

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

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30 minutes

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).

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