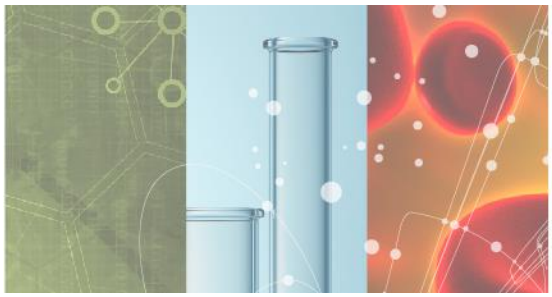

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American Chemistry Council

ACC Science and Research Highlights

Cellular Networks and Threshold Responses: addressing the challenge of understanding the shape of dose-response curves at low exposure levels

One of the core activities of the American Chemistry Council's Long-Range Research Initiative (LRI) Research Strategy (2015-2019) is aimed at actualizing the vision of the 2007 NRC report *Toxicity Testing in the 21st Century (TT21C)*. At the Hamner Institutes of Health Sciences (Hamner), supported in part by the ACC LRI, significant progress has been made toward utilizing advanced approaches such as *in vitro* systems, high-throughput screening (HTS), computational modeling, and exposure forecasting for risk-based priority setting and safety assessment screening. As the vision laid out in TT21C grows closer to realization, the need for effective methods of interpreting results from the advanced techniques of HTS and computational profiling becomes more pressing and continues to be a priority for the LRI program.

Just as in traditional toxicity testing and risk assessment, **applying knowledge of dose-response will be key for setting safe exposure levels** from HTS and computational profiling results:

- In traditional toxicity testing using lab animal models, substances are administered at several doses, responses are measured, and, from the dose-response relationship, a point of departure is derived. Typically the point of departure is either a No Adverse Effect Level or a Benchmark Dose. To set safe exposure levels for non-cancer toxicities from lab animal studies, a threshold extrapolation approach is then used, based on the principle that exposure below the threshold dose will not produce sufficient concentrations in the body (at the toxicity target site) to produce any adverse effects. However, thresholds cannot be proven or disproven from typical animal toxicity studies – due to biological and experimental variability, these studies lack the statistical power to distinguish thresholds from shallow linear dose responses.

- The TT21C report does not deal with thresholds directly. Instead it describes a safety evaluation process based on understanding biologically significant perturbations in key toxicity pathways. But this has begged the question, what is "perturbation"? And how do we go about determining when toxicity pathways are "perturbed" sufficiently to lead to adverse health effects? Is every possible change that can be measured in HTS assays a "sufficient perturbation"?

The recent scientific research article, "Molecular Signaling Network Motifs Provide a Mechanistic Basis for Cellular Threshold Responses," addresses the challenge of understanding the shape of dose-response curves at low exposure levels and determining thresholds in toxicity pathways. Led by Hamner researchers, this collaboration, which included scientists from the U.S. Environmental Protection Agency and Michigan State University, used computational methods to evaluate the complex biological pathways that underpin cellular functions and which regulate cellular-level responses to chemical stressors. The authors show that **the intricate connections in these pathways,**

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similar to the wiring found in integrated circuits, are constructed from ubiquitous biochemical building blocks referred to as "network motifs."

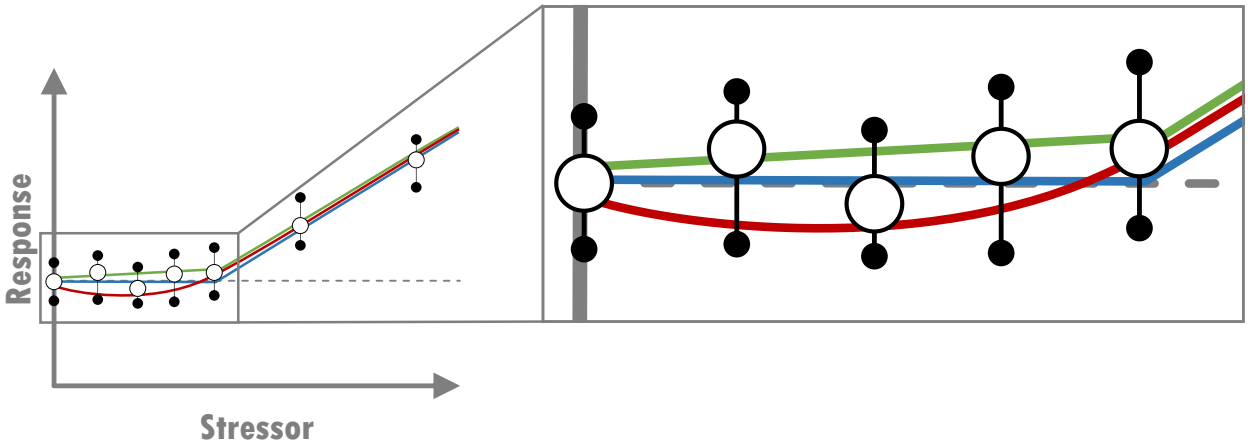
The cellular networks involved in homeostasis – the pathways that allow cells, organs and the body "to resist or adapt to moderate levels of external perturbations and maintain a relatively stable internal environment" – were shown to have **"clear-cut, mechanistically definable thresholds."** This circuitry means that at doses below the threshold, even though there may be exposure, "responses are identical to those in the nonstressed controls." These researchers also describe dose-response curves from other networks, some of which have well-defined thresholds, while others exhibit different behaviors (e.g., a discontinuous change in response at a particular degree of stress, or subthreshold shallow dose response).

This research brings us a step closer to operationalizing the concept of biologically significant perturbations in determining safe exposure levels. For example, it should now be **feasible to attach the circuitry of these network motifs to the connections between key events in Adverse Outcome Pathways (AOPs)**. This will enable AOPs to more realistically evaluate dose-response behaviors, including thresholds.

Changing the paradigm from lab animal testing to new approaches, using HTS, computational profiling, and exposure forecasting, creates both challenges and opportunities. The ACC LRI focus on supporting research and collaborations, such as the research summarized above, is helping to advance the development and application of innovative, scientifically sound approaches for risk-based priority setting, screening safety evaluations, and eventually risk assessments.

Click here to learn more about the research programs at the Hamner Institutes for Health Sciences.

Figure 1. Dose-Response Curves at Low Doses. This figure was adapted from Figure 1 presented in *Molecular Signaling Network Motifs Provide a Mechanistic Basis for Cellular Threshold Responses* (Zhang et al., 2014).



At low doses, a variety of dose-response curves could be used to fit the same data points.

Non-zero slope dose–response curve: small non-zero slope in the low-dose region and significant increase at higher doses.
Threshold dose–response curve: flat slope in the low-dose region and significant increase when stressor exceeds threshold.
Hormetic dose–response curve: decrease and then increase in the low-dose region and significant increase at higher doses.

ACC's Regulatory and Technical Affairs Department is continuing to support applied research projects to improve methods for interpreting human biomonitoring studies. For more information on these activities please contact Sarah Brozena (sarah_brozena@americanchemistry.com) or Rick Becker (rick_becker@americanchemistry.com).