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Modeling Biotransformation Using In Vitro Data on Parent-Metabolite Pairs within the ToxCast Phase I Chemical Set

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Office of Research and Development
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Overview

- ToxCast Overview
- ToxCast Biotransformation Overview
- Parent/Metabolite Analysis
- Assessment of Current Technology
- Conclusions & Next Steps

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ToxCast

- Objectives
  - In Vitro & In Silico Endpoints to Predict In Vivo Outcomes
  - Use Resulting Predictions for Chemical Prioritization

- Challenges
  - Accounting for METABOLISM / BIOTRANSFORMATION
  - Extending from Animal to Human Toxicity Potential

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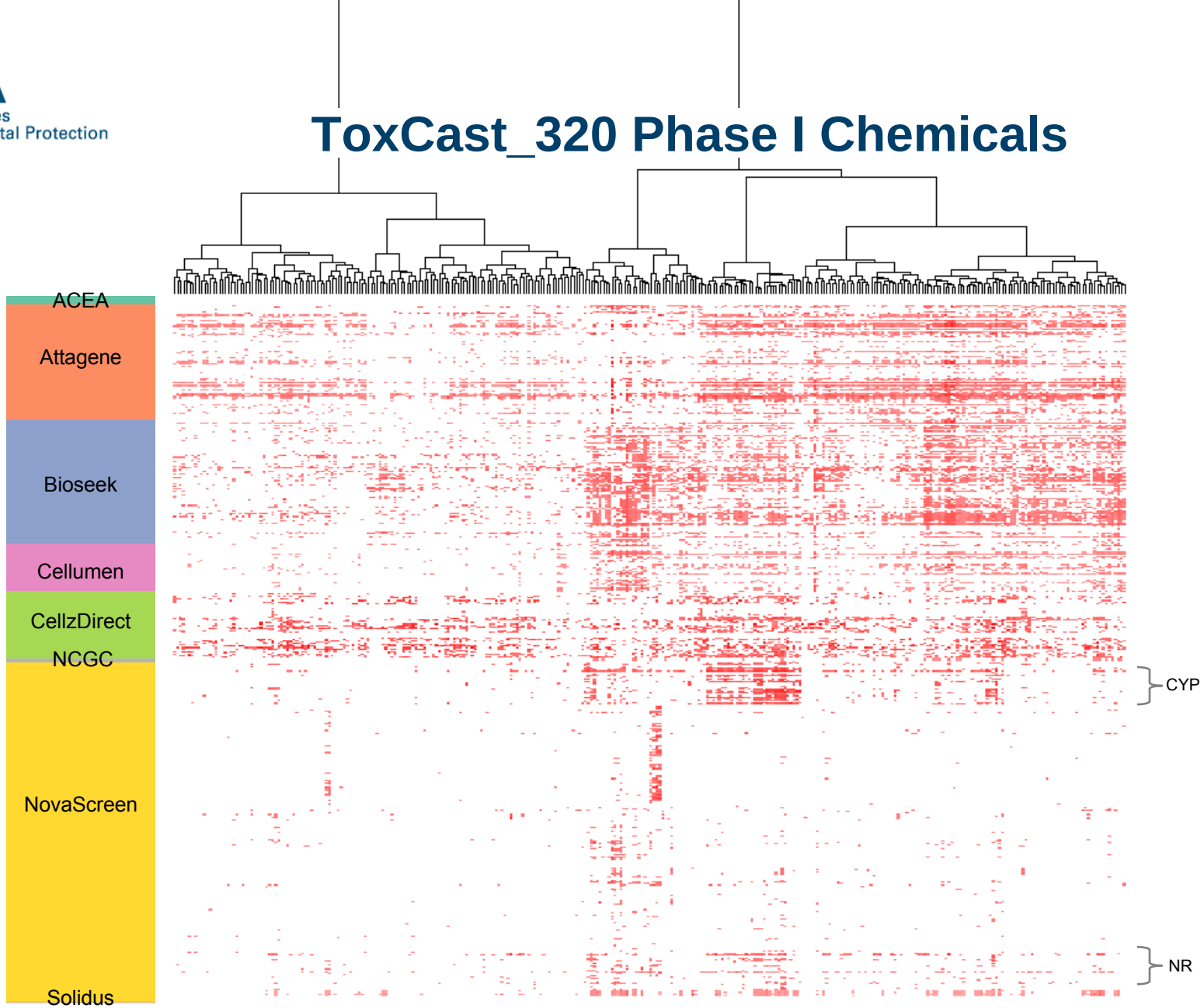
ToxCast_320 Phase I Chemicals

ToxCast Phase I Assays 500 endpoints



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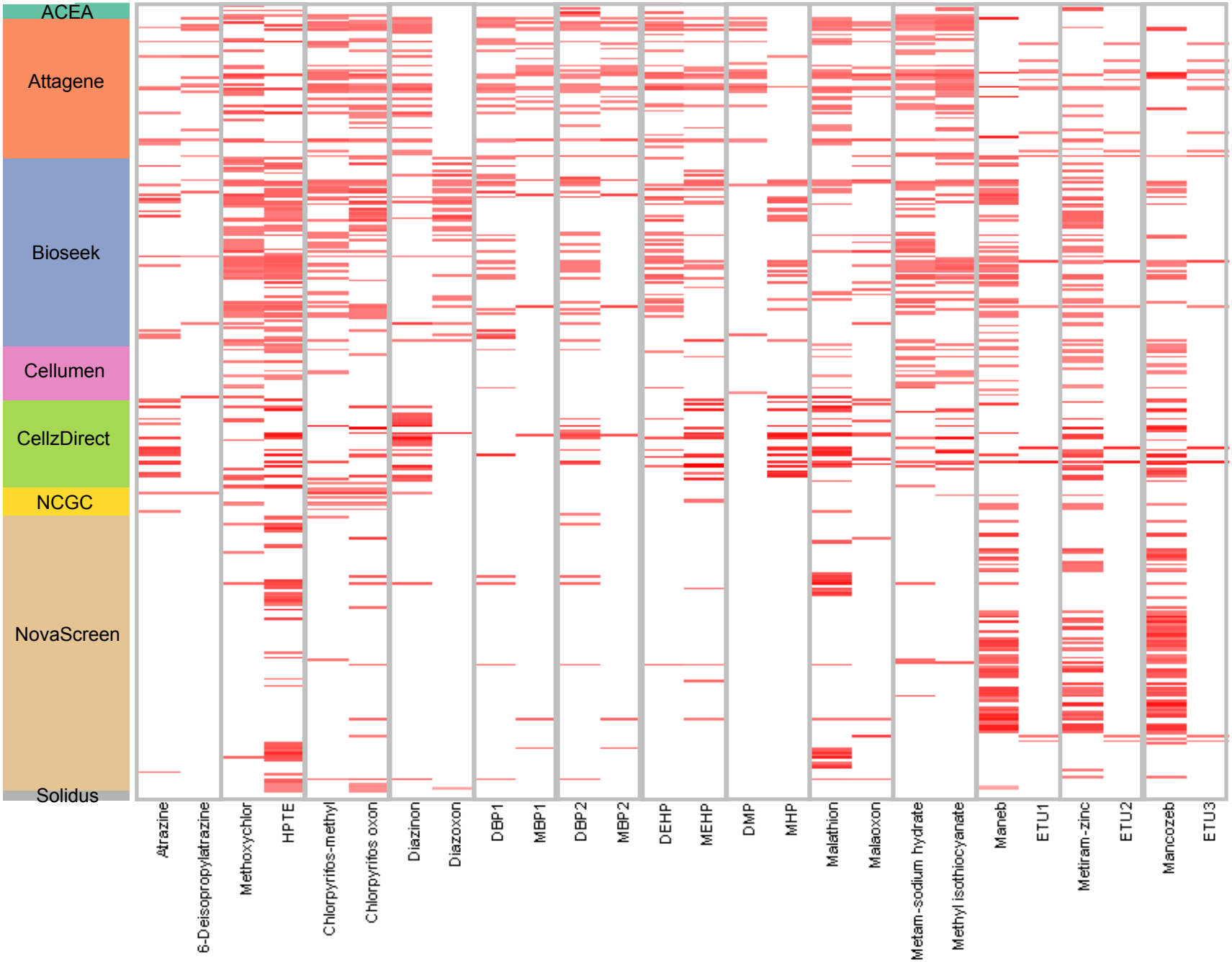
ToxCast - Biotransformation

- ToxCast_320
  - 309 Unique Chemical Structures
  - 13 Parent-Metabolite Pairs
    - 1 Replicate (DBP)
    - 3 Parent Chemicals Share Common Metabolite (ETU)
- ToxCast Assays (500 Endpoints)
  - Cell-Based
    - HCS & Cytotoxicity
    - w/ & w/o Metabolic Competency
  - ADME (CYP Inhibition & Induction/Suppression)
  - NR (Binding & Transcription Factor Activation)

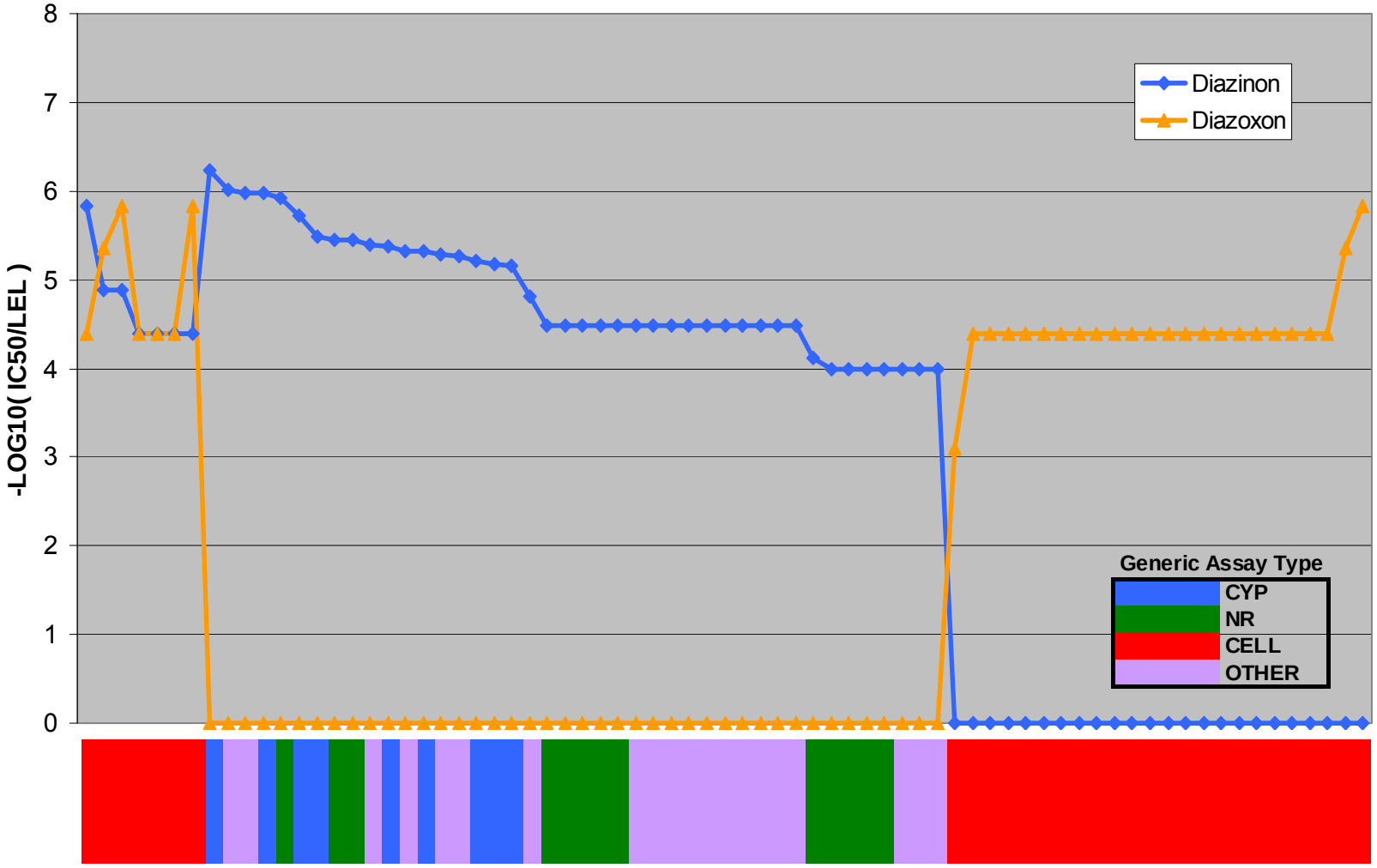
Profile of Parent/Metabolite Hits Across 320 Endpoints



Data Interpretation

- Parent Activity
  - Are Positives Related to Parent Toxicity or Indicative of Metabolism?
    - Cyp Inhibition & Induction (associated w/ Downstream Toxicities & Metabolic Activation)
  - Can Cytotoxicity Endpoints in Assays w/ & w/o Metabolic Capacity Serve as an Indicator of Metabolic Activation?
    - Sensitive Enough?
    - Specific Enough?
- Metabolite Activity
  - Which Metabolite?
  - Is Metabolite Activity >, <, or ≠ Parent Activity?

Comparing Parent & Metabolite Bioactivity Profiles



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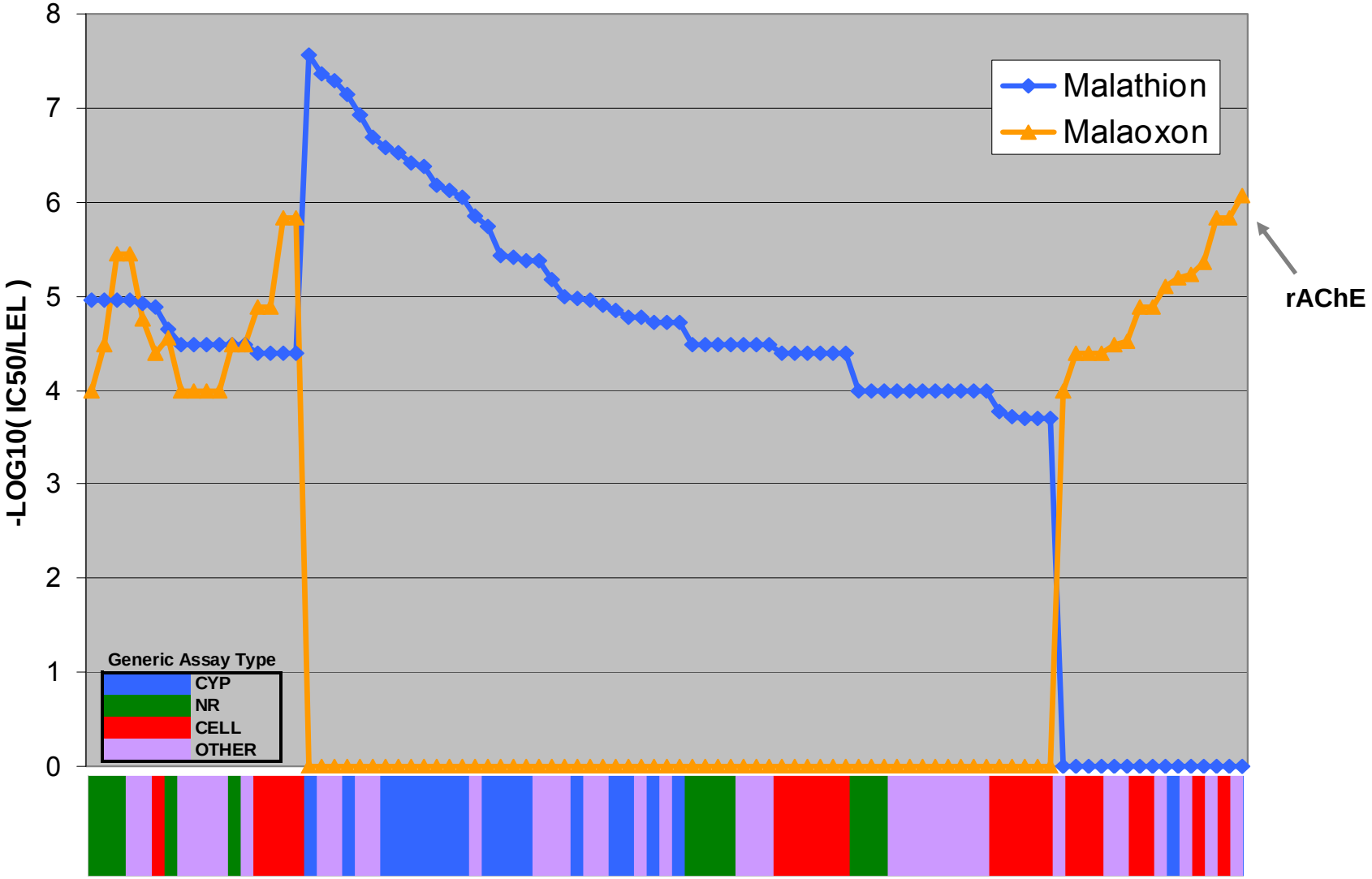

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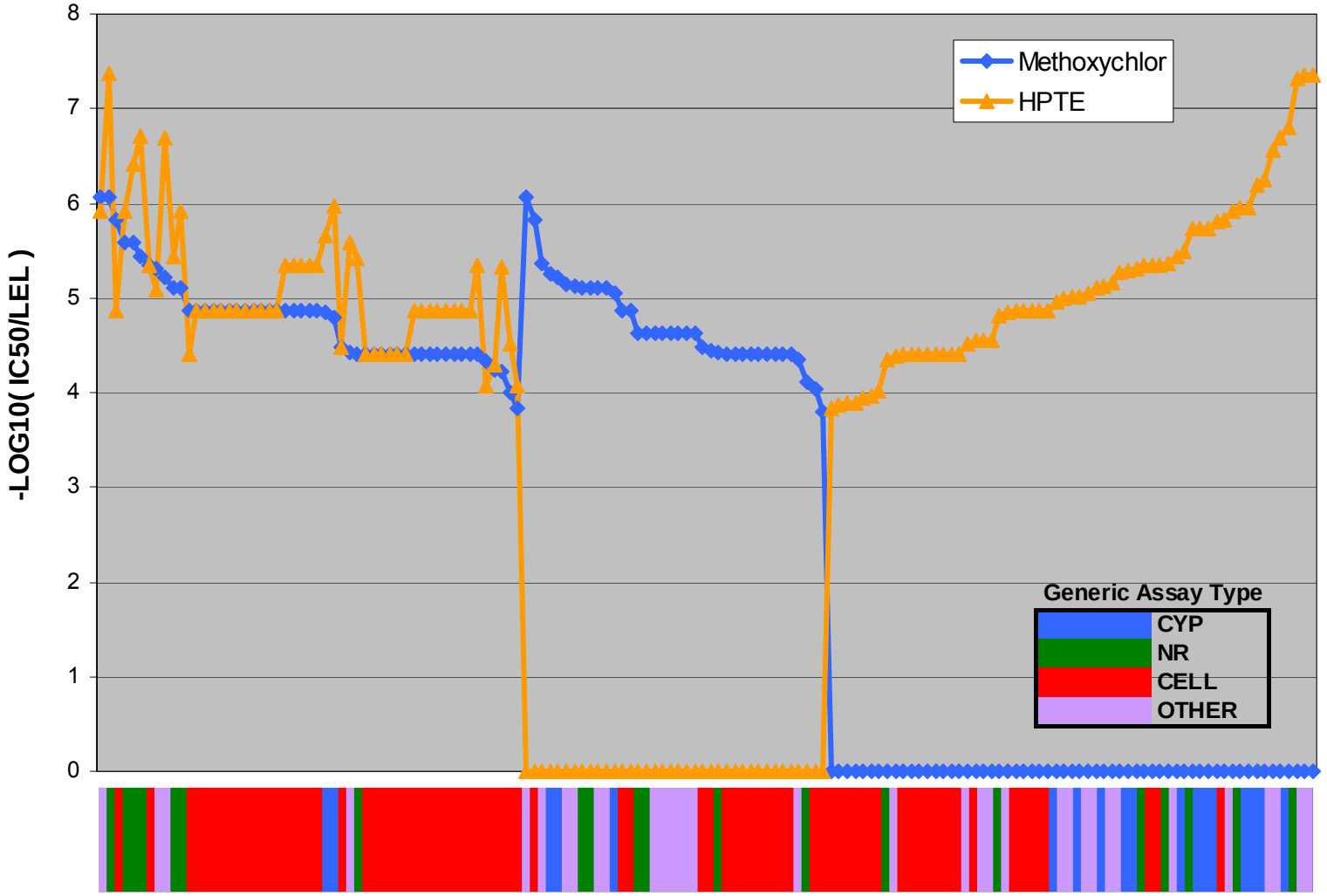

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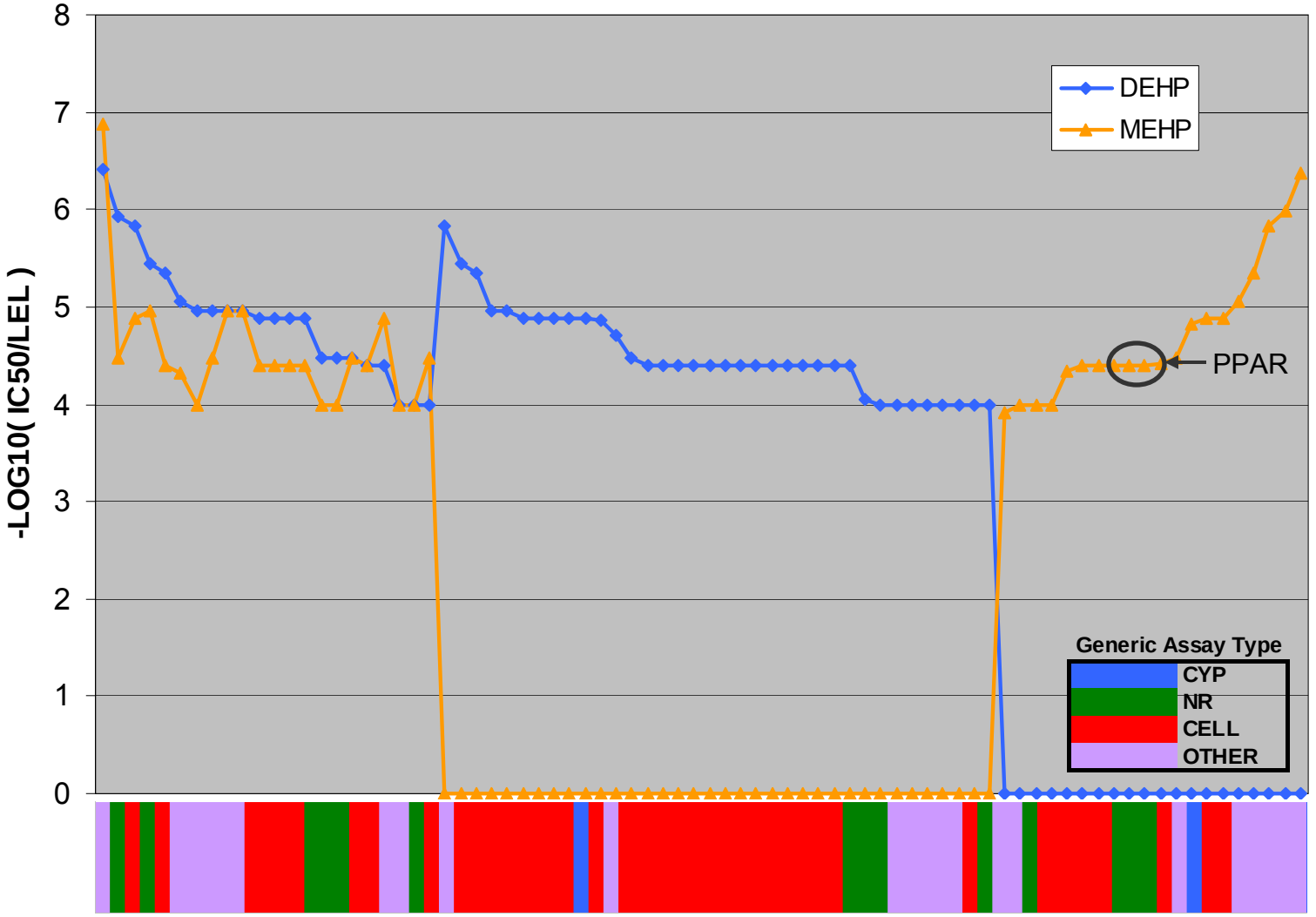
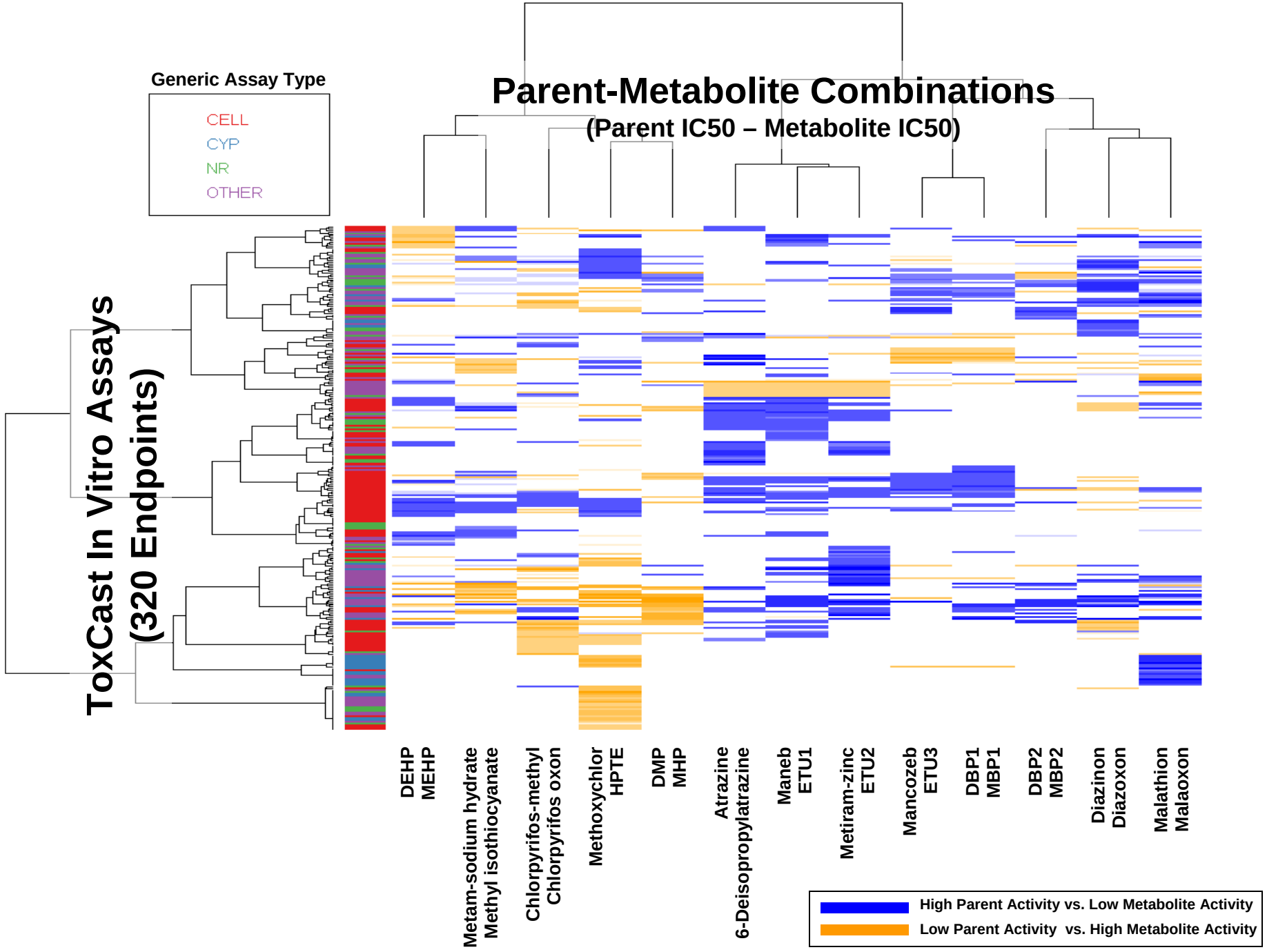



Generic Assay Type
CELL
CYP
NR
OTHER
Parent-Metabolite Combinations
(Parent IC50 – Metabolite IC50)
ToxCast In Vitro Assays
(320 Endpoints)
DEHP
MEHP
Metam-sodium hydrate
Methyl isothiocyanate
Chlorpyrifos-methyl
Chlorpyrifos oxon
Methoxychlor
HPTE
DMP
MHP
Atrazine
6-Deisopropylatrazine
Maneb
ETU1
Metiram-zinc
ETU2
Mancozeb
ETU3
DBP1
MBP1
DBP2
MBP2
Diazinon
Diazoxon
Malathion
Malaoxon
High Parent Activity vs. Low Metabolite Activity
Low Parent Activity vs. High Metabolite Activity

Highly Discriminating Assays

Parent vs. Metabolite

Top Assays w/ High Parent Activity & Low Metabolite Activity

- CLZD_CYP2B6_6
- NVS_ADME_hCYP2C19
- ATG_PXR_TRANS
- ATG_VDRE_CIS

} Xenobiotic Metabolism

- BSK_3C_Proliferation
- BSK_LPS_PGE2
- CLMN_CellLoss_72hr
- CLMN_MitoticArrest_72hr

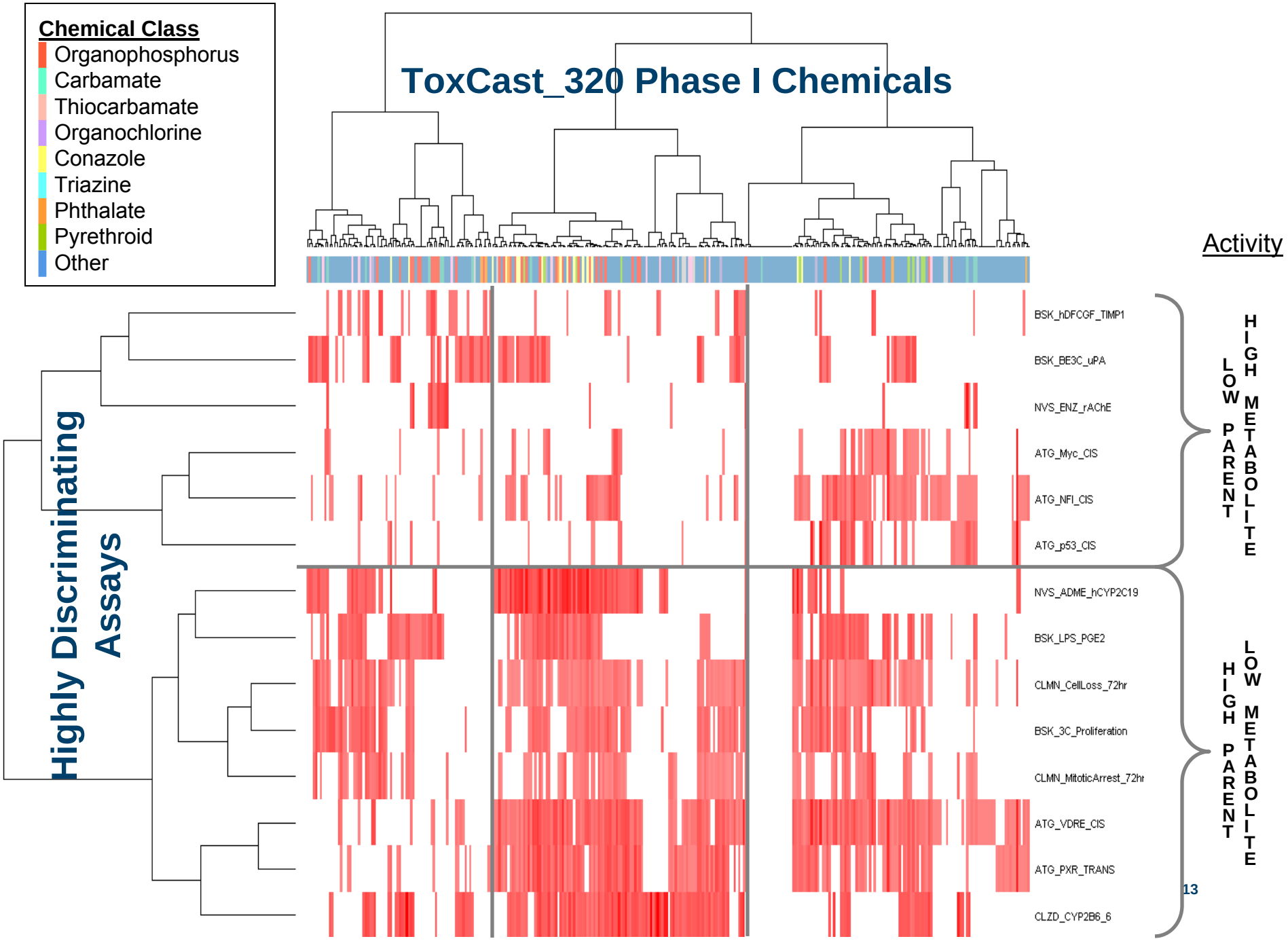
} HepG2

Top Assays w/ Low Parent Activity & High Metabolite Activity

- NVS_ENZ_rAChE

} AChE Inhibition

- BSK_BE3C_uPA
- ATG_NFI_CIS
- ATG_Myc_CIS
- ATG_p53_CIS
- BSK_hDFCGF_TIMP1


Chemical Class
Organophosphorus
Carbamate
Thiocarbamate
Organochlorine
Conazole
Triazine
Phthalate
Pyrethroid
Other
ToxCast_320 Phase I Chemicals
Activity
Highly Discriminating Assays
BSK_hDFCGF_TIMP1
BSK_BE3C_uPA
NVS_ENZ_rAChE
ATG_Myc_CIS
ATG_NFI_CIS
ATG_p53_CIS
NVS_ADME_hCYP2C19
BSK_LPS_PGE2
CLMN_CellLoss_72hr
BSK_3C_Proliferation
CLMN_MitoticArrest_72hr
ATG_VDRE_CIS
ATG_PXR_TRANS
CLZD_CYP2B6_6
LOW PARENT HIGH METABOLITE
HIGH PARENT LOW METABOLITE

Conclusions

- Chemical Perspective
  - Clear Differences in Parent-Metabolite Bioactivity
  - Both Increased & Decreased Metabolite Activity
  - Difficult to discern between:
    - Potentially Adverse Interaction
    - Metabolism-Related Interaction
    - Combination of Both
- Assay Perspective
  - Identified assays as indicators of metabolic activity
  - Identified assays susceptible to 'false negatives' if parent only tested
  - Parent chemical cytotoxicity results w/ metabolic capacity do not have similar results to metabolite cytotoxicity
- Next Steps
  - Identify and procure larger set of parent-metabolite pairs for ToxCast Phase II & Tox21
  - Further analyze data from existing cell-based systems w/ metabolic capacity
  - Explore new methods & technologies w/ metabolic components
  - Develop predictive models of biotransformation & subsequent activity/toxicity

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Conceptual Model for Predicting Biotransformation & Subsequent Toxicities