**Is the Mechanism of APAP Toxicity In Vivo & In Vitro really the same? A model mechanism based explanation of the in vitro–in vivo disconnect**

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In vitro methods are being increasingly relied upon to predict by extrapolation in vivo pharmacological and toxicological phenomena even though the in vitro–in vivo (IVIV) disconnect persists as the major impediment to effective translation. Continued reliance on correlation and extrapolation is evidence that there is a pressing need for explanatory theories about those disconnects. Predictions of acetaminophen (APAP) metabolism, hepatic clearance, and toxicity made using data from in vitro studies employing specialized cell lines, isolated rodent and human hepatocytes serve as exemplary case studies.

The operating hypothesis behind IVIV extrapolations is that the mechanism of APAP toxicity in hepatocytes in vivo and in vitro is sufficiently similar to justify making predictions from one to the other. Quantitative IVIV extrapolations assume that mappings exist between key features of the in vitro and in vivo mechanism. Also, extrapolators recognize that some in vivo features do not have an in vitro counterpart and vise versa. Identification and explanation of those differences is expected to account for the translational disconnect. By better understanding the sources—the causes—of those differences, we can begin closing the disconnect. The objective of this work is to demonstrate the feasibility of using virtual experiment methods to identify and explain differences.

We started with a virtual Mouse Analog that uses a multi-level model mechanism of APAP hepatotoxicity in mice that achieved multiple qualitative and quantitative validation targets (<http://journals.plos.org/ploscompbiol/article?id=10.1371/journal.pcbi.1005253>). We simulated isolating all analog Hepatocytes (aHPCs) from an analog Mouse Liver, while keeping the intracellular parameterization of each aHPC unchanged, and configuring them into a Culture Analog that mimics commonly used 2D culture systems. The toxicity feature on which we focused is a measure of necrosis. Although the aHPC in the two systems are the same, Mouse and Culture Analogs have different dose-response relationships. That difference means that the mechanisms are different in particular ways. Spatial and temporal features of the Mouse Analog’s mechanism are absent in the Culture Analog. Spatial and temporal mechanisms shared by both Analogs *behave* the same. However, the collections of mechanisms end up behaving differently. These organizational differences provide a concrete explainable example of how and why IVIV translations of drug effects can be problematic; they provide the first plausible concrete quantitative explanation for the observed in vitro–in vivo APAP hepatotoxicity disconnect.