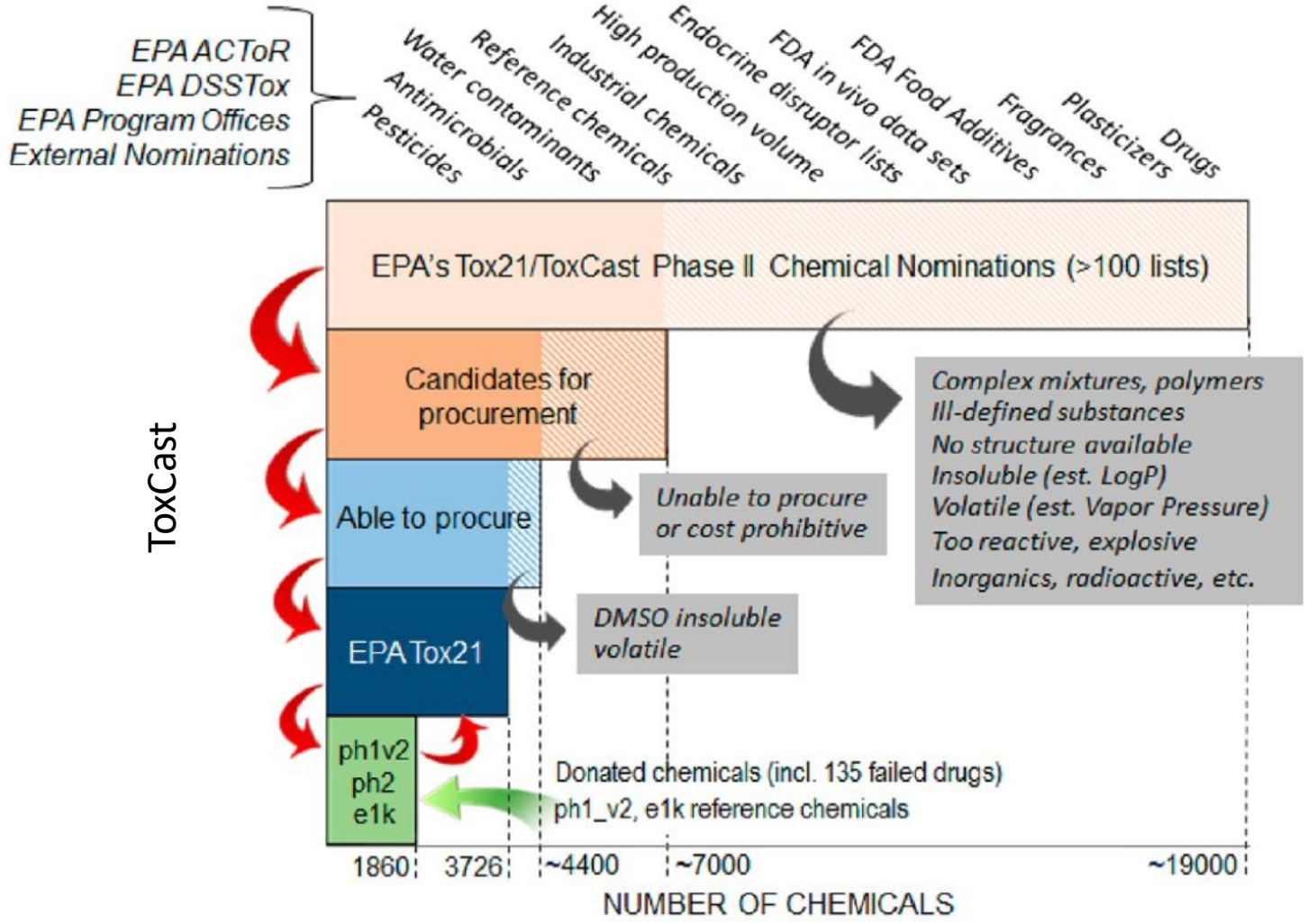
n assays = ~1500
n chemicals = ~10K



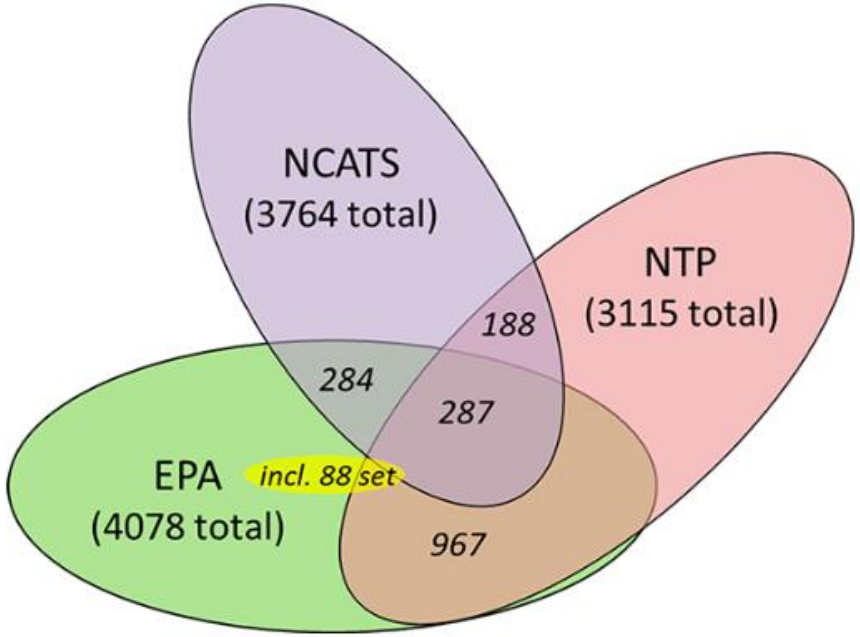
Richard et al. 2020

NTP = National Toxicology Program
EPA = Environmental Protection Agency
NCATS = National Center for Advancing Translational Sciences

Chemical Selection



Richard et al., 2016



Richard et al., 2020

HTS Assays/ Analysis (non-exclusive list)

Assay	Assay End Point*	Cell Type	Assay Readout
Cell viability Apoptosis	Intracellular ATP, caspase-3/7	Hek293, Jurkat, HepG2, Sh-SY5Y, SK-N-SH, BJ, HUV-EC-C, MRC-5, mesangial, kidney proximal tubules, N2a, H-4-II-E, NIH3T3	Luminescence
Membrane integrity	LDH release, protease release	HEK293, mesangial	Fluorescence, luminescence
Mitochondrial toxicity	Membrane potential	HepG2	Fluorescence
DNA damage	Micronucleus	CHO	Fluorescence
Cytokine	IL-8, TNF-α	THP-1	Homogenous time-resolved fluorescence
Nuclear receptor	AR, ERα, FXR, PPARδ, PPARγ, RXR, TRβ, VDR, GR, hPXR, AhR, rPXR, CAR, ERR	Hek293, HeLa, HepG2	β-Lactamase reporter, luciferase reporter
Toxicity pathway	AP-1, HIF-1α, SIE, NF-κB, HSR, ESRE, ARE/Nrf2, CREB, p53, real-time cytotoxicity and viability	ME-180,HeLa, HepG2, Hek293, CHO, HCT-116, HepG2	β-Lactamase reporter, luciferase reporter
hERG channel	Thallium influx	U-2OS	Fluorescence
G-protein-coupled receptor signaling	TRHR, TSHR	Hek293	Fluorescence, homogeneous time-resolved fluorescence
Enzyme activity	AChE	SH-SY5Y	Fluorescence
Developmental pathway	Retinol signaling, Hedgehog/Gli, SBE/Smad	C3H10T1/2, 3T3, HEK 293T	Luciferase reporter, fluorescence

https://ncats.nih.gov/research/research-activities/Tox21/assays

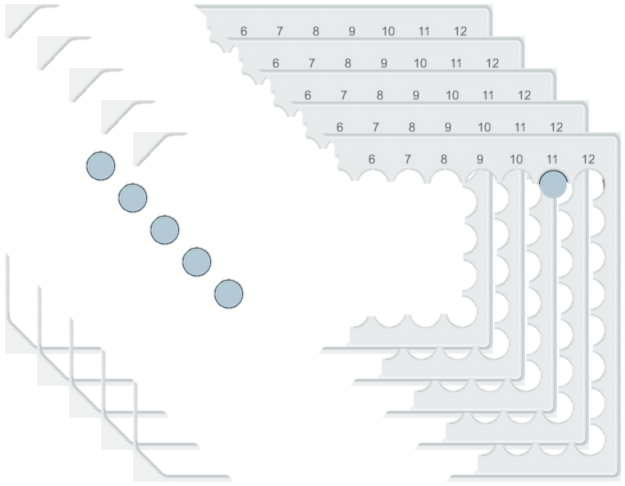
- Nomination process
  - Assays are selected based on their biological and toxicological relevance and adaptability to miniaturization and qHTS screening

Understanding assay naming convention

- TOX21_CAR_Agonist
- LTEA_HepaRG_CYP1A1_dn (DMSO)
- LTEA_HepaRG_CYP1A1_up (DMSO)
- CCTE_Simmons_AUR_TPO_dn (methimazole)
- APR_HepG2_MitoticArrest_24h_up
- APR_HepG2_MitoticArrest_24h_dn
- APR_HepG2_MitoticArrest_72h_dn
- APR_HepG2_MitoticArrest_72h_up

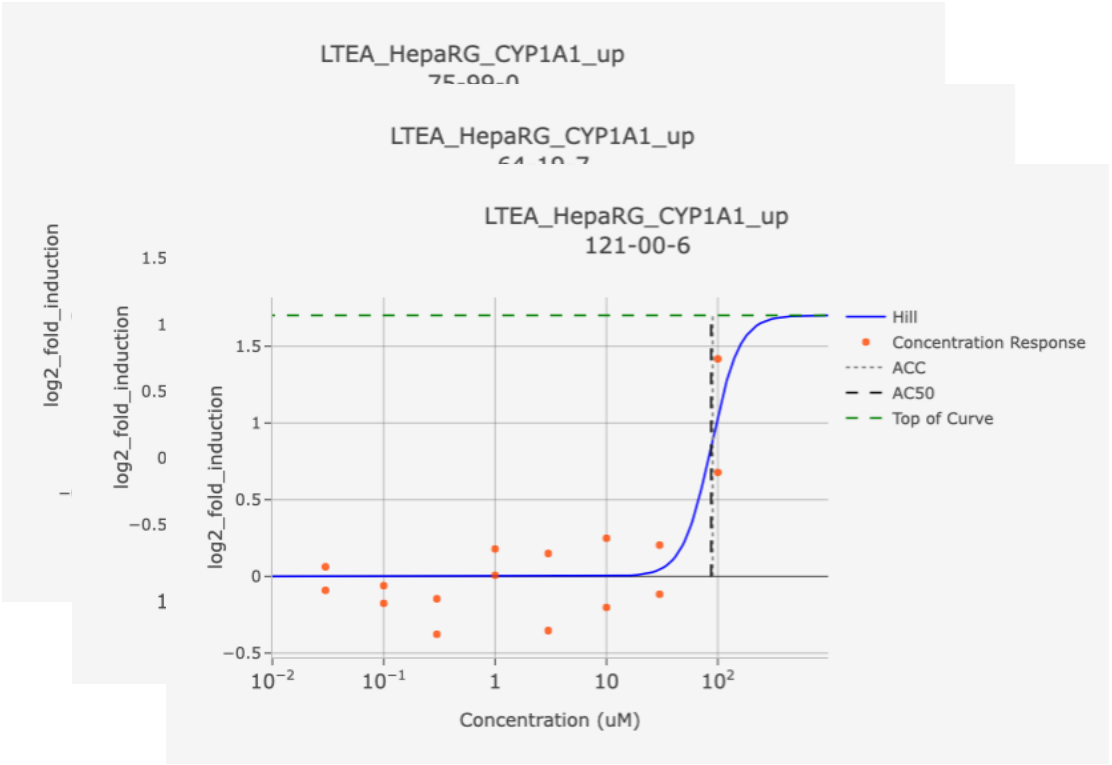
Data Processing

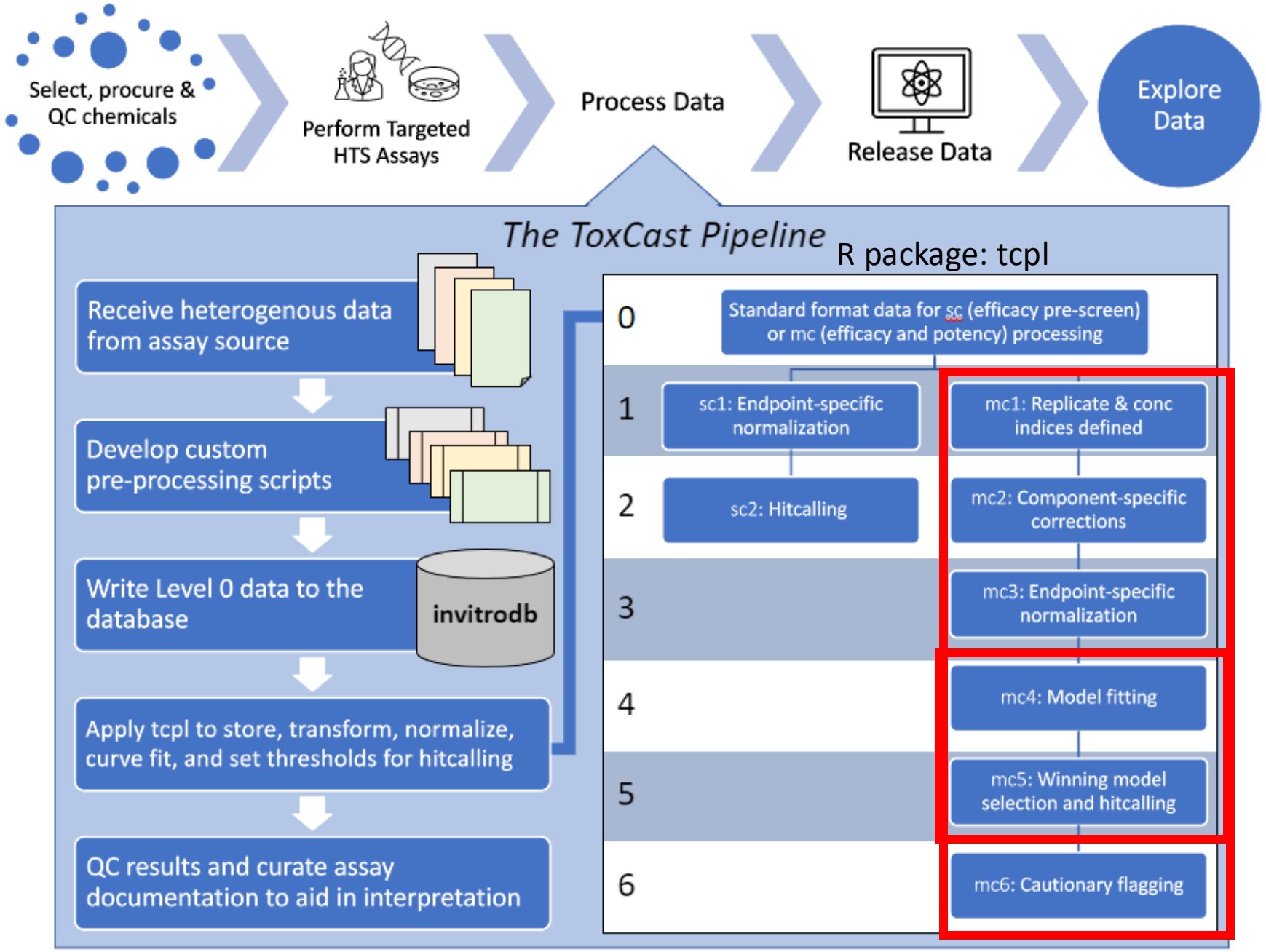
- Turning plate read outs into concentration-response curves



High Throughput Screening Assays

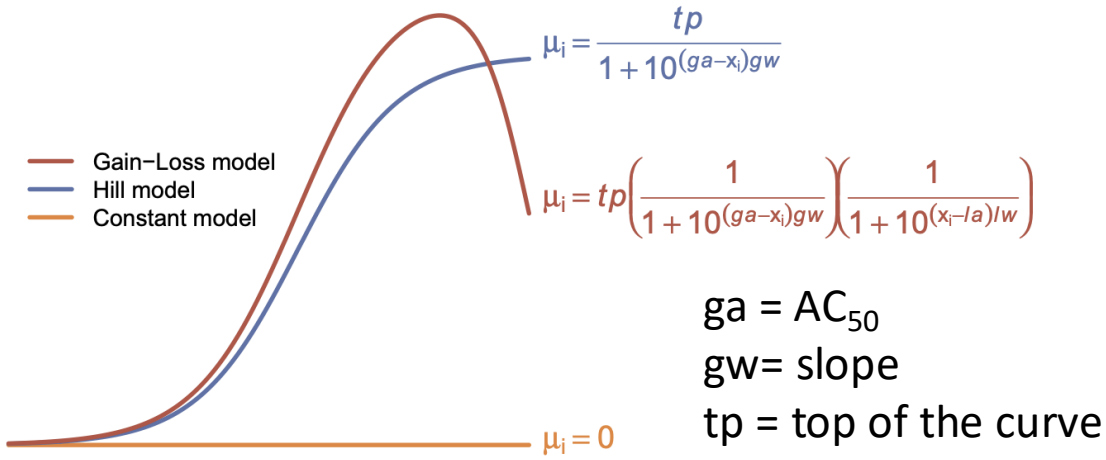
R: EPA's tcpl pipeline





Source: https://cran.r-project.org/web/packages/tcpl/vignettes/Introduction_Appendices.html

Mode fitting: Concentration-response modeling



ga = AC_{50}
gw= slope
tp = top of the curve

Fig. 1 The three models utilized by the tcpl package. The constant model and its associated formula for μ_i is shown in orange, the Hill model and its associated formula for μ_i is shown in blue, and the gain-loss model and its associated formula for μ_i is shown in blue

- best model is chosen using AIC

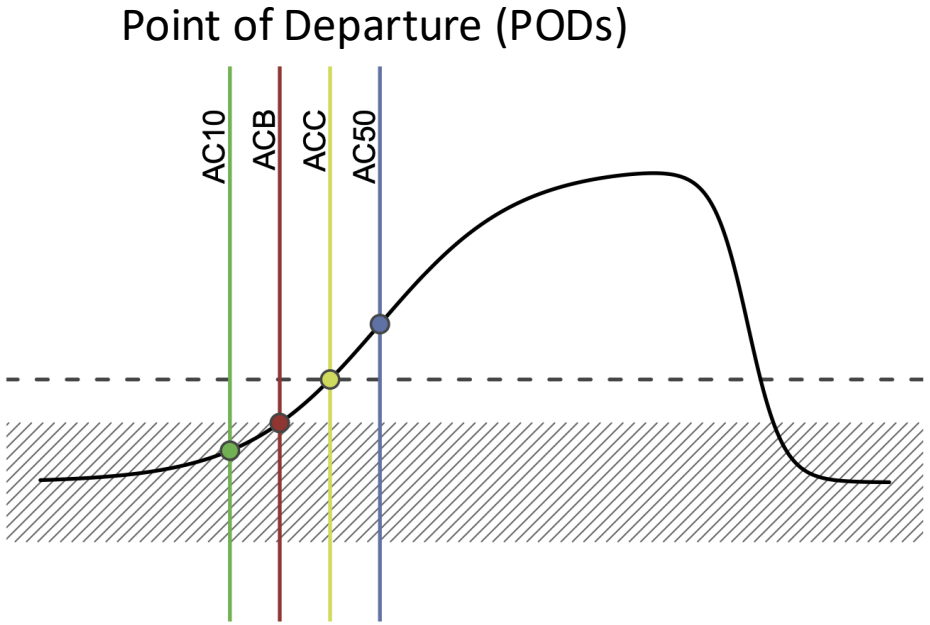
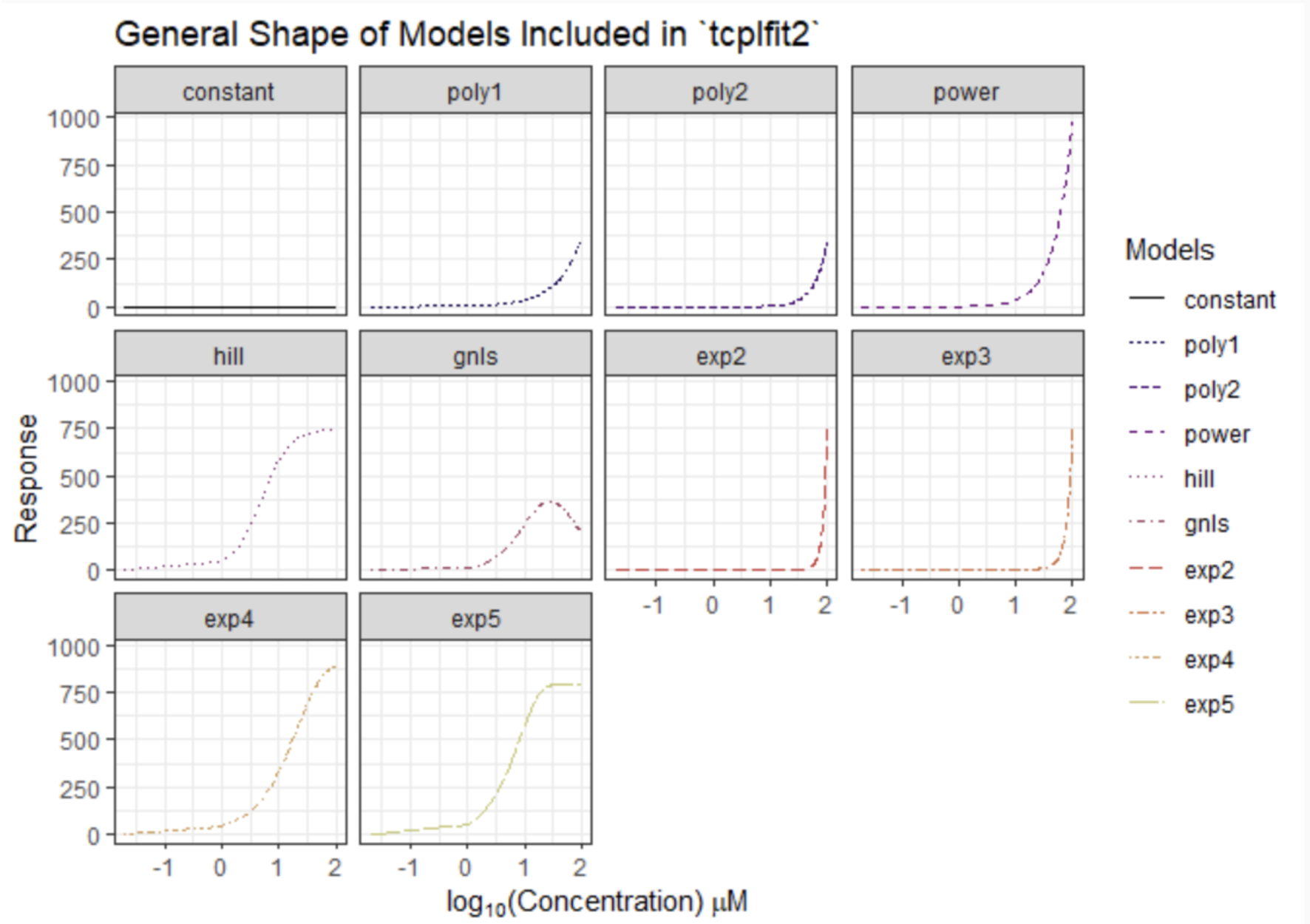


Fig. 3 The point-of-departure estimates calculated by the tcpl package. The shaded rectangle represents the baseline region, $0 \pm 3bmad$. The dark stripped line represents the efficacy cutoff. The vertical lines show where the point-of-departure estimates are defined: the activity concentration at baseline (ACB) in red, activity concentration at cutoff (ACC) in yellow, and activity concentration at 50% maximal activity (AC_{50}) in blue

bmad = baseline median absolute deviation

Filler et al., 2016

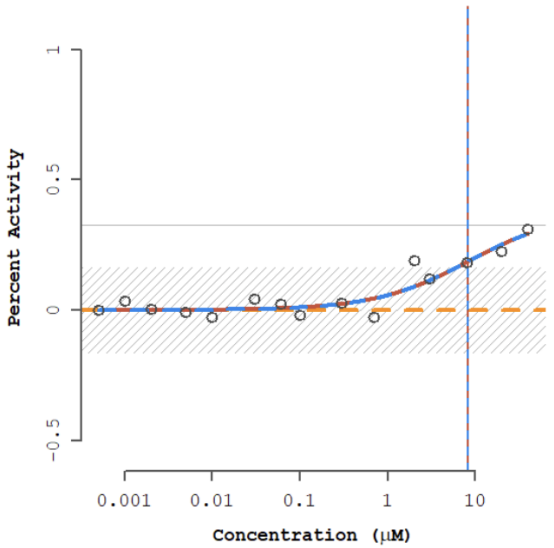
tcplfit2



https://cran.r-project.org/web/packages/tcpl/vignettes/Data_processing.html

Hit call/ Flagging

- Hitc: interpret the continuous hitc into active or inactive
  - Must meet activity thresholds
- Flag – omit
  - QA/QC
  - Removed the least reproducible curve-fits
    - Very low AC_{50} (below the screened conc range)
    - Low efficacy (within 1.2-fold of the cutoff)
    - Low n of tested concentrations
    - library(tcpl): ?MC6_Methods(lvl = 6)




```
ASSAY:    AEID1 (TOX21_ERa_BLA_Agonist_ratio_gain)

NAME:     Phenylalanine
CHID:     23463    CASRN: 150-30-1
SPID(S): Tox21_110011
M4ID:     3

HILL MODEL (in red):
          tp        ga        gw
val:   0.375     0.915     0.81
sd:    0.119     0.411     0.294

GAIN-LOSS MODEL (in blue):
          tp        ga        gw        la        lw
val:   0.375     0.915     0.81      3.1       5.19
sd:    0.119     0.412     0.295     670       2340

       CNST         HILL         GNLS
AIC:   -18.94       -49.21       -45.21
PROB:  0            0.88         0.12
RMSE:  0.13         0.04         0.04

MAX_MEAN: 0.313      MAX_MED: 0.313      BMAD: 0.0546

COFF: 0.328  HIT-CALL: 0     FITC: 21    ACTP: 1
```

The hill_tp and gnls_tp parameters are equal and greater than coff; however, the maximum median value (max_med) is not greater than the cutoff making the series inactive.

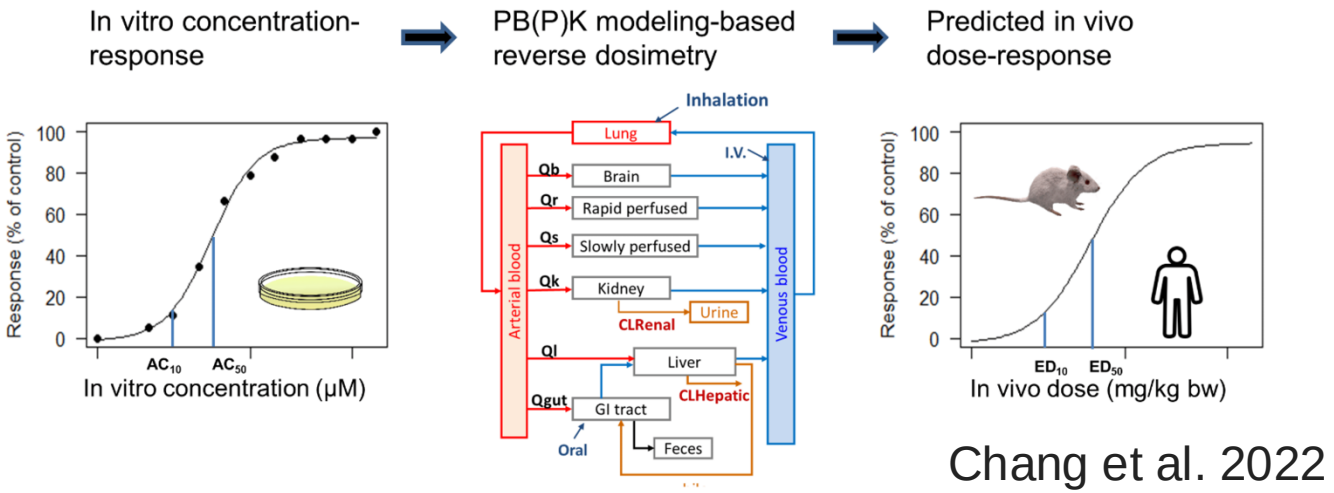
https://cran.r-project.org/web/packages/tcpl/vignettes/Data_processing-Archive_tcpl_v2.html

Data Release/ Data Exploration

- **Tox21 Toolbox**
  - The Tox21 toolbox contains useful tools for accessing and visualizing the Tox21 quantitative high throughput screening (qHTS) 10K library data, as well as integrating with other publicly available data.
  - Integrated Chemical Environment (ICE): https://ice.ntp.niehs.nih.gov
    - Has extra curation step
- **EPA CompTox Chemicals Dashboard**
  - The EPA Computational Toxicology (CompTox) Chemicals Dashboard is a one-stop-shop for chemistry, toxicity and exposure information for over 760,000 chemicals. Data and models within the Dashboard also help with efforts to identify chemicals of most need of further testing and reducing the use of animals in chemical testing. The CompTox Chemicals Dashboard integrates data from various sources including:
  - ToxCast and Tox21 - High-throughput chemical screening data
  - General Use – Assay Information: https://comptox.epa.gov/dashboard/
  - ToxCast: https://www.epa.gov/comptox-tools/exploring-toxcast-data

Data Interpretation/ Utilization

- Translating *in vitro* data into real world meaning
  - Using in pathway analysis (e.g., Estrogen and Androgen Receptors)
  - Input into *in silico* models
    - QSAR/ physiochemical properties for read-across
  - *in vitro* to *in vivo* extrapolation (IVIVE) (Dr. Marc Beal's Lecture)



Chang et al. 2022

Future Directions for Tox21 (Phase III: 2014–present)

- **Toxicodynamic Variability in Developmental Neurotoxicity**
  - *Goal: Incorporate genetic variation into cell-based test systems to better understand potential population differences in response to chemicals that may cause toxic neurological effects.*
- **Retrofitting Existing Tox21 High-Throughput Screening Assays with Metabolic Capability**
  - *Goal: To add xenobiotic metabolism capability to existing Tox21 assays so they provide more accurate and informative data regarding in vivo activity.*
- **Cell Line Selection for High Throughput Transcriptomics (HTTr)**
  - *Goal: Develop a strategy for selecting maximally diverse cell types/lines to maximally cover biological targets and pathways for high-throughput chemical screening using gene expression (i.e., transcriptomics).*
- ***In Vitro* Chemical Disposition**
  - *Goal: Understand the impact of chemical disposition within in vitro test systems across a broad range of chemical categories and develop a computational model to predict differences between the "nominal" concentration of a chemical compared with "true" concentration in the media and cells.*

Wednesday Hands-On Activity

Concentration-response modeling in R with ToxCast/ Tox21 Data

1. Install R from https://cran.rstudio.com
2. Install RStudio from: https://www.rstudio.com/products/rstudio/download/#download
3. If you have not used R before read over Intro to R: https://github.com/kristineccles/setac_intro_to_r_2020/blob/main/intro_to_r_lecture.pdf
   - Try out some of the scripts in this repo: https://github.com/kristineccles/setac_intro_to_r_2020
4. Download (or clone) the repository with the materials for Wednesday: https://github.com/kristineccles/BIM4103-HTS-Dose-Response
   1. R scripts
   2. Data

References

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Vinken, M., Knapen, D., Vergauwen, L., Hengstler, J. G., Angrish, M., & Whelan, M. (2017). Adverse outcome pathways: a concise introduction for toxicologists. *Archives of toxicology*, *91*, 3697-3707.