Future Platforms for *in vitro*-based Toxicity Testing

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Toxicity testing typically involves studying adverse health outcomes in animals subjected to high doses of toxicants with subsequent extrapolation to expected human responses at lower doses. The system relies on the use of a 40+-year-old patchwork of animal tests that are expensive (costing more than $3B per year), time-consuming, low-throughput and often provide results of limited predictive value for human health effects. The low-throughput of current toxicity testing approaches (which are largely the same for industrial chemicals, pesticides and drugs) has led to a backlog of more than 80,000 chemicals to which humans are potentially exposed whose potential toxicity remains largely unknown**.** In 2007, the National Research Council (NRC) released the report "Toxicity Testing in the 21st Century: A Vision and a Strategy", that charted a long-range strategic plan for transforming toxicity testing. The major components of the plan include the use of predictive, high-throughput cell-based assays (of human origin) combined with high-content multi-omics measurements, computational systems biology models and pharmacokinetic tools to evaluate perturbations in key cell-signaling pathways and to conduct targeted testing against those pathways. By integrating all of these tools —termed "integrated systems toxicology" — it may be possible to map and annotate toxicity pathways, conduct systems analysis of pathway function, and link pathway perturbations to cell and tissue responses thereby enabling both dose-response modeling and *in vitro* to *in vivo* extrapolation. This approach will greatly accelerate our ability to test the vast "storehouses" of chemical compounds using a rational, risk-based approach to chemical prioritization, and provide test results that are far more predictive of human toxicity than current methods.

Toxicity pathways are simply normal cell signaling pathways that are susceptible to chemically-induced perturbations. Typical toxicity pathways include stress responses – such as DNA damage, oxidative stress, hypoxia, endoplasmic reticulum damage, metal stress, *etc.* – and receptor-mediated responses – such as that occurring through nuclear hormones, among others. Although a number of toxicity pathways have already been identified, most are only partially known and no common annotation exists. Mapping the entirety of these pathways (i.e. the Human Toxome) will be a large-scale effort, perhaps on the order of the Human Genome Project.

Agilent Technologies has partnered with key toxicology thought leaders to establish a research consortium comprised of life science tools providers, industrial companies, academics and not-for-profit organizations to conduct a project intended to demonstrate how a deep knowledge of cell signaling pathways could be directly used to conduct human health risk assessments. Using a case study approach focused on a few key prototype nuclear receptor and stress-response pathways, we are applying an integrated systems toxicology approach to map and model these pathways. The goals of this program are to: 1) develop *in vitro* assays for relevant compounds in appropriate cells/tissues; 2) use a suite of tools to create a dense data

stream on dose response; 3) apply bioinformatics tools to infer pathway circuitry while generating a computational systems biology model of the circuitry, and 4) create dose-response curves for the various assayed endpoints. In a related effort, a consortium of researchers led by Dr. Thomas Hartung at The Johns Hopkins University, have begun to map estrogenic pathways in human breast cancer cells using a combination of transcriptomics and metabolomics (http://altweb.jhsph.edu/news/current/caatnihgrant.html).

In this talk, we describe these research consortia in more detail. We also describe the suite of technology platforms used in these studies and show how these tools are being and have been applied in our studies. We can now identify specific technologies and experiments that will accelerate completion of the first-phase of pathway mapping and modeling. We also discuss how these integrated data packages are shaping, informing and modifying our conventional views of toxicity pathways.