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Behavioral toxicology in the 21st century: Challenges and opportunities for behavioral scientists
Summary of a symposium presented at the annual meeting of the Neurobehavioral Teratology Society, June, 2009

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ABSTRACT

The National Research Council (NRC) of the National Academies of Science recently published a report of its vision of toxicity testing in the 21st century. The report proposes that the current toxicity testing paradigm that depends upon whole-animal tests be replaced with a strategy based upon *in vitro* tests, *in silico* models and evaluations of toxicity at the human population level. These goals are intended to set in motion changes that will transform risk assessment into a process in which adverse effects on public health are predicted by quantitative structure–activity relationship (QSAR) models and data from suites of high-throughput *in vitro* tests. The potential roles for whole-animal testing in this futuristic vision are both various and undefined. A symposium was convened at the annual meeting of the Neurobehavioral Teratology Society in Rio Grande, Puerto Rico in June, 2009 to discuss the potential challenges and opportunities for behavioral scientists in developing and/or altering this strategy toward the ultimate goal of protecting public health from hazardous chemicals. R. Kavlock described the NRC vision, introduced the concept of the 'toxicity pathway' (a central guiding principle of the NRC vision), and described the current status of an initial implementation this approach with the EPA's ToxCast® program. K. Crofton described a pathway based upon disruption of thyroid hormone metabolism during development, including agents, targets, and outcomes linked by this mode of action. P. Bushnell proposed a pathway linking the neural targets and cellular to behavioral effects of acute exposure to organic solvents, whose predictive power is limited by our incomplete understanding of the complex CNS circuitry that mediates the behavioral responses to solvents. B. Weiss cautioned the audience regarding a pathway approach to toxicity testing, using the example of the developmental toxicity of phthalates, whose effects on mammalian sexual differentiation would be difficult to identify based on screening tests *in vitro*. Finally, D. Rice raised concerns regarding the use of data derived from toxicity screening tests to human health risk assessments. Discussion centered around opportunities and challenges for behavioral toxicologists regarding this impending paradigm shift. Opportunities include: identifying and characterizing toxicity pathways; informing the conditions and limits of extrapolation; addressing issues of susceptibility and variability; providing reality-checks on selected positives and negatives from screens; and performing targeted testing and dose-response assessments of chemicals flagged during screening. Challenges include: predicting behavior using models of complex neurobiological pathways; standardizing study designs and dependent variables to facilitate creation of databases; and managing the cost and efficiency of behavioral assessments. Thus, while progress is being made in approaching the vision of 21st century toxicology, we remain a long way from replacing whole-animal tests; indeed, some animal testing will be essential for the foreseeable future at least. Initial advances will likely provide better prioritization tools so that animal resources are used more efficiently and effectively.

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1. Introduction

Growing awareness of the disparity between the rate of deployment of new anthropogenic chemicals and assessment of their potential risks to public health prompted the EPA and NIEHS to

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request advice from the National Research Council on how to address this issue. In response, the NRC convened a Committee on Toxicity Testing and Assessment of Environmental Agents, which reported in January 2007 a "vision and strategy" for "toxicity testing in the 21st century" [67] (http://www.nap.edu/openbook.php?record_id=11970&w=1). In this new paradigm, the current practice of extensive animal-based characterization of chemical hazard, dose–response relationships, and extrapolation to human health is replaced by high-throughput *in vitro* tests, *in silico* models and evaluations of efficacy at the human population level.

The new paradigm raises substantial questions regarding the role of toxicologists concerned about the effects of chemicals on the behavior of intact animals. These questions are particularly acute given that one specified goal of the vision is to eliminate the use of whole animals in assessing chemical risk. Certain questions naturally arise about this vision, including:

- What are the implications of this proposed paradigm shift for behavioral toxicology?
- What roles can behavioral toxicology play in this new paradigm?
- What challenges do behavioral toxicologists face in applying their skills and expertise to this paradigm?
- What opportunities are presented by this paradigm shift?

A key component of this vision involves the concept of the "toxicity pathway", which may be thought of as a biologic process perturbed beyond homeostasis by exposure to a chemical. That is, normal "signaling motifs, genetic circuits, and cellular-response networks" that make up the fundamental biochemical processes of life are presumed to function within a homeostatic range of operating characteristics. In this view, exposure to toxic chemicals perturbs these pathways and, with sufficient perturbation, produces toxicity in the whole organism. Theoretically, then, toxicity can be detected by changes in such pathways using *in vitro* tests and computational models, thus obviating the need for exposing test animals to substances of unknown biological activity.

This symposium was therefore convened to present these concepts to the community of behavioral toxicologists and teratologists at the annual meeting of the Neurobehavioral Teratology Society, and to encourage discussion of their implications for behavioral toxicology in the twenty-first century. Because of the central role of the toxicity pathway in the testing strategy of the NRC vision, it became the focus of the presentations in this symposium. The concept was introduced by Robert Kavlock, in the context of current efforts in the EPA to identify pathways useful for implementing the NRC vision. Kevin Crofton then described a developmental toxicity pathway based on disruption of thyroid hormone status, and Philip Bushnell followed with a description of potential pathways based on disruption of ion channels in the CNS. Bernard Weiss then raised cautionary flags with a description of the developmental toxicity of phthalate esters, and Deborah Rice discussed concerns about the NRC vision within the current regulatory framework. Finally, after discussion of the pros and cons of the vision, some possible constructive roles for behavioral toxicologists were suggested. The following sections of this report summarize the presentations of each of the speakers in the symposium.

2. *Robert Kavlock*: the NRC framework and EPA's ToxCast program

The NRC vision proposes an approach to toxicity testing based upon the idea that exposure to a toxic chemical activates one or more toxicity pathways in the organism (Fig. 1). A toxicity pathway may be considered to arise from perturbation of a sequence of inherent biological processes that generate normal biological functions. In this concept, exposure to a toxicant causes early cellular changes that induce a deviation from normal output of the pathway. Of course, exposure leads to an internal dose of the chemical via kinetic mechanisms, and the chemical then interacts with a biological target to induce some perturbation of cellular function. At low levels of toxicant exposure, adaptive stress responses arise to restore the pathway to produce its normal outputs. At high enough levels of exposure and internal dose, perturbation exceeds the homeostatic capacity of the adaptive responses, and cell injury occurs, leading eventually to morbidity and mortality.

The potential utility of the pathway concept in toxicity testing rests upon the detection of early cellular changes that are linked to adverse outcomes *in vivo*. That is, in a pathway in which clear mechanistic links between early cellular changes and subsequent downstream events that lead to irreversible injury have been demonstrated, testing for toxicity can be accomplished at the level

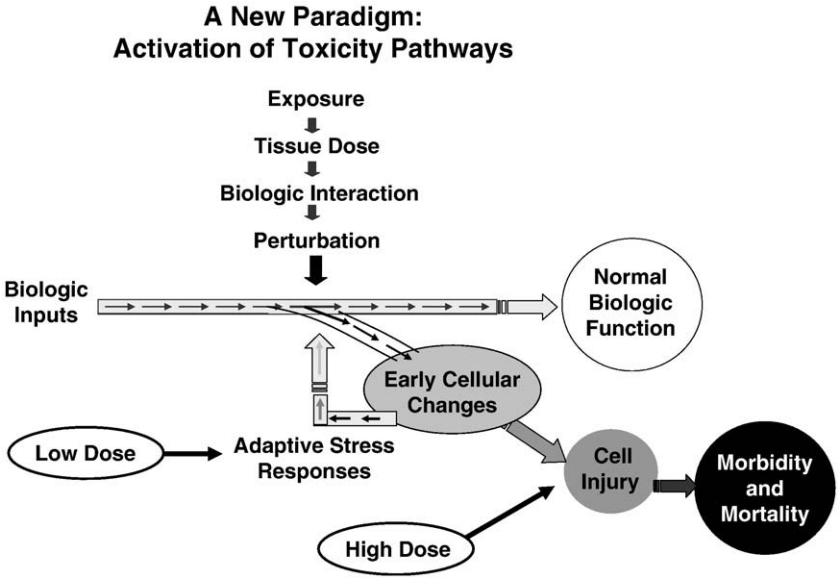

Fig. 1. The toxicity pathway concept as proposed by the National Academies. The horizontal line depicts a biological process, or signaling pathway, that generates a normal biologic function from an input. When a xenobiotic chemical is introduced (vertical arrows descending toward the pathway), interaction of the chemical with some molecular target perturbs the pathway and leads to early cellular changes. At low levels of exposure (low internal dose), homeostatic mechanisms generate adaptive responses that maintain or restore the function of the pathway. At high doses, these adaptive responses are overcome, leading to cell injury and, eventually, to morbidity and mortality. Adapted from NRC (2007). Reprinted with permission from the National Academies Press, Copyright 2007, National Academy of Sciences.