Comparing Toxicological Tipping Points from High-Content Imaging to Rat Subchronic Hepatoxicity Doses

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Background

Objective: **A major challenge to using *in vitro* high-throughput screening (HTS) data in risk assessment is the identification of toxicological "tipping points" between adaptation and adversity. *Toxicological tipping points represent a systems threshold, or critical point, beyond which biological pathways invoke permanent perturbations that eventually lead to adverse effects*.** Previously, we have proposed a formal approach to utilize time-course high-content imaging (HCI) data to identify tipping points *in vitro*. Here, we analyzed toxicological tipping points for chemicals in rat primary hepatocytes and used quantitative *in vitro* to *in vivo* extrapolation (qIVIVE) to compare them with rat subchronic hepatic lowest observed adverse effect levels (LOAELs).

Approach: First, we selected 88 chemicals from ToxRefDB that produced subchronic effects in rats. Next, we treated rat primary hepatocytes with 10 concentrations (0.2 to 100μM) of these 88 chemicals. We used HCI to measure endoplasmic reticulum stress (ES), mitochondrial function (MF), lysosomal mass (LM), steatosis (St), apoptosis (Ap), DNA texture (DT), nuclear size (NS) and cell number (CN) at 6 time points (1, 3, 6, 24, 48 and 72h). After processing and normalizing the data to calculate cell-state trajectories produced by each chemical treatment, we examined the occurrence of tipping points. For chemicals that produced tipping points, critical concentrations (C_{cr}) were estimated and extrapolated to oral equivalent doses using (qIVIVE), and were compared rat subchronic LOAELs for liver effects.

Methods

For every chemical, endpoint, and time we calculated:

- Endpoint perturbation: $x=log_2(r/r^*)$; r– smoothed data; r^*- plate median.
- Z-score standardized perturbation: $z=(x-x^*)/\sigma_x$;
  x^*- median of x; σ_x standard deviation of x.
- The lowest effective concentration (LEC) by numerically solving: $|z|=3$.

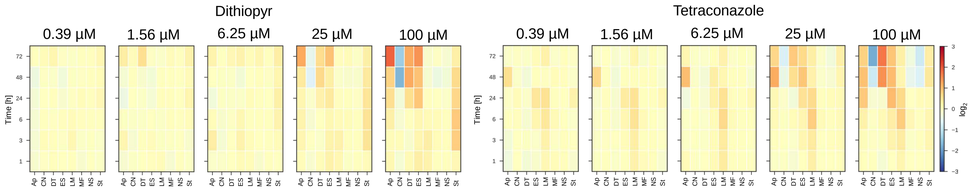
For each concentration and time point we defined:

- Systems perturbation vector: $\boldsymbol{X}=(x_{DT}, x_{MF}, x_{LM}, x_{NS}, x_{CN}, x_{ES}, x_{Ap}, x_{St})$.
- Scalar perturbation: $X=|\boldsymbol{X}|=(\sum x_i^2)^{1/2}$, and trajectory $T=(X^0,X^1,...,X^t,...X^N)$.
- Velocity as the rate of change of the scalar perturbation across time: $V = \frac{\partial X}{\partial t}$.

  At a given time point the trajectory may undergo:
  recovery ($V<0$), no recovery ($V>0$), or no change ($V=0$).
- The rate of change of velocity across concentration: $\partial_c V = \partial V/\partial c$.
  $\partial_c V < 0$ for concentrations that show recovery,
  $\partial_c V > 0$ for concentrations that do not show recovery, and
  $\partial_c V = 0$ for critical concentration that defines "tipping point"

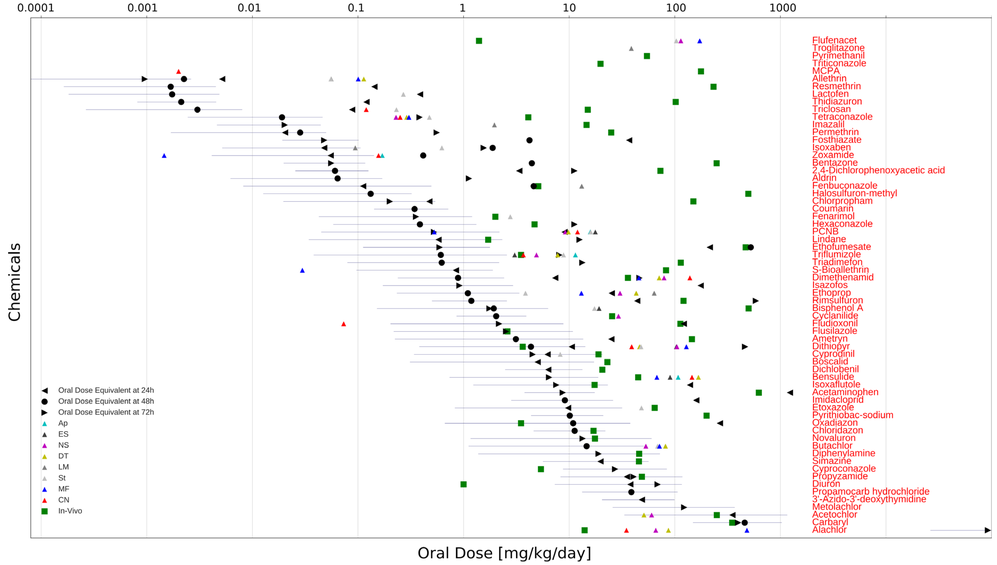
Concentration and time-dependent perturbations cause by chemicals

We interpret temporal trends as the chemically-induced stresses that cause endpoints to deviate from the homeostatic value. Below, each heatmap is a trajectory $T=(X^0,X^1,...,X^t,...X^N)$, and each row is a systems perturbation ($\boldsymbol{X}=(x_{DT}, x_{MF}, x_{LM}, x_{NS}, x_{CN}, x_{ES}, x_{Ap}, x_{St})$).

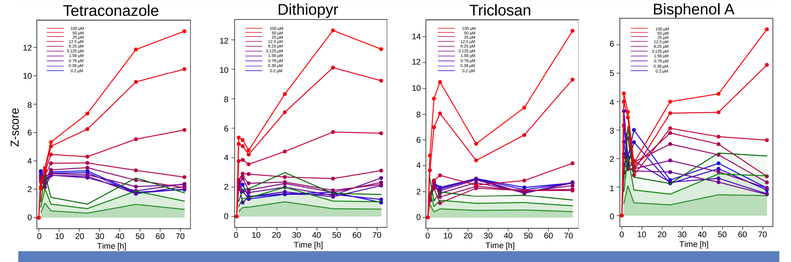


qIVIVE extrapolation of C_{cr} and comparison with *in Vivo* data

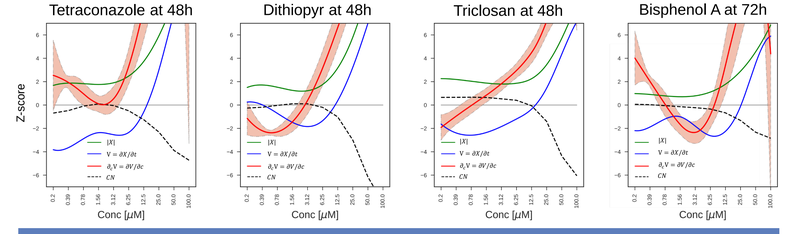
HTTK package was used to perform *In vitro to in vivo extrapolation* of critical concentrations and LEC. Obtained oral equivalent doses were compared with subchronic rat data.



Scalar Perturbation: $X=|\boldsymbol{X}|=(\sum x_i^2)^{1/2}$



Trajectory analysis and critical concentrations (C_{cr})



Results

1. 81/88 chemicals produced tipping points (C_{cr}) between 24h and 72h.
2. 7/88 chemicals showed transient perturbations followed by system recovery.
3. Data for IVIVE analysis was available for 63/88 chemicals (60 Ccr, and 3 with LEC only)
4. Oral doses for C_{cr} were the lowest in 50/60 cases, 4/60 for LEC, and 6/60 animal data.
5. 15 C_{cr} oral equivalent doses did not have corresponding LOAELs.
6. On average, C_{cr} equivalent oral doses were 18 times lower than the lowest extrapolated LEC, and ~250 times lower than in-vitro data
7. Our results show the utility of *in vitro* tipping points as a sensitive estimate of a systems threshold between adaptation and adversity that is supported by *in vivo* data.

References

1. Shah, Imran, et al. "Using ToxCast™ Data to Reconstruct Dynamic Cell State Trajectories and Estimate Toxicological Points of Departure." *Environmental health perspectives* (2016) .
2. Pearce, Robert G., et al. "httk: R package for high-throughput toxicokinetics." *J Stat Softw*79.1 (2017): 1-26.