As mentioned in Workflow Part 1, we are interested in liver damage cause by APAP metabolism, specifically the observation of necrosis initially near the CV and spreading outward towards the PV over time. A mechanistic hypothesis for this AILI phenomenon is the differential production across the three liver lobule zones of the reactive APAP metabolite, NAPQI, which causes enough macromolecular damage to induce necrosis. Within hepatocytes, the amount of cytochrome P450 enzymes that metabolize APAP are higher in Zone 3 near the CV than in Zone 1 near the PV. This zonation is common from many hepatocyte functions. Wet-lab experiments directly on lobule zonation are impractical. Therefore, we utilized in silico liver lobule analogs, composed of software objects and agents, to perform virtual experiments to challenge these mechanistic hypothesizes. Longer term, we want to discover multi-scale explanatory mechanisms (i.e. causal cascades), which when measured, achieve multiple, multi-scale validation targets for AILI.

Results of some virtual experiments are described here and refer to a poster presented at the MSM meeting at the NIH in 2013 (see Workflow Part 2 Part B). The referent experiment (i.e. wet-lab) is a perfused mouse liver lobule, in which blood containing APAP flows from the PV to the CV crossing the three zones with hepatocytes having different capabilities to metabolize APAP (poster panel 1). The hypothesis for this work was that there is a critical level or tipping point of accumulated macromolecular damage product that when reached irreversibly triggers necrosis. In experimenting on our analogs, we seek biomimetic, coarse-grained mechanisms that achieve our validation target, which is that PV-to-CV damage differential enables a tipping point to be reached in Zone 3, but not in Zones 1 and 2, with the constraint that previously achieved multi-scale validation targets are not significantly changed (poster panel 2). The previous quantitative and qualitative validation targets include APAP hepatic clearance, single pass hepatic disposition profiles, measures of two inactive and one reactive (NAPQI) metabolite, responsible enzymes exhibit different PV-to-CV patterns, and NAPQI production increases PV-to-CV. In addition, we include in our analogs some biologically constraints, such as normal GSH levels decline PV-to-CV, APAP metabolism and NAPQI production increases PV-to-CV, NAPQI depletes GSH after which NAPQI creates disruptive damage products, and considerable heterogeneity at all levels, from within and between lobules to individual mice. Part of our methodology in performing virtual experiments is the implementation of the hypothesized mechanisms, or causal cascade of events, into the lobule analog, specifically in the hepatocyte analogs (i.e. “Hepatocytes”) where APAP metabolism occurs (poster panel 3). The simplest tipping point scenario requires accumulation of considerably more Damage Product (D) in Zone 3 “Hepatocytes”. We started with Lobule 0 (poster panel 4) and explored consequences of adding two mechanism features parsimoniously (i.e. no more complicated than needed): GSH depletion and repair of damage produced by NAPQI. After implementation, we sought parameterizations that would enable achieving the preceding scenario.

Lobule 0 (poster panel 4): From the initial analog containing APAP metabolism we added the mechanism that NAPQI or N produces Damage or D. The bottom four plots are the spatial (i.e. PV-to-CV) parameterizations for APAP metabolism, including probability that APAP is metabolized, the fraction of APAP metabolites that are A & B (map to sulphation and glucuronidation), and the fraction of APAP metabolites that are N, and the probability that N→D, which has no zonation. The amount of Damage in Zone 3 > Zone 2 > Zone 1, but not significantly to support the tipping point scenario.

Lobule 1 (poster panel 5): This Lobule contains the same parameterizations for APAP metabolism and Damage production as Lobule 0 but with the inclusion of the glutathione (GSH) depletion mechanism, in which an N depletes a pool of GSH objects or threshold. This number of GSH objects is constrained to decrease from PV to CV, and we parsimoniously chose a linear decline. If the number of GSH depletion events is < threshold, then an N object “reacts” with a GSH object with a specific probability

(90%) and gets eliminated. After the threshold, N produces D. With this GSH depletion mechanism and parameterization, the amount of Damage in Zone 3 > Zone 2 > Zone 1, but insufficient for the tipping point scenario.

Lobule 2 (poster panel 6): This Lobule has the same mechanisms as Lobule 1 but with a different GSH depletion parameterization. Again, the amount of Damage in Zone 3 > Zone 2 > Zone 1, but insufficient for the tipping point scenario.

Lobule 3 (poster panel 7): This Lobule has the same mechanism for APAP metabolism, Damage production, and GSH depletion as Lobule 2, but with the additional mechanism of the Repair of Damage. The Repair mechanism is that D “produces” R with a certain probability, which has a spatial parameterization. We chose the parameterization to decrease PV-to-CV because hepatocytes close to PV have greater resources (e.g. Oxygen) and thus presumably greater repair capabilities than hepatocytes close to CV, and we parsimoniously chose a linear decline. The additional Repair mechanism qualitatively achieve a validation target in that one Zone contained much more Damage than the other two; however, the amount of Damage in Zone 2 >> Zone 1 > Zone 3, which is not the tipping point scenario sought after.

Lobule 4 (poster panel 8): This Lobule has the same mechanisms as Lobule 3, but the spatial parameterization of Repair was a decreasing reverse sigmoid distribution and not linear. These parameterizations achieved the tipping point scenario in that the amount of Damage in Zone 3 >> Zone 2 > Zone 1.

Towards understanding APAP induced spatial and temporal necrosis patterns in liver lobules, we performed virtual experiments on in silico lobule analogs containing mechanistic hypotheses to explain this phenomenon. Both mechanisms for GSH depletion and Repair of Damage caused by NAPQI were necessary to generate the significantly greater amount of Damage in Zone 3 than in Zones 2 & 1 consistent with a tipping point scenario. In addition, the spatial parameterization or zonation of Repair needed to decrease PV-to-CV nonlinearly, which seems counterintuitive because damage thus necrosis most often occurs first near the CV; therefore, one might expect macromolecular Damage and Repair mechanisms to be enhanced near the CV. Future experiments will include mechanisms for Damage amplification, which is proposed to occur when Damage causes mitochondrial dysfunction and the accumulation of reactive oxygen species, and the actual death of hepatocytes.