ToxCast approaches to high throughput risk assessments: Pathways-based TK/TD

Richard JUDSON
(*US EPA*, *National Center for Computational Toxicology*, *RTP NC 27711*)

Abstract: A significant challenge in toxicology is the "too many chemicals" problem. Humans and environmental species are exposed to as many as tens of thousands of chemicals, few of which have been thoroughly tested using standard *in vivo* test methods. This talk will discuss several approaches to dealing with this problem being developed by the U.S. EPA, under the umbrella of the ToxCast program (http://epa.gov/ncct/toxcast/). The overall problem is broken into several tasks: (1) identifying biological pathways, that when perturbed can lead to toxicity; (2) developing high-throughput *in vitro* assays to test chemical perturbations of these pathways; (3) identifying the universe of chemicals with likely human or ecological exposure; (4) testing as many of these chemicals as possible in the relevant *in vitro* assays; (5) developing hazard models that take the results of these tests and identify chemicals as being potential toxicants; (6) generating pharmacokinetic data on these chemicals to predict the doses at which these hazard pathways would be activated; and (7) developing exposure models to identify chemicals for which these hazardous dose levels could be achieved. This overall strategy will be described and briefly illustrated with examples from the ToxCast program. Further details of these steps are as follows: 1. Candidate pathways of toxicity, also referred to as Modes of Action (MOA) or Adverse Outcome Pathways (AOPs), were derived from surveys of the literature and discussions with experts. Many of the pathways tested involve pharmaceutical targets, which could lead to adverse effects if improperly activated (off-target toxicity) (Ankley *et al* 2010; Boobis *et al* 2008; Meek *et al* 2003). 2. *In vitro* assays were obtained from commercial testing laboratories, from in-house labs at the EPA, from collaborators at the U.S. NIH Chemical Genomics Center (NCGC) and from academic partners. In total, there are over 700 assays being used as part of the ToxCast program. These cover a large range of technologies, including cell-free biochemical assays; assays targeting nuclear and other receptors and other molecular targets; assays measuring downstream integrated cell processes; and model organisms (especially zebrafish) (Chandler *et al* 2011; Dix *et al* 2007; Houck *et al* 2009; Judson *et al* 2010; Knight *et al* 2009; Knudsen *et al* 2011; Rotroff *et al* 2013; Sipes *et al* 2013). 3. Chemicals for testing were nominated by U.S. agencies: EPA, NIH, FDA; various stakeholder groups (industry, academia and non-governmental organizations); international governmental agencies; and working groups of the OECD. These chemicals include pesticides, pharmaceuticals, food additives and food-contact substances, cosmetics ingredients, personal care ingredients and industrial chemicals (Judson *et al* 2012). 4. A total of 1800 chemicals are in the ToxCast library. These, plus an additional 6400 chemicals are also being tested by the NCGC in a selected subset of assays. This complete data set is being released publicly by the EPA in Fall 2013. The data consists of concentration-response profiles for each chemical-assay pair, as well as a "hit-call", or determination of whether or not the chemical was active in the assay (http://epa.gov/ncct/toxcast/chemicals.html). 5. The *in vitro* data from ToxCast is being combined with *in vivo* toxicity data from guideline studies in the EPA Toxicity Reference Database (ToxRefDB, http://epa.gov/ncct/toxrefdb/). Using these two data sets, we are developing models that predict *in vivo* effects from *in vitro* assay measurements. Several preliminary models have been published, including ones for reproductive and developmental endpoints and cancer (Kleinstreuer *et al* 2013; Kleinstreuer *et al* 2011; Martin *et al* 2011; Reif *et al* 2010; Sipes *et al* 2011). These models use a combination of statistical and biologically-based modeling approaches. Currently, these models are being tested and refined using the newest ToxCast data. 6. In order to quantitatively predict *in vivo* toxicity, it is necessary to have an appropriate pharmacokinetic model. Here, we are

using a method called Reverse Toxicokinetics (RTK) to make first order predictions of the scaling from ingested dose to blood concentration of the chemical. This approach requires that two experimental *in vitro* measurements be carried out: clearance of the parent chemical in primary hepatocytes, and the fraction unbound in the presence of plasma protein. These measurements have been carried out using both human and rat hepatocytes and plasma. The end result of the RTK process is a prediction of the oral dose at which each biological pathway will be activated (Rotroff *et al* 2010; Thomas *et al* 2013; Wetmore *et al* 2013; Wetmore *et al* 2012). 7. These biological pathway activating dose values (BPAD (Judson *et al* 2011)) can then be compared with estimated exposure levels. If individuals are exposed to levels in excess of the BPAD, then one could prioritize that chemical for further toxicity testing. On the other hand, if there is a wide safety margin (exposure is much less than the BPAD), then the chemical is of less concern. We are developing high-throughput exposure models for this type of application, under the EPA ExpoCast program. An important aspect of these models accurate estimation of uncertainty (Wambaugh *et al* 2013).

E-mail: Judson. richard@epa. gov

Future platforms for *in vitro*-based toxicity testing

Mary MCBRIDE
(*Agilent Technologies*, *Inc*)

Abstract: 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 (ie 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 organ-