Using ToxCast™ *In Vitro* Screening Data for Chemical Prioritization

Incorporating Human Exposure and Dosimetry

April 30, 2010

Impact Statement

Tens of thousands of chemicals are currently in commerce, and hundreds more are introduced every year. Only a small fraction of chemicals have been adequately assessed for potential risk. To reduce the number of untested chemicals and prioritize limited testing resources, EPA and other government agencies are exploring the use of technologies that can examine the effects of thousands of chemicals against many key biological processes. These technologies use non animal (*in vitro*) assays to help understand what might happen when a human is exposed to a chemical. Interpreting the relevance of *in vitro* data to predict toxicity remains a challenge for a number of reasons. This study looks into the key question about what amount of human exposure is required to cause an effect measured in the *in vitro* tests. To provide insights into this question, this study experimentally measured human hepatocyte clearance rates and plasma protein binding for 35 of the 309 ToxCast Phase 1 chemicals. ToxCast is a multi-year, multi-million dollar effort that uses innovative technologies to efficiently ($20K per chemical) study the key biological processes impacted by chemicals which then lead to adverse health effects. ToxCast includes over 400 *in vitro* high-throughput screening assays. This study indicates that understanding relevant exposure conditions is important when using high-throughput *in vitro* data to identify the highest priority chemicals for further testing and risk management.

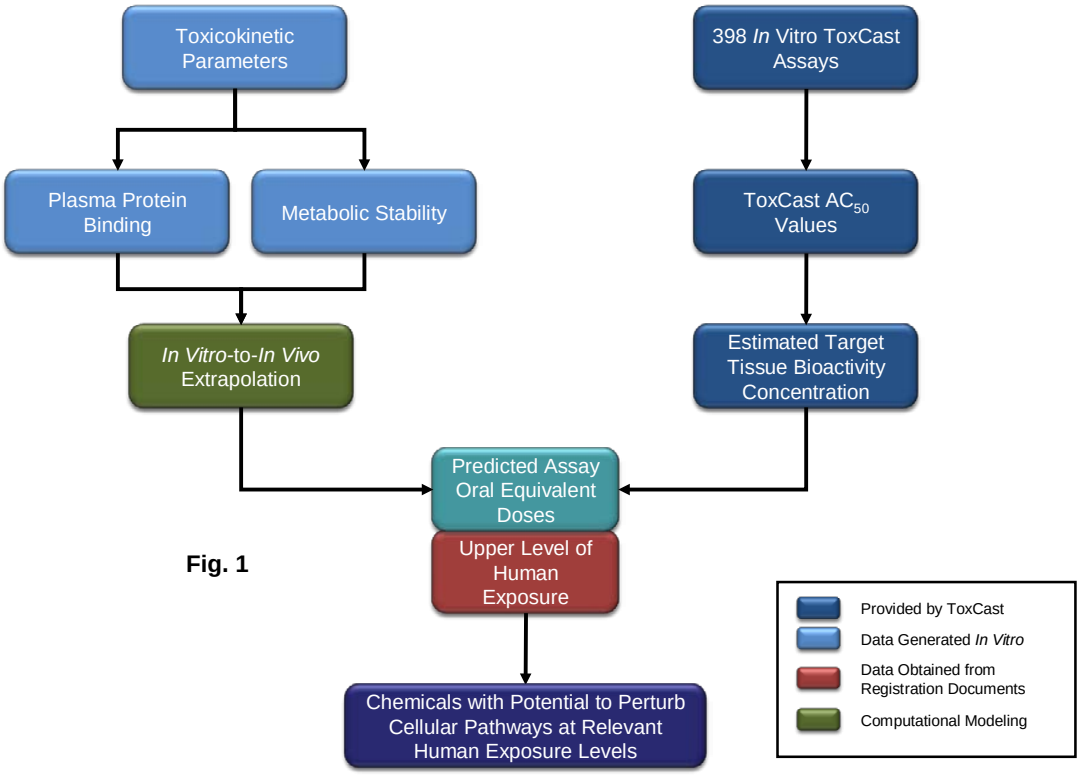

Fig. 1

Study Description

This study used a combination of the ToxCast data, new human *in vitro* data, and computer modeling to identify chemicals with the potential to perturb cellular pathways at relevant human exposure levels. Below is a summary of how the study combined and assessed the data (See *Figure 1*):

- The selected chemicals had bioactivity in many, sometimes hundreds, of ToxCast assays. The amount of a chemical that caused a 50% change in an assay was chosen as an estimate of the blood concentration needed for bioactivity.
- In order to relate chemical exposure to blood concentration, two measurements were made for each chemical – the ability of human plasma to bind the chemical and the ability of human hepatocytes to metabolize the chemical. Computational modeling (with Simcyp© simulation software) was used to predict the amount of chemical that would have to be ingested in order to have blood levels that were similar to those that caused effects in the ToxCast assays.
- Human oral equivalents of ToxCast *in vitro* results were compared to official EPA estimates of chronic

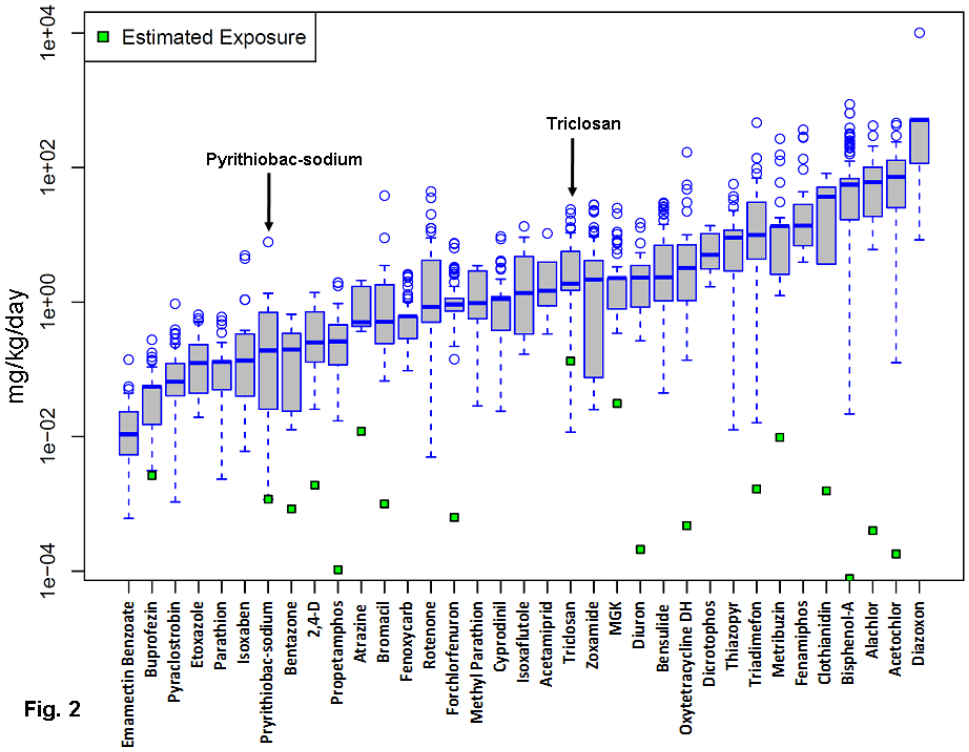

Fig. 2

human oral exposure. To be protective, the sub-population with the largest estimated exposure was used. EPA estimates of human exposure were available for 24 of the 35 chemicals.

Conclusions

Only two chemicals (See Figure 2), Pyrithiobac-sodium and Triclosan, had estimated human oral exposures that overlapped with some oral equivalent doses. These two chemicals would not have been identified as higher priority for evaluation using ToxCast results alone, since those data do not assess the amount of chemical expected in the body due to environmental exposure. Triclosan is an antibacterial antifungal agent that is in many household items (soap, toothpaste, etc) with a high potential for human exposure. Studies have identified a potential role of Triclosan in thyroid and liver toxicity. Pyrithiobac-sodium is an herbicide with little research beyond the standard toxicological testing done for product registration.

Other conclusions include:

The pharmacokinetics approaches in this study have the potential to move beyond a hazard identification paradigm toward the use of *in vitro* data in a more realistic environmental context.

The integration of human dosimetry and exposure information with results from high-throughput screening efforts enhances our ability to make informed decisions on chemical testing priorities.

The use of only *in vitro* ToxCast values for prioritization could underestimate the hazard associated with other chemicals because of inadequate consideration to internal exposure potential and dosimetry estimates.

Using highly sensitive in vitro assays for predicting relevant responses in humans is debatable. Activation of these *in vitro* endpoints does not necessarily represent an adverse biological response, but should be regarded as a measure of potential biological changes caused by a chemical.

Further ToxCast analysis is underway to develop predictive signatures consisting of different *in vitro* assays to predict *in vivo* responses. This analysis is needed to settle the ongoing toxicological debate about the difference between adverse and adaptive responses.

Background

Most chemicals in commerce have only undergone the minimum safety testing that is required by law. Toxicity testing is time-consuming, complex, and expensive. In an effort to improve existing chemical screening, US EPA developed ToxCast to develop a new method to efficiently screen chemicals and prioritize limited testing resources toward those that have the potential to cause greatest hazard to human health. Legislation to overhaul the existing Toxic Substance Act is currently under discussion in the US Congress.

This research was performed by the US EPA National Center for Computational Toxicology in collaboration with the Hamner Institutes for Health Sciences and CellzDirect.

REFERENCE: ROTROFF ET AL (2010) "Incorporating Human Dosimetry and Exposure into High Throughput *In Vitro* Toxicity." Toxicological Sciences.

WWW.EPA.GOV/NCCT/TOXCAST

CONTACT: Monica Linnenbrink, NCCT, Office of Res. + Dev., 919-541-1522 or linnenbrink.monica@epa.gov