Combining ToxCast™, Dosimetry and Human Exposure Research to Increase the Relevance of Rapid Chemical Toxicity Testing Results

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Impact Statement

Tens of thousands of chemicals are currently in commerce, and hundreds more are introduced every year. Because there are so many chemicals—and since traditional chemical toxicity tests using animals are expensive and time consuming—only a small fraction of chemicals have been fully assessed for potential risk.

In 2007, EPA scientists began working on ToxCast, a research project that identifies and prioritizes potentially toxic chemicals using rapid, automated tests called high-throughput screening (HTS) assays. ToxCast is currently assessing over 2,000 chemicals from a broad range of sources, including pesticides, industrial and consumer products, food additives, and failed drugs that were never released to the market.

The technologies included in ToxCast use non-animal tests called *in vitro* assays to help understand what might happen when a human is exposed to a chemical. However, it is difficult to determine the relevance of *in vitro* data when predicting toxicity from real-
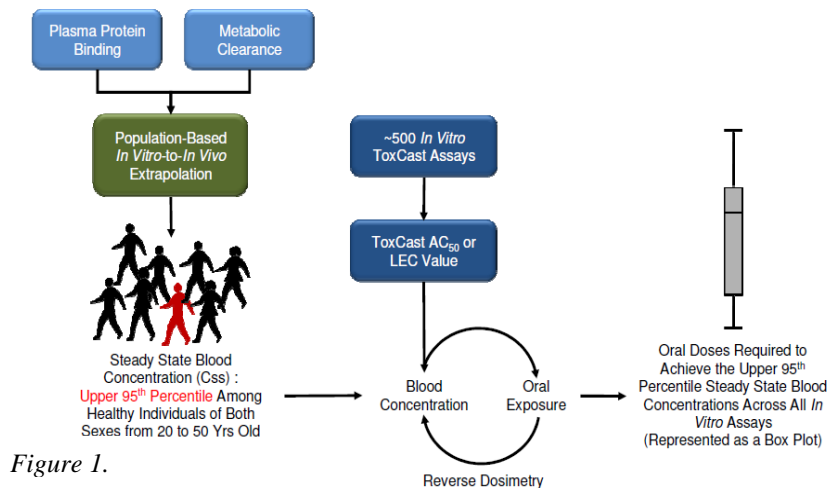

Figure 1.

world exposures. This study focuses on discovering what level of human exposure is required to result in the internal concentrations that caused effects in *in vitro* tests.

To provide insights into this question, this study made experimental measurements and calculated relevant human exposures for 239 of the 309 ToxCast Phase I chemicals. This study indicates that understanding relevant exposure conditions is important when using HTS *in vitro* data to prioritize chemicals for further testing and risk management.

Study Description

This study used a combination of ToxCast data, public exposure data, and new

dosimetry data to identify chemicals with the potential to disturb cellular pathways at relevant human exposure levels. Below is a summary of how the study combined and assessed the data (See *Figure 1*):

- 239 chemicals taken from ToxCast Phase I were tested for possible toxicity using over 500 HTS assays. The amount of a chemical that caused a 50% change in an assay (AC_{50}) 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 (plasma protein binding) and the ability of human liver cells to metabolize the chemical (hepatic clearance). These two parameters were used

bioactivity at environmentally-relevant concentrations. If these chemicals had been examined using only HTS methods, they may have been overlooked.

The pharmacokinetics

Figure 2.

to convert *in vitro* concentrations into estimated real world exposures. Chemicals can then be prioritized from most to least potent in terms of relevant human exposures (*See Figure 2, which displays the least potent 25% of chemicals tested*). Finally, publically-available exposure estimates were compared with the estimated exposures necessary for *in vitro* activity.

Conclusions
After examining data from the HTS assays, 18 chemicals were flagged as potentially causing

approaches in this study have the potential to move beyond simply identifying hazards, toward the use of *in vitro* data for realistic analysis of environmental risks.

However, using molecular and cellular *in vitro* assays for predicting toxicity and adverse outcomes in human biological systems continues to be a challenge requiring further research. 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.

ToxCast analysis is underway to develop predictive signatures consisting of different *in vitro* assays to predict adverse effects in animal models and humans. This analysis will help clarify the difference between adverse and adaptive responses.
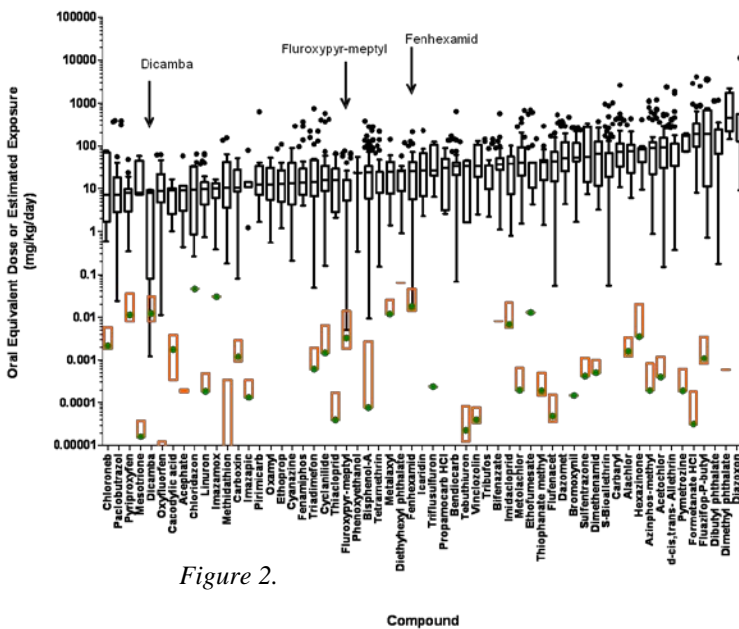
Background
Most chemicals in commerce have only undergone limited safety testing, and conventional toxicity testing is time-consuming, complex, and expensive. In an effort to improve existing chemical screening, U.S. EPA developed ToxCast as a novel way to efficiently screen chemicals and prioritize limited testing resources toward those that have the potential to cause greatest hazard to human health.

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Reference
Wetmore, *et al.* "Integration of Dosimetry, Exposure and High-Throughput Screening Data in Chemical Toxicity Assessment" *Toxicological Sciences* (2011). Accepted.

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