

Expansion of the EPA-Hamner MOU:

In October of 2007, the National Center for Computational Toxicology (NCCT) in EPA's Office of Research and Development entered into a 5 year Memorandum of Understanding (MOU) with The Hamner Institutes for Health Sciences to advance the development of the ToxCast research program. As noted in the MOU, the research programs and missions of the NCCT and The Hamner are very complementary, providing an excellent mix of expertise and interests for collaborating in use of high throughput screening assays for predictions of hazards of chemicals and prioritization for additional toxicological evaluation. The original areas of cooperation focused on two research projects. The first involved the assessment by the Hamner of the ToxCast library of chemicals in an *in vitro* system utilizing primary lung, liver and kidney cells that could be compared to results from the ToxRefDB that house traditional toxicological data for the ToxCast library. The second element involved research by the Hamner to extrapolate the concentrations of ToxCast chemicals that cause responses in the *in vitro* assays to comparable doses levels for the whole animal (a topic sometimes referred to as reverse dosimetry).

Given the successful interactions to date, efforts are now underway to expand collaboration between the US EPA ToxCast program and The Hamner Institutes for Health Sciences. Specifically, Hamner will conduct research on key issues associated with developing and applying results from rapid, high throughput *in vitro* cell assays for toxicity testing, chemical prioritization and risk assessment. The two specific projects are (1) identifying key toxicity pathways along with assessment of susceptibility factors for these pathways and (2) guiding strategies for using results from *in vitro* assays to support dose response assessments. The design phase for this research included discussions with between key Hamner and NCCT staff.

In the first project, a high data content imaging screen will examine eleven (11) integrated, *in vitro* cell response markers using a sub-set of the ToxCast Phase I chemicals. The testing will be conducted with a panel of mouse embryonic fibroblast (MEF) cells derived from 35 distinct genetic strains of mice. The use of cells from the panel of pure-bred mice allows mapping of the genetic characteristics that determine susceptibility to specific responses. This approach will identify a set of medium-throughput, integrated cell responses that could serve as the basis for defining key 'toxicity pathways'. Assays for these toxicity pathways need to be included in future toxicity testing strategies. Eventually, according to the NAS report, 'Toxicity Testing in the 21st Century', product testing would focus on results from a broad range of these *in vitro* toxicity pathway tests.

The second project will develop data to support a computational systems biology description of activation and cellular responses for human toxicity pathways. In this specific research, Hamner scientists focus on PPAR- α receptor activation. This type of information, eventually required for each toxicity pathway assay in the test suite, will guide dose response and risk assessments from *in vitro* test results. Laboratory methods for this multi-endpoint project include: protein-protein interactions, kinase/phosphatase contributions, gene expression analysis, transcription factor profiling, and extraction of functional circuitry motifs controlling

the PPAR- α mediated cellular responses. The more generic goal of the project is to provide the tools for pathway mapping and computational systems biology modeling and to identify the types of data that will be needed for dose response evaluations for human toxicity pathways. The two projects will be pursued over the next 2 years.