

Science Question

Can efficient screening-level assays be developed for adverse outcomes that are not (well) evaluated by current methods? These areas include:

- Neurotoxicity/Developmental neurotoxicity
- Cell stress pathways
- Immunotoxicity
- *Developmental toxicity* (Hunter et al. poster)
- *Reproductive Toxicity* (Hunter et al. poster)

Research Goals

- Develop assays for predicting chemical effects on developmental neurotoxicity, stress pathways and immunotoxicity.
- Adapt those assays to high-throughput/high-content platforms amenable to screening large numbers of compounds.
- As HTP/HCS assays are developed, apply them to the ToxCast₃₂₀ chemicals as part of assay evaluation.

Approach

Developmental Neurotoxicity (DNT)

- Toxicity pathways were identified based on the critical processes (e.g. proliferation, migration, differentiation, neurite growth, synaptogenesis, myelination, apoptosis) underlying brain development
- Cell-based assays were developed using High-Content Screening (HCS) technology
- Assay performance evaluated using *training set* of chemicals (positive and negative controls) and *test set* of chemicals (known developmental neurotoxicants)

Stress Assays

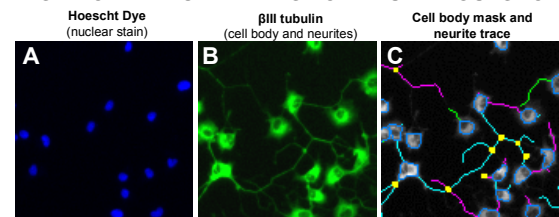
- Adaptive stress response pathways are a set of highly-conserved signaling pathways activated in response to environmental insults including toxicant exposure
- Battery of cell-based reporter gene assays that measures the transcriptional activation of the adaptive stress response pathway network (validated using pathway-specific controls)
- Stress assay battery can run in high-throughput mode to measure mechanistically-specific perturbations caused by toxicant exposure

Immunotoxicity

- Assessment of cytokine release from in vitro immune models

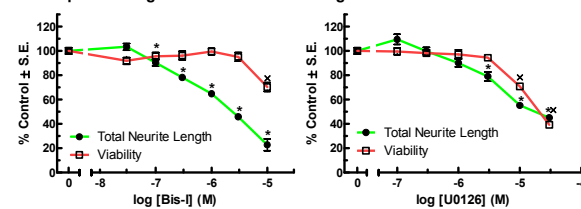
DNT Assays

HIGH-CONTENT SCREENING FOR NEURITE OUTGROWTH

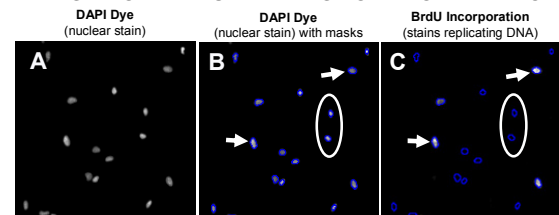


- Nuclei are labeled blue with Hoescht dye and used to identify valid cells (Channel 1).
- Cell bodies and neurites are labeled green with ICC for BIII tubulin (Channel 2).
- The image analysis algorithm identifies the cell body and measures a number of parameters (e.g. cell body size, neurite number, neurite length, branch points).

Example Training Set Data for Neurite Outgrowth

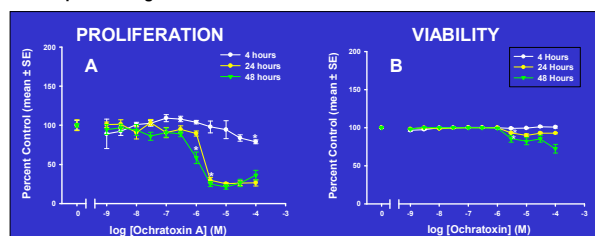


HIGH-CONTENT SCREENING FOR PROLIFERATION



- Gray-scale image of nuclei stained with DAPI dye (Channel 1).
- Objects were determined by computer algorithm and outlined with a blue mask (Channel 1).
- Nuclei positive for BrdU (arrows) were determined in Channel 2 based on objects detected in channel 1. The oval indicates objects that were negative for BrdU.

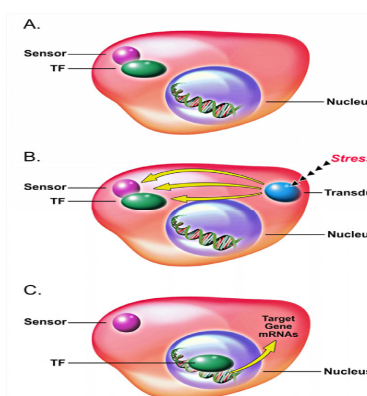
Example Training Set Data for Proliferation



Methods/Results

Cellular Stress Assays

PATHWAY NODES MEASURE CELLULAR PERTURBATIONS

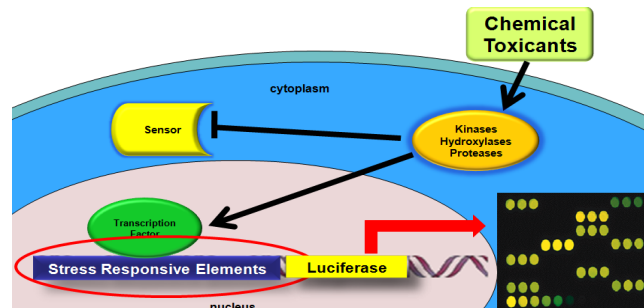


Under normal cellular conditions, the pathway transcription factor (TF) is negatively regulated and sequestered in the cytoplasmic compartment by the pathway sensor

Stress stimuli such as heat, UV radiation or chemical toxicants activate enzymatic transducers which transfer biochemical signals to the TF-sensor complex

Activated transducers destabilize the sensor and/or stabilize the TF which subsequently translocates to the nucleus where it triggers the expression of stress-relieving gene products

Reporter genes constructed to exploit the common architecture of key cellular stress response pathways provide a convenient tool to measure multiple parallel stress pathway activations in a rapid and cost-effective format



Immunotoxicity Assays

- Mode of action-based screening and prioritization
- Detect chemical-induced modulation of cytokine release (Indicators of immunosuppression or allergic disease)



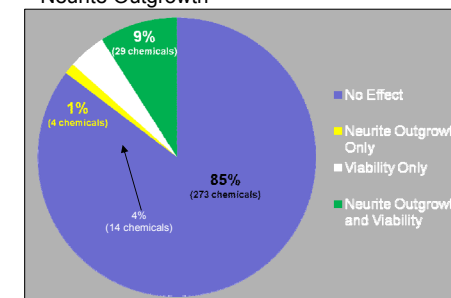
Antigen presentation induces cytokine (IL-2) release; reduced IL-2 associated with immunosuppression and reduced resistance to infection

Cytokine (IL-8) release by macrophages is stimulated by chemicals that cause allergic contact hypersensitivity and suppressed by pesticides that suppress immune function. Other cytokines (e.g., TNF α) release is stimulated by chemicals that cause inflammatory diseases

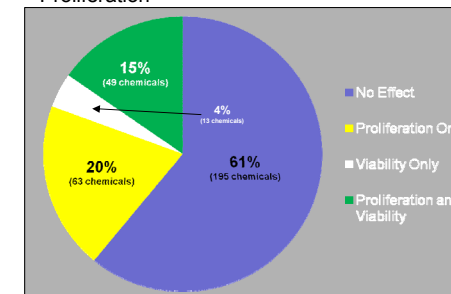
Application to ToxCast

DNT: The ToxCast₃₂₀ chemicals were assayed at a single concentration of 40 μ M. The pie charts illustrate the proportions of the ToxCast₃₂₀ that were considered "hits" (>3x s.d.) in the assays.

Neurite Outgrowth

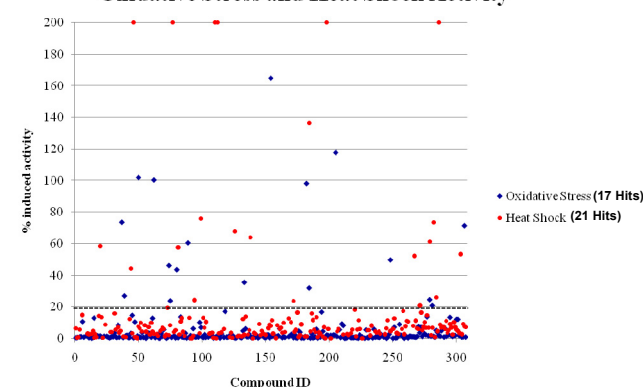


Proliferation



Stress: The ToxCast₃₂₀ chemicals were assayed for oxidative stress and heat shock responses. HepG2 cells stably expressing reporters for oxidative stress- and heat shock-responsive reporter genes were treated with the ToxCast₃₂₀ at 15 concentrations. Graph shows the relative peak biological activities. Compounds that elicited a response greater than (>3x s.d.; dashed line) of vehicle-treated cells were considered "hits" in the assays.

Oxidative Stress and Heat Shock Activity



Conclusions

- High-throughput assays can be implemented at the EPA to screen large numbers of chemicals for toxicity endpoints
- There was a high degree of consistency of results from both within plate and between plate repeated chemicals for all endpoints
- The Stress assay battery quantitatively measured adaptive transcriptional changes in specific cellular stress networks in response to chemical insults

Impact and Outcomes

- Results demonstrate that an approach focusing on processes critical for cell development and function will provide successful screening assays.
- Cell based assays for DNT and cell stress were applied to the ToxCast₃₂₀.
- This data provides a more complete characterization of potential chemical hazards utilizing in vitro approaches.

Future Directions

- Concentration-response characterization of hits for all endpoints is completed for DNT and cell stress assays
- Further analysis of potency and efficacy of ToxCast₃₂₀ is ongoing
- Endpoints for high-throughput screening of immune function are currently under development and will be evaluated using the ToxCast₃₂₀ chemicals

References

- Breier, J.M., Radio, N.M., Mundy, W.R. and Shafer, T.J.: Development of a high-throughput screening assay for chemical effects on proliferation and viability of immortalized human neural progenitor cells. *Toxicol. Sci.* 105:119-133, 2008
- Radio, N.M., Breier, J.M., Shafer, T.J. and Mundy, W.R.: Assessment of chemical effects on neurite outgrowth in PC12 cells using high content screening. *Toxicol. Sci.* 105:106-118, 2008
- Simmons, S.O., Fan, C.Y., Ramabhadran R.: Cellular Stress Response Pathway System as a Sentinel Ensemble in Toxicological Screening. *Toxicol Sci.*, in press, 2009.
- Oostingh et al.: A high-throughput screening method based on stably transformed human cells was used to determine the immunotoxic effects of fluoranthene and other PAHs. *Tox. in Vitro* 22:301-310, 2008