Title: Finding Toxicological Tipping Points from High-Content Imaging Data

Abstract: A key challenge to using in vitro data in risk assessment is differentiating between chemical-induced adaptive versus adverse cellular responses. To further investigate this issue, we studied the effects of hundreds of chemicals in HepG2 cells using high-content imaging (HCI). HCI measured chemical concentration and time-dependent perturbations in p53, JNK, oxidative stress, cytoskeleton, mitochondria, and cell cycle progression. We developed a novel computational models to analyze these multidimensional HCI datastreams, and used this model to interpret the dynamic responses to chemicals as cell-state trajectories. By analyzing trajectories for each chemical we found three concentration-dependent trends in HepG2 cell behaviors including: (a) adaptation followed by complete recovery, (a) adaptation followed by partial recovery, and (c) adaptation without recovery leading to irreversible injury. We consider the concentration-dependent transition from adaptation to injury a "tipping point" of the system. Using Boolean network (BN) reconstruction to systematically analyze all trajectories, we found putative regulatory programs that could explain the basis of cellular resilience. We believe that multidimensional and dynamic *in vitro* data for chemicals can be interpreted with computational models to find tipping points, and gain unique mechanistic insight into the threshold for homeostatic adaptation. With additional work, tipping points could also be used as the point of departure for risk assessment.

This abstract does not necessarily reflect U.S. EPA policy.

Impact:

Current toxicological tests are based on identifying apical adverse effects to define a point of departure for risk assessment. This research uses high-content imaging (HCI) datastreams from the ToxCast project to identify the "toxicological tipping point" of cells. These tipping points offer a novel approach for interpreting the role of early molecular and cellular perturbations in the context of adverse outcomes.