Chemical risk assessment: How well do *in vitro* and *in silico* data predict the *in vivo* situation?

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New Approach Methods (NAMs)

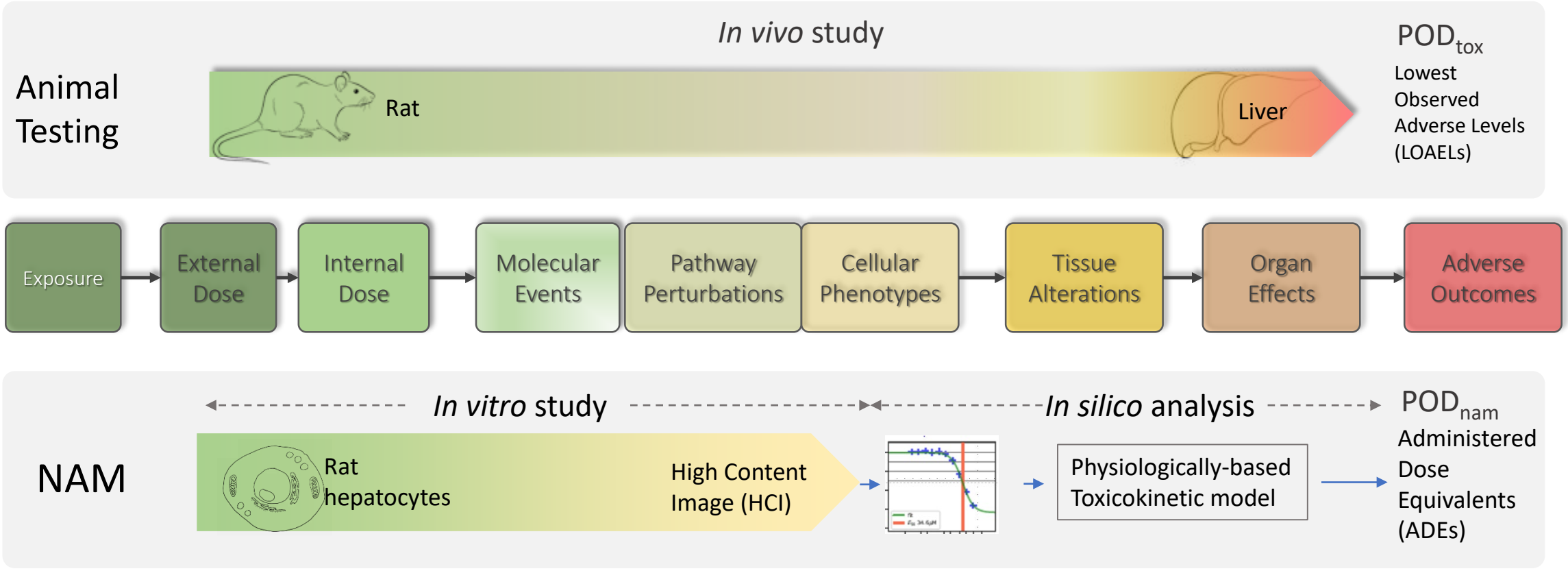
- New approach methods (NAMs) are important for screening thousands of untested chemicals.
- NAMs can be used to predict point of departure (POD) using *in vitro* and *in silico* methods
- How do NAM-based PODs compare with *in vivo* PODs?

NAM = in vitro | in silico

Any technology, methodology, approach, or combination of methods that can provide information about chemical hazard and risk assessment without using whole animals.

Kavlock, et al 2018

A NAM for Repeat-Dose Rat Liver Toxicity Testing



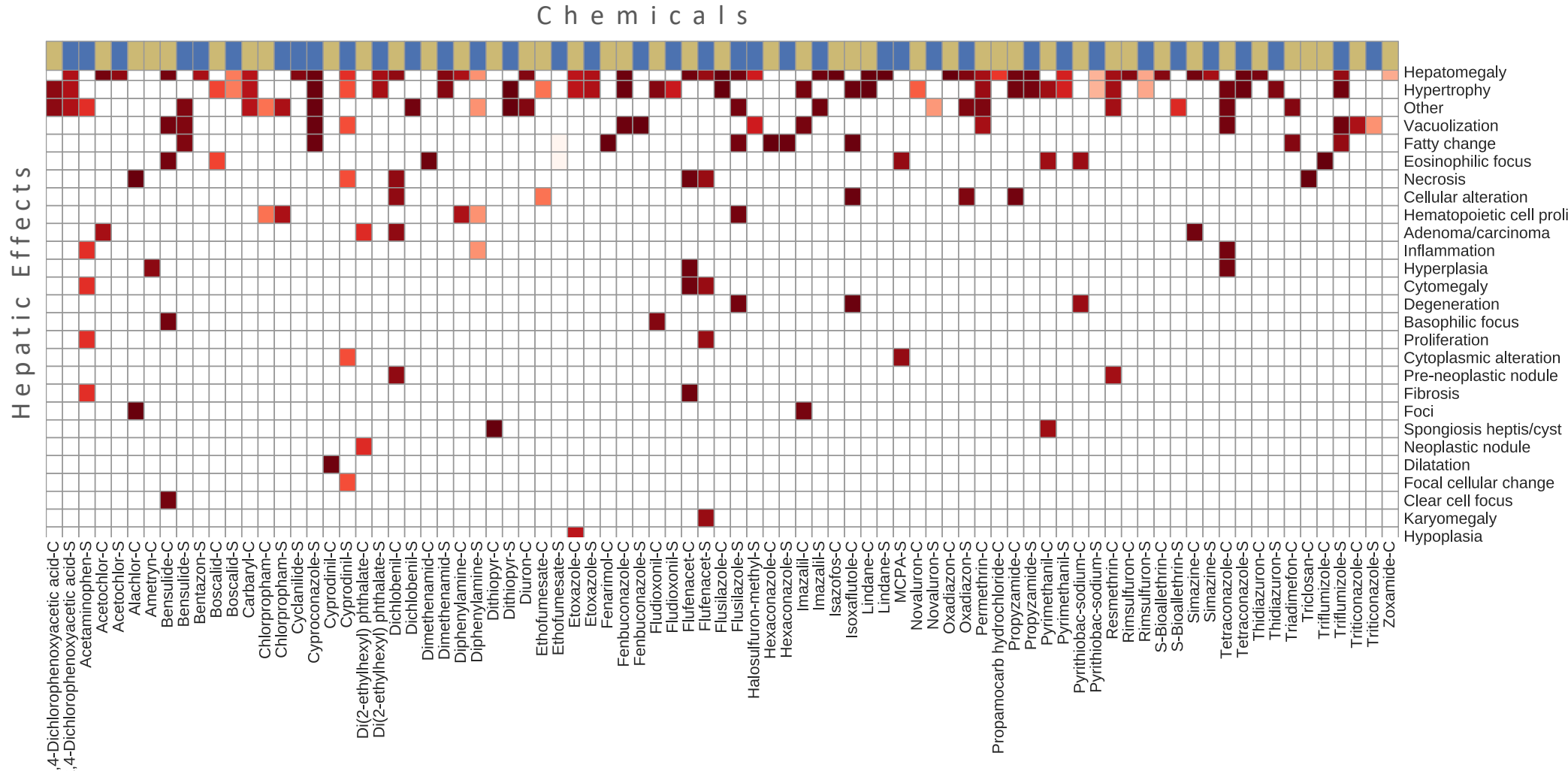
Approach: Retrospectively compare POD from NAMs (POD_{nam}) with POD from traditional toxicity testing (POD_{tox})

Repeat-Dose Toxicity Data

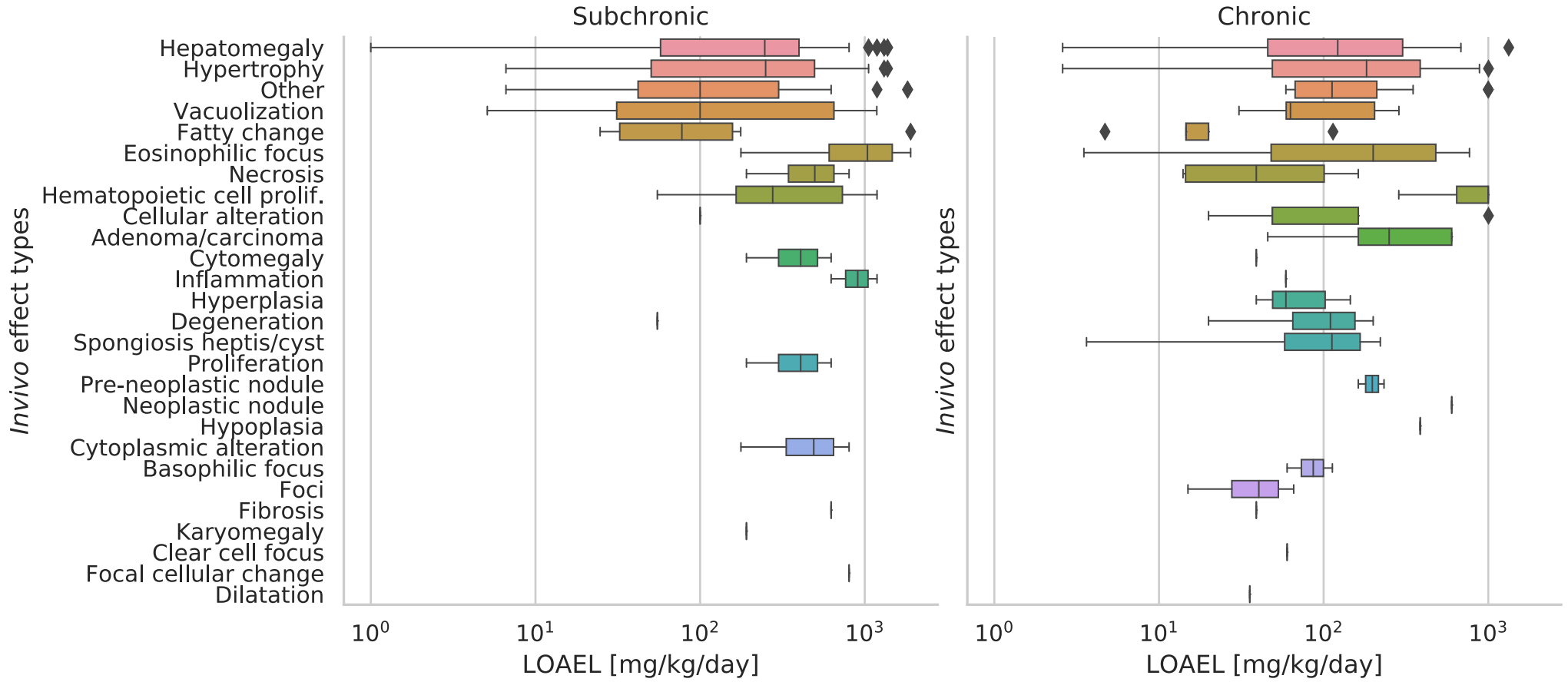
POD_{tox} values for 51 chemicals

51 chemicals with *in vivo* repeat-dose toxicity data from guideline subchronic and/or chronic studies

Data obtained from ToxRefDB v2.0 using species=rat, administration route=oral, study type = (subchronic |chronic) and target organ = Liver



Distribution of POD_{tox} values

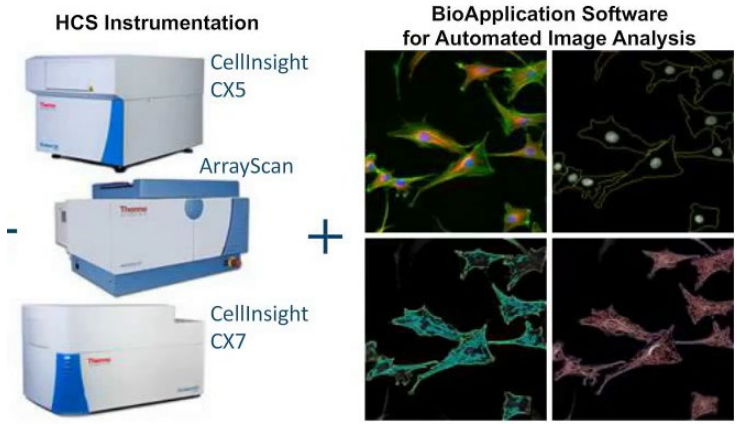


Identify 51 chemicals with *in vivo* repeat-dose toxicity data from guideline subchronic and chronic studies

Data obtained from ToxRefDB v2.0 using species=rat, administration route=oral, study type = (subchronic |chronic) and target organ = Liver

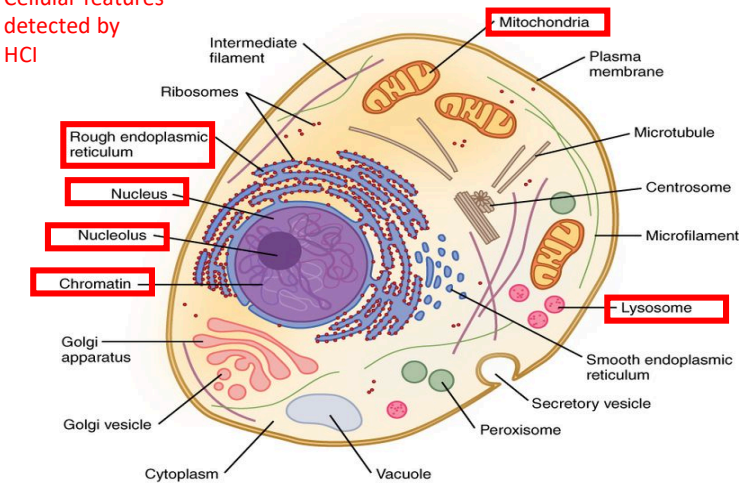
In vitro Rat Hepatocyte HCI Assay (HepRn)

- Cell model: Rat primary hepatocytes
- Assay: High-content imaging (HCI)
  - Steatosis: LipidTox®
  - ER Stress: GADD153 (CHOP)
  - Mitochondrial function: MitoTracker Red
  - Lysosomal Mass: LysoTracker Red
  - Apoptosis: Cytochrome C
  - DNA texture: Hoechst 33342
  - Nuclear size: Hoechst 33342
  - Cell number: Hoechst 33342
- Chemical treatments
  - Controls: (-) DMSO; (+) CCCP, Bupivacaine, Tamoxifen, Nimesulide
  - Conc: 0.2, 0.39, 0.78, 1.56, 3.12, 6.24, 12.5, 25, 50 and 100 µM
  - Duration: 24, 48 and 72 h.
  - Reps: 2 on plate



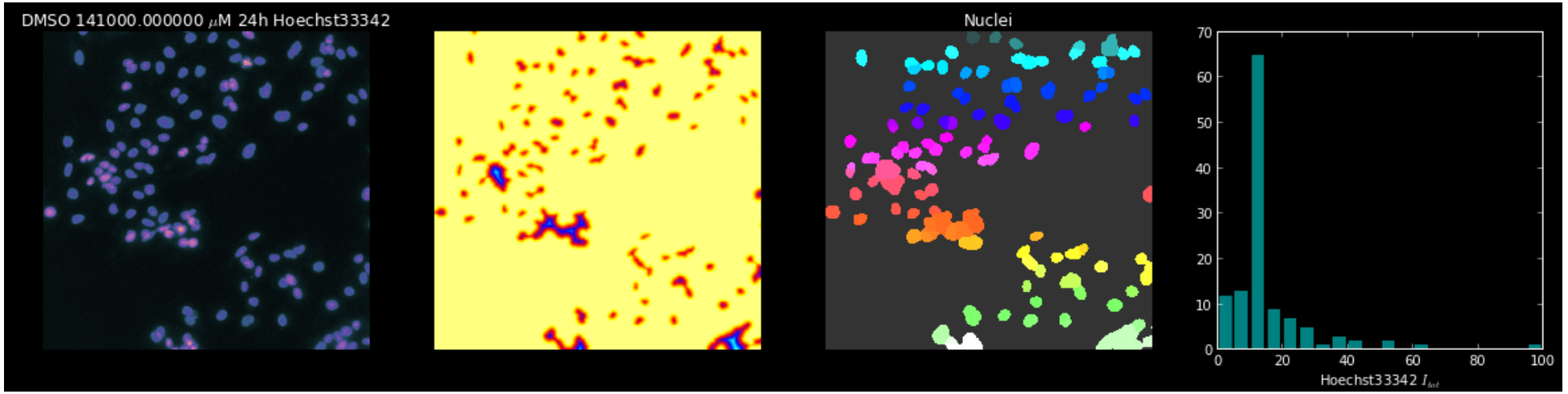
Thermofisher.com

Cellular features detected by HCI



Wikimedia.org

HCI Data Based on Cytomorphological Features



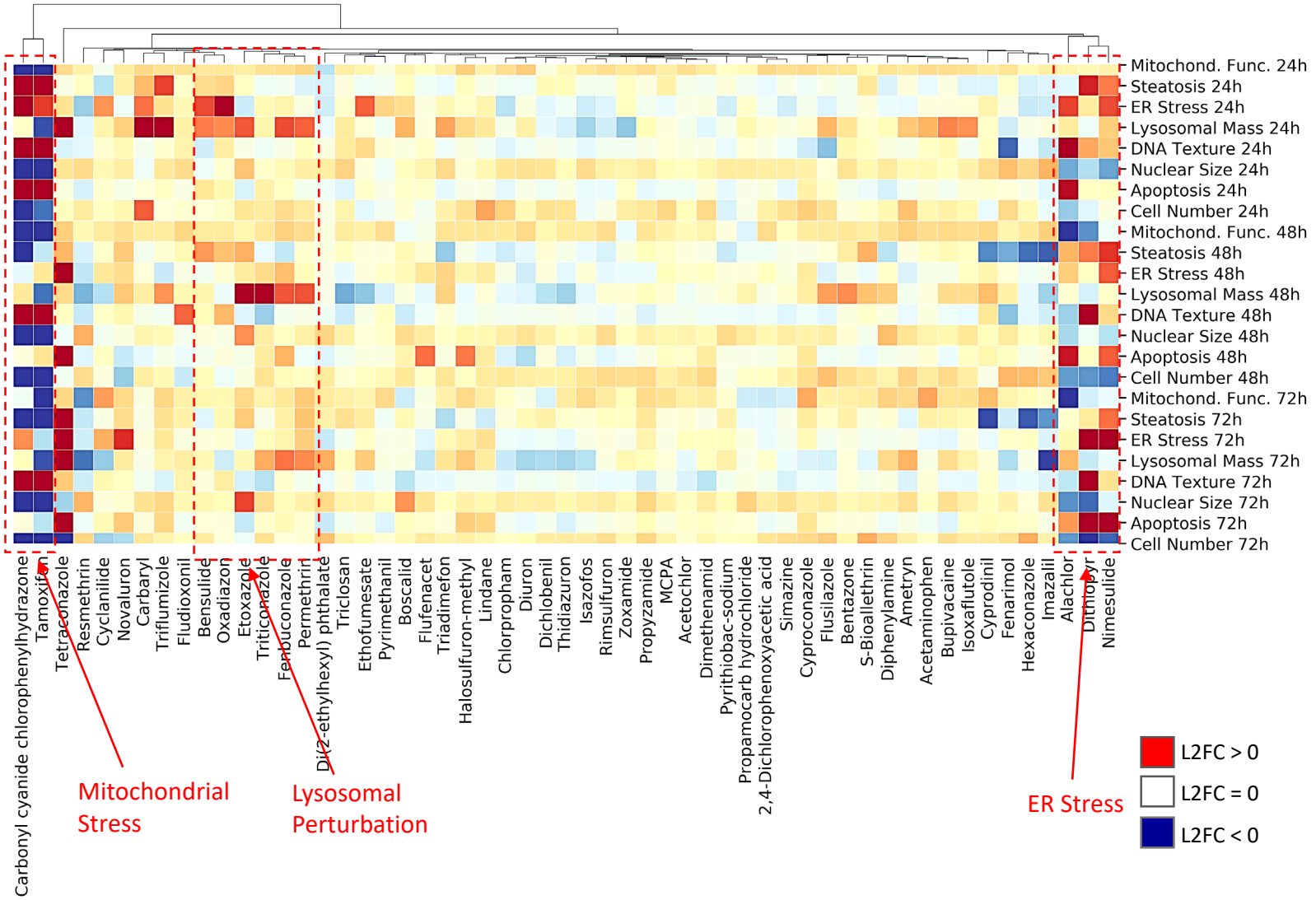
Raw Image (Hoechst) → Intensity Analysis → Cell compartment Analysis → Cell compartmental intensity Distribution → Well level aggregation

Rat hepatocyte HCI endpoints (HepRn assay)

- Mitochondrial function (MF)
- Lysosomal mass (LM)
- ER stress (ES)
- Steatosis (St)
- DNA texture (DT)
- Nuclear size (NS)
- Apoptosis (Ap)
- Cell number (CN)

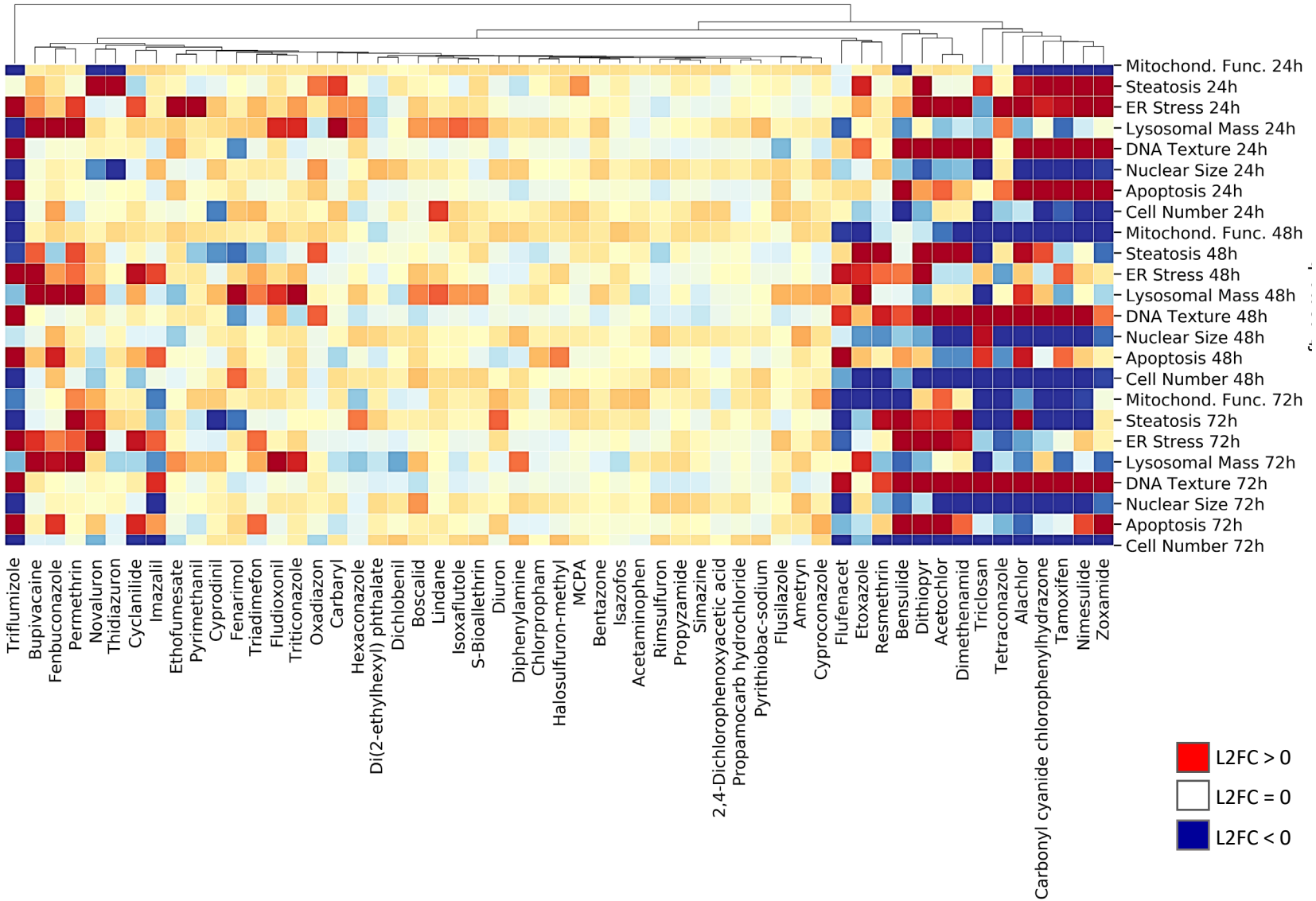
In vitro Effects – Measured by HCI (50µM)

- Log2 Fold Change (L2FC) by comparison with DMSO controls
- Summarize L2FC of all chemicals at 50µM
- Heatmap shows chemicals (columns) vs HCI features at 24, 48 and 72h and L2FC values (blue=decrease and red=increase)
- Phenotypic response categories
  - Mitochondrial stress ± cell death
  - Lysosomal ± cell death
  - ER Stress ± cell death
  - No effect
- Cross-talk between stress-responses

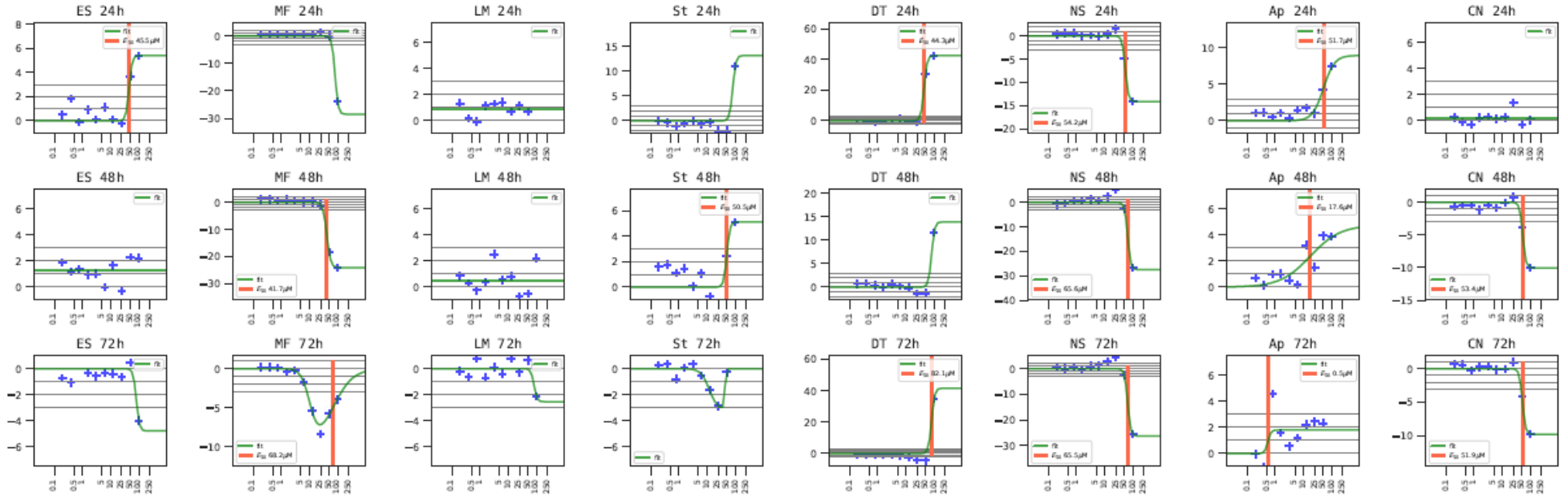


In vitro Effects – Measured by HCI (100µM)

- Summarize L2FC of all chemicals at 100µM
- Heatmap shows chemicals (columns) vs HCI features at 24, 48 and 72h and L2FC values (blue=decrease and red=increase)
- Phenotypic response categories
  - Increased cell death
  - Increased perturbations
  - Some chemicals still produce no effects



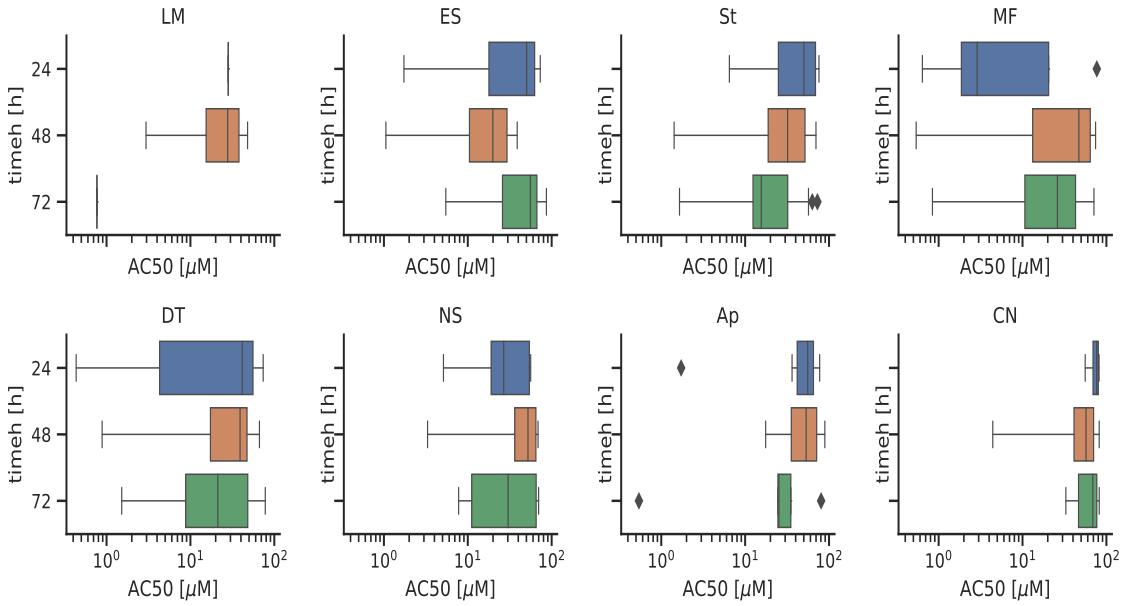
Conc-Response Analysis: E.g. Alachlor



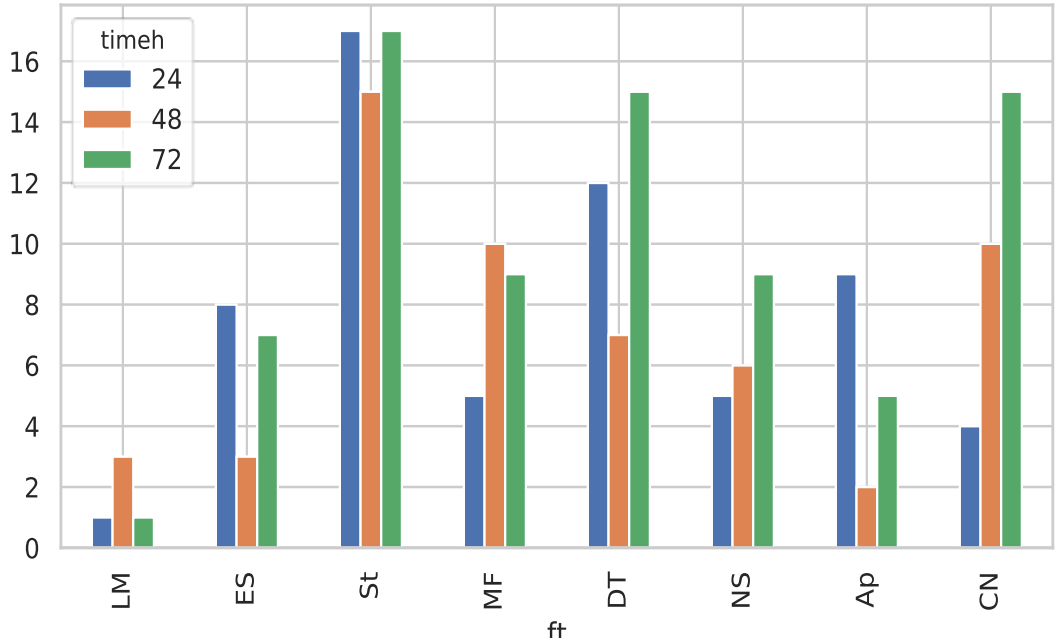
- Conduct concentration-response analysis of all chemicals using the R/tcpl (Filer, 2014) package
- Standardize the data using DMSO controls and identify the best curve-fits (constant, exponential, hill and gainloss)
- For non-constant fits find the AC_{50} for each chemical, assay, time point

- Filter 51*8*3 curve-fits for quality manually:
  - AC_{50} within range of test conc.
  - More than one response |Z|>3
  - Maximum response |Z|>3
  - Noisy fits

HepRn HCI Potency values and Hits



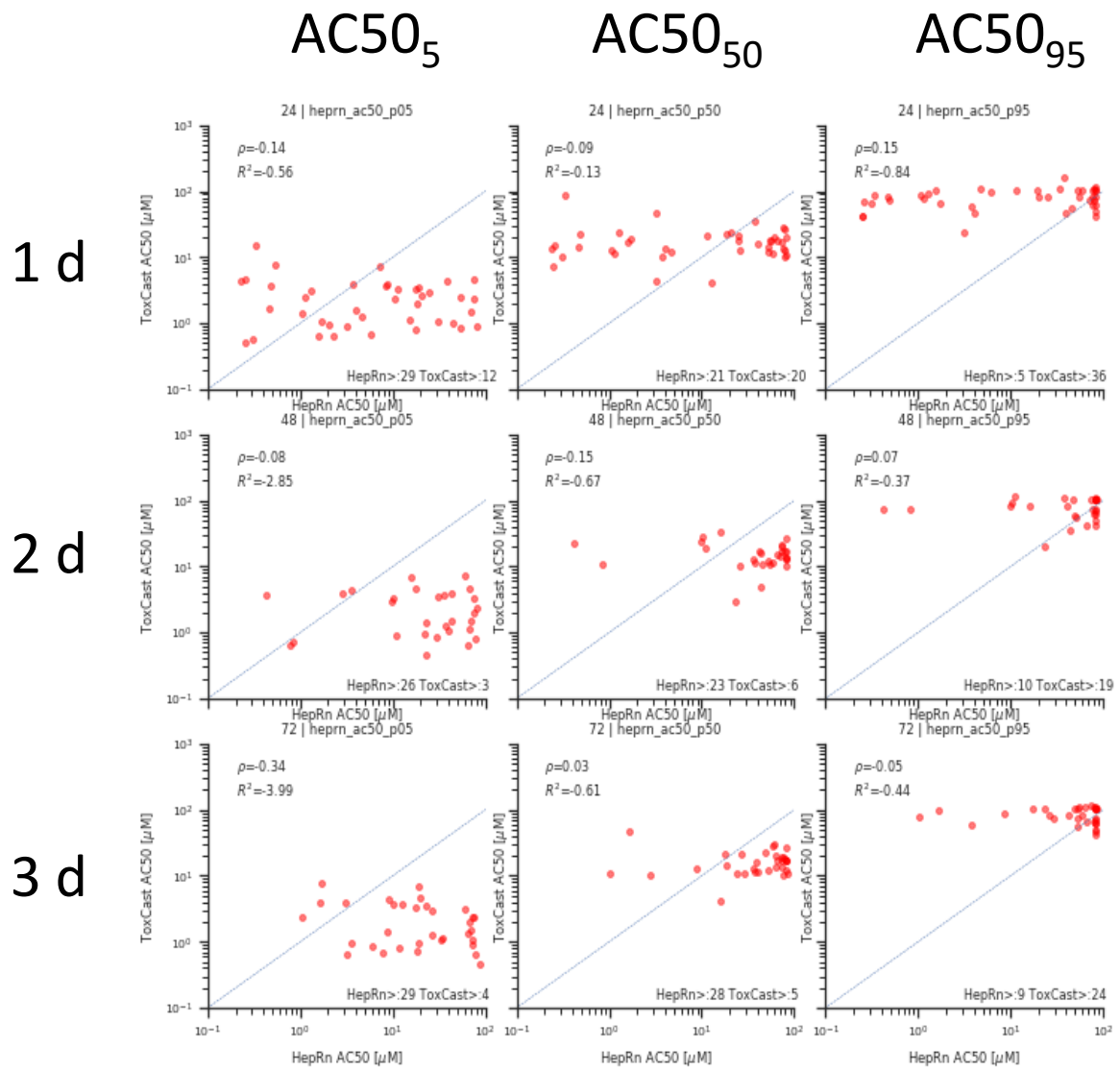
Mitochondrial function and DNA texture most sensitive assays



Hit = chemical & endpoint has high quality curve-fit

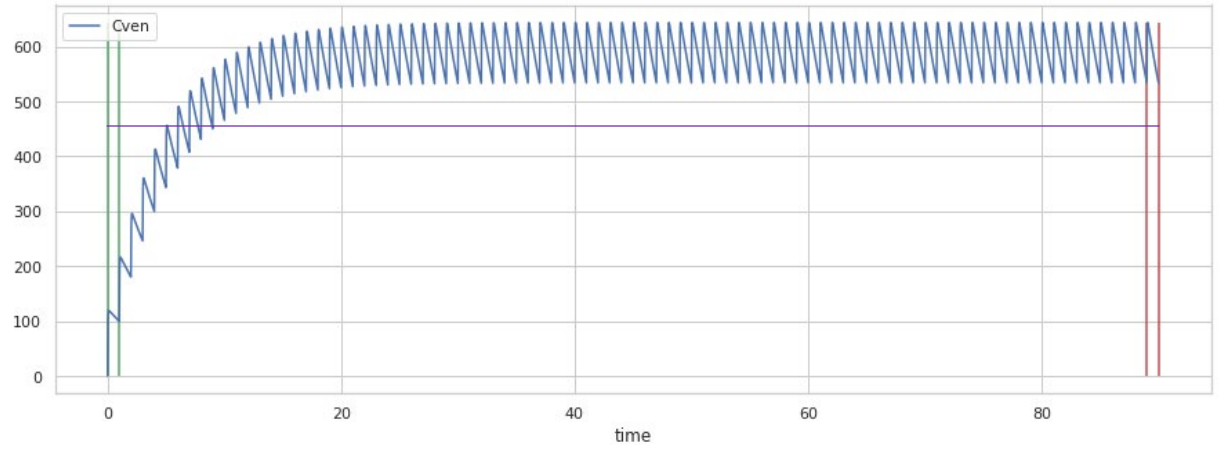
Compare HepRn with ToxCast assays

- Get the AC_{50} values for the 51 chemicals from ToxCast using NCCT InVitroDB v3.0
- Compare the 5^{th},50^{th} and 95^{th} percentiles of AC_{50} values between HepRn and ToxCast assays
- The ToxCast AC_{50} values are generally lower than corresponding HepRn values



In Vitro to *In Vivo* Extrapolation

- Use physiologically-based toxicokinetic modeling (PBTK) extrapolate AC_{50} values to administered dose equivalents (ADEs)
- Used the following approaches:
  A. Concentration at steady state (Css)
  B. Physiologically-based pharmacokinetic modeling (PBTK)
     - Clearance: restrictive
     - Dose metrics: Cmax, Cave, AUC
     - Comparing *in vitro* conc. to *in vivo* dose
       - time-initial-matched
       - time-averaged
       - time-final-matched
- Implemented using R/httk package (Pearce et al. 2017)



time-initial-matched: *In vitro* conc of hepatocytes = venous conc to *in vivo* at the same time
time-averaged: In vitro conc of hepatocytes = average venous conc in rats across time
time-final-matched: *In vitro* conc of hepatocytes is venous conc *in vivo* at the final time

POD_{tox} vs POD_{nam}

POD_{nam} based on:

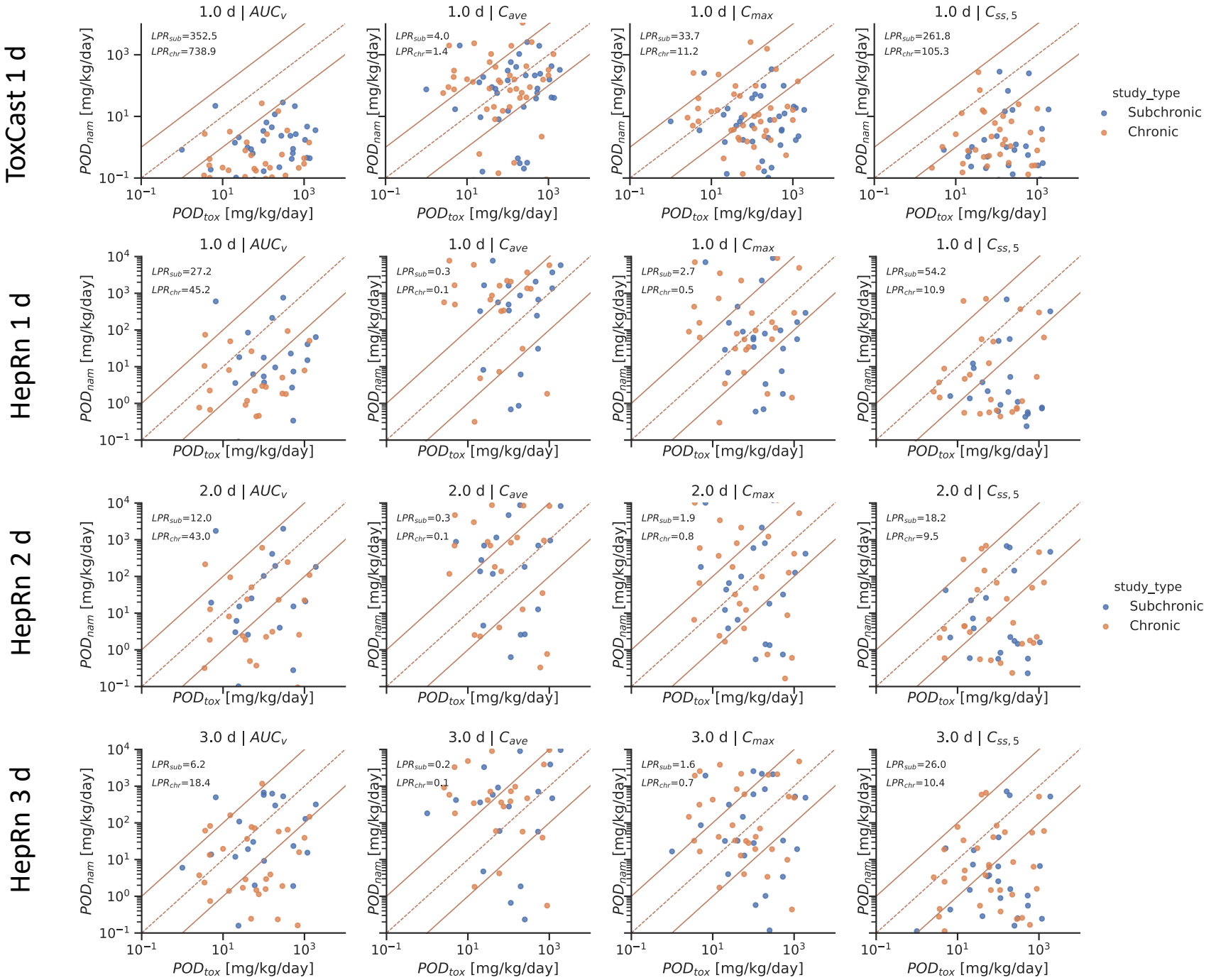
- $AC_{50,5}$ *: In vitro* potency using 5th percentile of AC_{50} from two assays:
  - HepRn: 1, 2 and 3 d exposures
  - ToxCast: using 1 d exposure
- ADE: PBTK:time-averaged using Css 5th percentile ($C_{ss,5}$) , AUC_v, C_{ave} and C_{max}

POD_{tox} based on:

- $LOAEL_5$: 5th percentile of lowest observed adverse effect levels in liver
- Study types: Chronic and subchronic

Compare using the “POD Ratio”:

- PR = mean(POD_{tox}/POD_{nam})
  - PR>1 is health protective
  - PR<1 not health protective



HepRn Subchronic POD_{nam} vs POD_{sub}

POD_{nam} = the ADE based on 5th percentile of *in vitro* AC_{50}

POD_{tox} = 5th percentile of lowest observed adverse effect levels ($LOAEL_5$) for subchronic rat liver effects

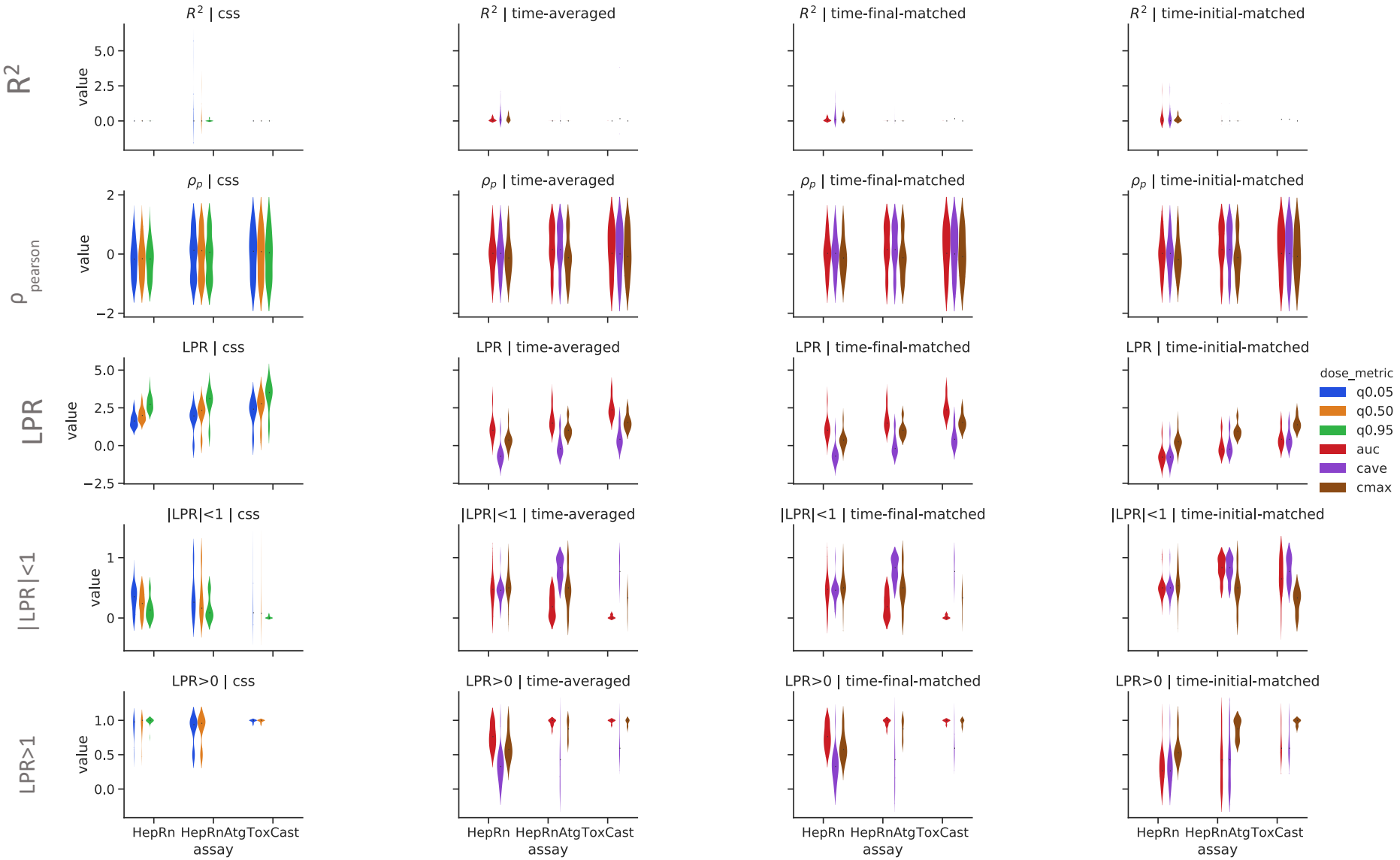
LPR = log10(mean(POD_{tox}/POD_{nam}))

|LPR|<1 = fraction of chemicals for which POD_{nam} i10x greater or less than POD_{tox}

LPR>1 = fraction of chemicals underpredicted (health-protective)

ρ_p = Corr(POD_{tox}/POD_{nam})

R^2 = Coefficient of determination



HepRn Chronic POD_{nam} vs POD_{chr}

POD_{nam} = the ADE based on 5th percentile of *in vitro* AC_{50}

POD_{tox} = 5th percentile of lowest observed adverse effect levels ($LOAEL_5$) for subchronic rat liver effects

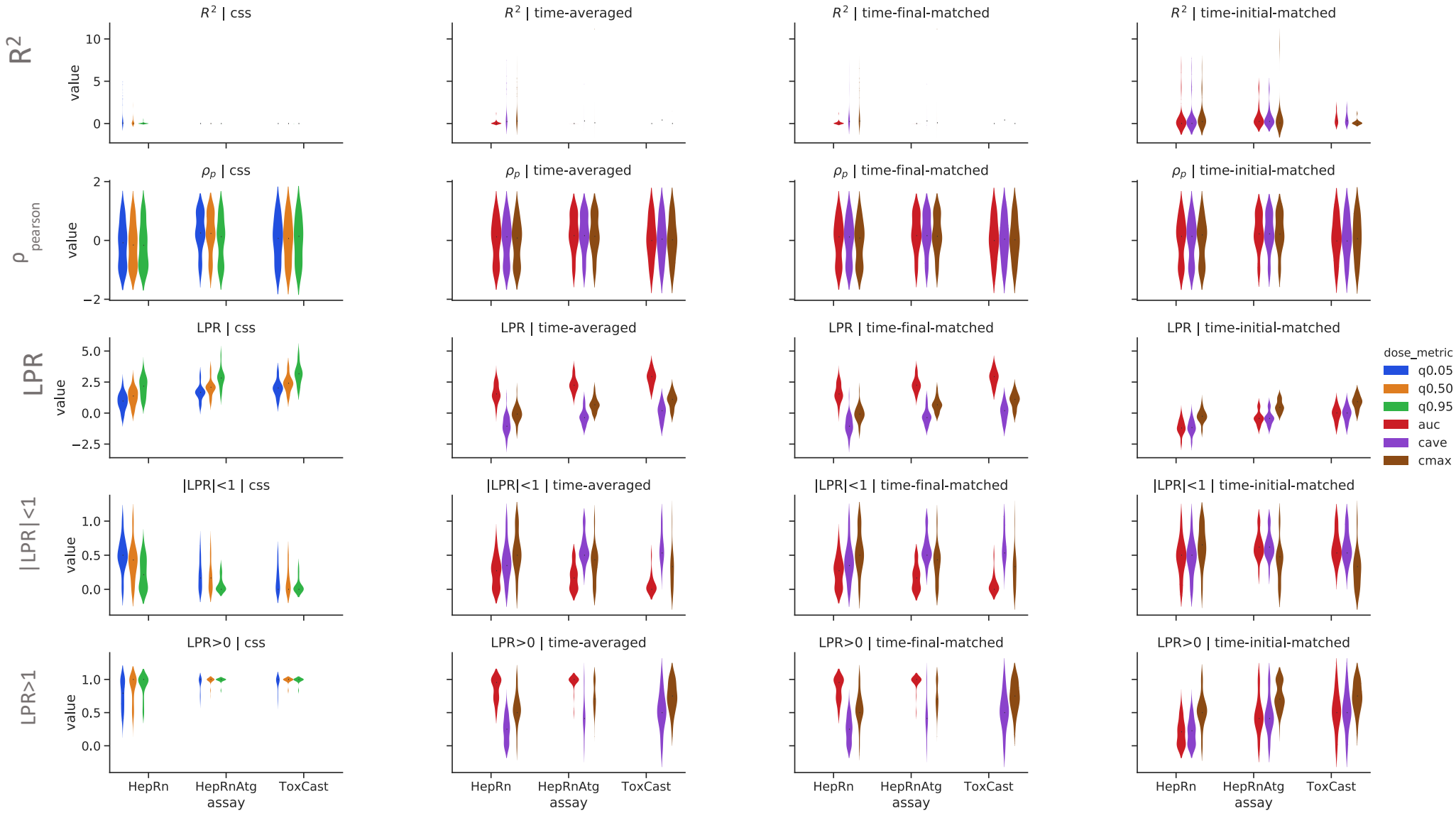
LPR = $\log10(\text{mean}(POD_{tox}/POD_{nam}))$

|LPR|<1 = fraction of chemicals for which POD_{nam} ¡10x greater or less than POD_{tox}

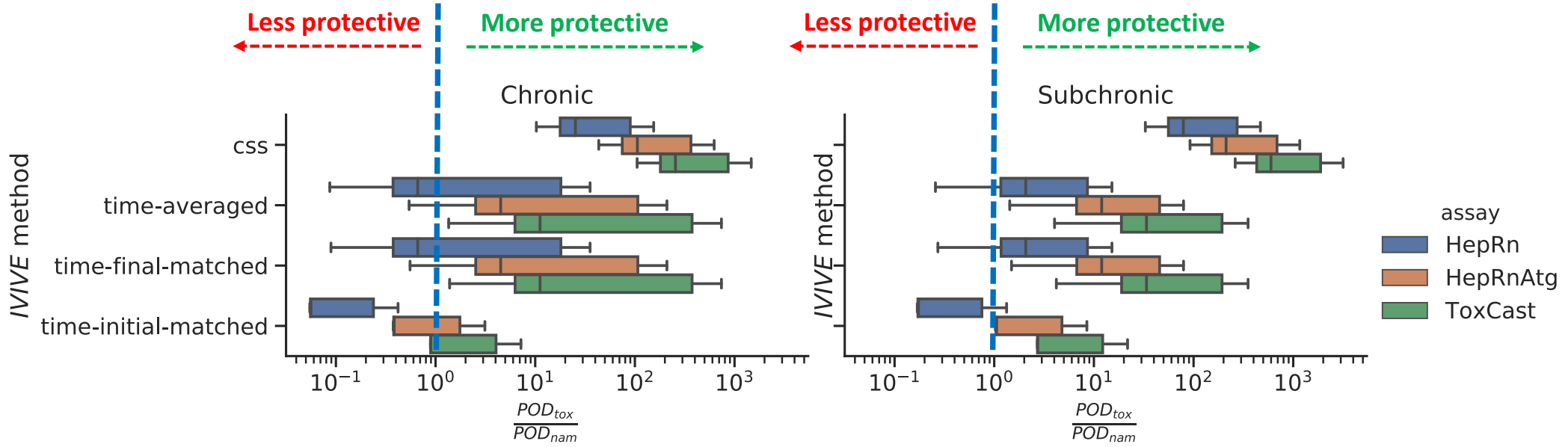
LPR>1 = fraction of chemicals underpredicted (health-protective)

ρ_p = Corr(POD_{tox}/POD_{nam})

R^2 = Coefficient of determination



How does HepRn Compare with ToxCast?



- Compare the POD ratios (PR) across different IVIVE approaches and assays (averaged over dose-metrics)
- HepRnAtg: Combine HepRn and ToxCast:Attagene to capture non-specific (general stress responses) as well as specific chemicals (receptor-mediated pathways

- IVIVE approach matters for
  - PR for Css >> PR for PBTK
  - PR for time-averaged similar to PR for time-final-time-matched
  - PR for time-initial-matched << PR for time-averaged
- On average, POD_{nam} from HepRn are 15-fold less than ToxCast (findings are consistent with Paul-Friedman et. al.)

Integrate Chronic *in vivo* & *in vitro*

Visually integrate all information about the chronic rat hepatotoxicants:

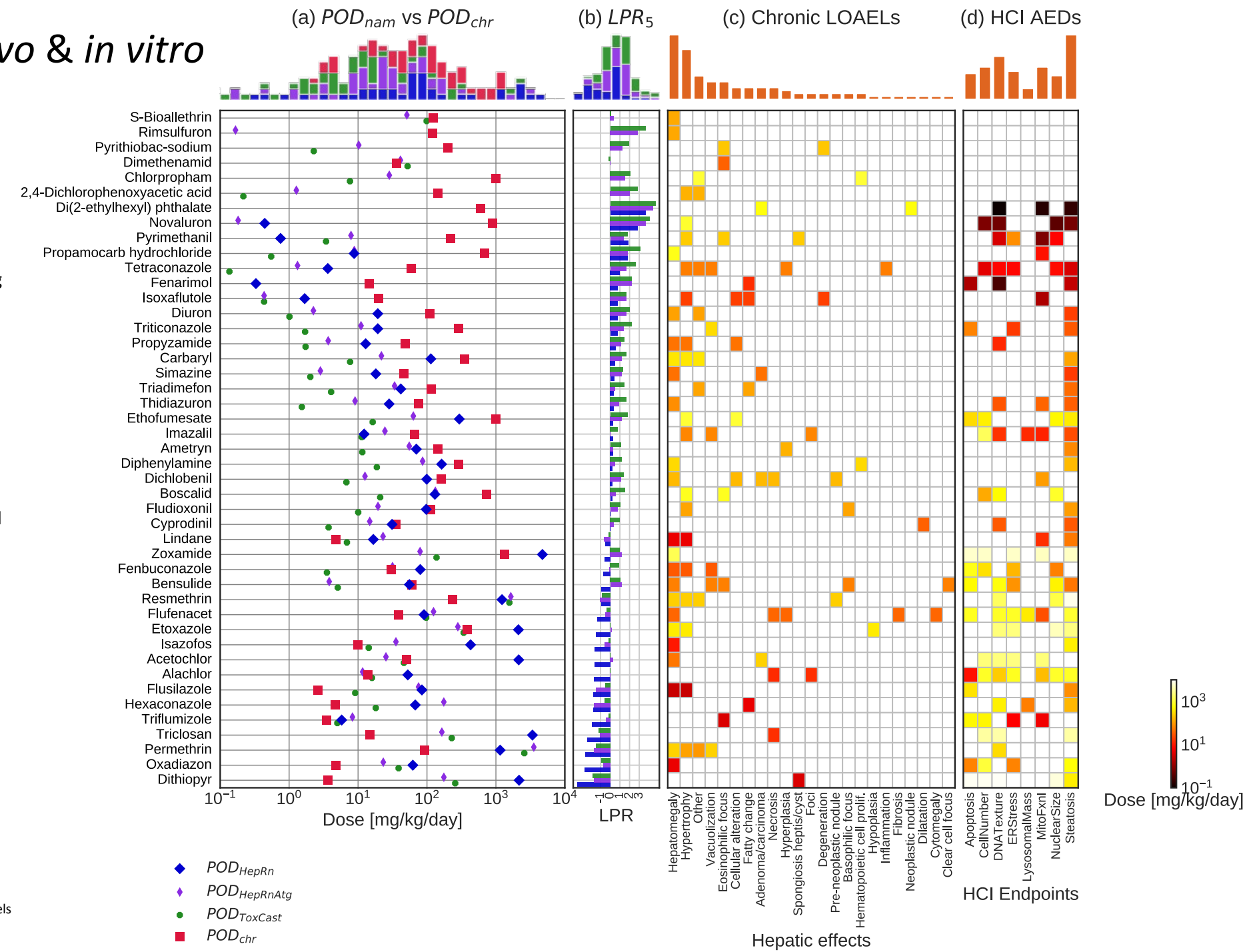
- Chronic POD values (POD_{chr})
- POD_{nam}
  - Assays: HepRn, ToxCast, HepRnAtg
  - IVIVE: time-averaged, C_{max}

- LPR for each assay type
- Chronic hepatic effects and corresponding LOEAL values
- HepRn assay endpoints and corresponding ADE_5

Highlight chemicals for which HepRn assay is protective (LPR>0), not protective (LPR<0) and reasonably predictive (|LPR|<1).

Examine the relationship between *in vivo* hepatic effects and *in vitro* cytomorphological changes

Overpredictions (LPR<0) of the HepRn assay could be due to issues with PBTK or due to specific receptor mediated mechanisms



POD_{nam} = the ADE based on 5th percentile of *in vitro* AC_{50}
POD_{tox} = 5th percentile of lowest observed adverse effect levels ($LOAEL_5$) for chronic rat liver effects
LPR = log10(mean(POD_{tox}/POD_{nam}))

Summary

- *In vitro* and *in silico* NAM-based PODs are generally health-protective
- IVIVE assumptions matter: Steady state concentrations are more health-protective than those based on PBTK models
- *In vitro* assays matter: PODs produced using the same species (rat) cell type (hepatocyte) are closer to PODs from animal testing
- Poor correlation between NAM-based PODs from animal testing could be due to:
  - *In vitro* assays: using single cell type, narrow biological coverage, or data analysis, etc.
  - TK: *In vitro* partitioning, PBTK gut absorption, etc.
  - Toxicodynamics (TD): biology is complex!

Acknowledgements and Questions

Todor Antonijevic
Rusty Thomas
Josh Harrill
Thomas Knudsen
Katie Paul-Friedman
John Wambaugh
Greg Honda

Additional slides