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Introduction to the Special Issue on Emerging High Throughput and Complementary Model Screens for Neurotoxicology

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The articles of this special issue "Emerging High Throughput and Complementary Model Screens for Neurotoxicology" were assembled to elucidate a spectrum of new approaches to neurotoxicology, which hold promise for rapidly garnering important information concerning a broad array of potential neurotoxicants. These model systems including high-throughput cell-based assays as well as invertebrate, aquatic and avian species can be used together with the classic mammalian models and epidemiology to provide a comprehensive approach to neurotoxicity hazard determination.

The National Research Council, in its 1984 report, *Toxicity Testing: Strategies to Determine Needs and Priorities*, summarized the availability of health hazard assessment data for chemicals (NAS, 1984). This report concluded that data for adequate hazard characterization was lacking for approximately 80% of the estimated 13,000 chemicals with production volumes of more than 1 million pounds per year. Over the past 25 years this backlog of chemicals lacking adequate hazard information has not diminished. Indeed, the size of this chemical universe [estimated to be approximately 30,000 chemicals with wide commercial use (Muir and Howard, 2006)] clearly precludes the use of standard toxicity assays that rely on animal testing that cost millions of dollars per chemical. In 2008, the National Research Council published a strategic plan entitled "*Toxicity Testing in the 21st Century: A Vision and a Strategy*". This plan encourages a bold new approach to chemical hazard assessment that utilizes novel in vitro approaches to chemical toxicity characterization and prediction (NRC 2007). An ongoing collaboration between the National Institutes of Health (NIH) and the U.S. EPA aims to realize the goals of the NRC report (Collins et al. 2008).

The long-term vision of the 2007 NRC report is to use high-throughput cell-based assays and quantitative structure activity relationships (QSAR) to predict adverse outcomes. While this is highly laudable, it will not occur overnight. More importantly, it will require a long-term research effort to connect key initiating molecular events to adverse outcomes. This will require the development of high-throughput approaches that can screen thousands of chemicals for these key events using time- and cost-effective models. Critical to determining the predictive

efficiency of these high throughput approaches, as well as important to tiered testing strategies, is the development of complementary approaches.

The replacement of standard in vivo neurotoxicity assessments with QSAR and high-throughput in vitro methods is not currently feasible, and may not occur for decades. Yet, we cannot continue in the interim, to rely on the standard in vivo approaches that have been used successfully in the past to assess the backlog of thousands of untested chemicals. These approaches are too resource and time intensive and will never get the job done of screening thousands of chemicals for neurotoxic potential. An alternative approach that may help is the use of true tiered testing schemes, where the first tiers rely on high-throughput methods that test for interactions of chemical with the key biological receptors that initiate pathways of changes that lead to adverse outcomes. The prime purpose of this tier is to identify chemicals for future testing. The papers by Radio et al, Price et al, and Breier et al are examples of this kind of testing. Second tier tests could involve the use of alternative species that will allow more moderate throughput, but in an intact or developing nervous system. Small fish or invertebrate species are possibilities and are highlighted in articles by (Levin et al, Boyd et al., Fan et al., Helmcke et al., Rand, and others). Chemicals identified as having neurotoxic properties could then be tested in intact mammalian models necessary for generation of data to be used in risk assessment. These lower tier mammalian testing models should be tailored to the data generated in the higher tiers. For example, if higher tier data suggest chemical-induced changes in myelin formation during development, then mammalian models appropriate to test myelin related endpoints (e.g., histopathology) should be used. This approach involves a general paradigm shift in toxicity testing, where the results from early tiers are used to prioritize chemicals for further testing, not for hazard assessment as we now know it. The general approach is to screen for prioritization at the higher tiers and test for hazard at the lower tiers.

In order for such a new neurotoxicity testing strategy to be useful, neurotoxicology as a field needs to develop new approaches to screening and characterizing the neurotoxic potential of chemicals. Critical to this process are complementary approaches that will allow assessment of the impact of chemical-induced alterations on key events in intact multi-cellular organisms. These tests should not just be supplementary in the sense of merely adding another category of information. Rather these new approaches should be driven by knowledge of how xenobiotics interfere with basic neurobiological processes. And, importantly, the results of these methods should inform and refine how and when the classical mammalian model approach are used. Each method of analysis will contribute unique information to the overall picture of neurotoxicity and inform the process and interpretation of the other avenues of investigation. Just as non-biologic analysis can contribute important information about important chemical properties of compounds (pH, partition coefficients, etc.), cell-based assays can provide critical information regarding toxicant effects on intracellular signaling and cell lifecycle processes. Non-mammalian whole animal models can provide important understanding of toxicant effects on intercellular signaling in an anatomically and temporally intact biologic system.

We must recognize that our current understanding of biology in general and neurobiology in particular is far from complete and will remain so for the foreseeable future. We will need to continue lines of research with mammalian models concerning functional effects of neurotoxicants and the complex mechanisms for these functional effects. The emerging high throughput and complementary models can help direct the best use of the classic mammalian models as the work with the mammalian models has and will continue to provide high through-put systems with the relevant measures to assess (e.g. thyroid or estrogenic effects). The integrated and tied use of the spectrum of neurotoxicity approaches provides a rationale

approach to decreasing the vast uncertain risk posed by thousands of chemicals with no available hazard information.

The extraordinary conservation of both genetic elements and differentiation processes between mammals and non-mammalians has been revealed during the last two decades. Animal studies are essential for testing drug efficacy and safety as well as the potential neurotoxic risks posed by chemicals in the environment. However, established animal models are expensive and time consuming, which often limits their use to in depth analysis of a relatively few chemicals. Emerging model organisms like the nematode, fruit fly and fish are making it possible to assess the effects of small molecules rapidly, inexpensively, and on a miniaturized scale. By combining the economy and high throughput of in vitro screens with the physiological complexity of whole animal studies, these model systems can enable rapid progress in the risk assessment process. The utility of the models is largely driven by the functional conservation seen between them and higher organisms, including humans so that knowledge obtained using non-mammalian model systems can often provide a better understanding of equivalent processes, pathways, and mechanisms in man.

One of the obstacles to understanding toxicity has been the inability to study toxicity in an intact organism where cell-cell interactions and complex metabolic milieus influence and modify xenobiotic-induced neurotoxic potential. *Caenorhabditis elegans* (C. elegans), zebrafish (*Danio rerio*) and *Drosophila melanogaster* (*D. melanogaster*). Each of the above provide powerful model organism for dissecting the components of neurodegeneration. The genome, biosynthesis and metabolic pathways are highly conserved with mammalian systems. Moreover these organisms are readily amenable to genetic manipulations. These species are also transparent, thereby allowing for easy real time examination of the neuronal morphology and direct viewing of protein expression patterns. The genomic sequencing of *C. elegans*, *D. rerio and D. melanogaster* has been completed, which allows for performance of whole animal PCR and provides a high-density polymorphism map of related strains. High throughput approaches include genome-wide screening for molecular targets or mediators in toxicity and rapid, high-content chemical screens to detect potential toxicants. Genome-wide screening is important for studying any toxicant with a poorly understood mechanism of action. This screening has been accomplished using RNA interference (RNAi), DNA microarrays, and gene expression analysis. Similarly, chemical screening has been shown to be a quick and inexpensive method for toxicity testing in several of these complimentary platforms.

These and other features have provided the impetus for the pursuit of non-mammalian systems in drug discovery and toxicity studies and the establishment of methods for high throughput analysis of signal pathways and mechanisms of cell toxicity, with special emphasis on neurotoxicological end-points.

Evaluation of cytotoxicity, particularly in a heterogeneous system such as the brain, is difficult in the intact animal because numerous factors (neural, hormonal, and hemodynamic) are not under experimental control. A simplified model, such as tissue culture (see reviews by Breier et al., Bal-Price et al., Barhoumi et al., Galofré et al., Radio et al., and others), has been therefore indispensable as a tool for understanding of basic physiology and molecular mechanisms that govern neurotoxic responses. Neural cultures offer numerous advantages over *in vivo* techniques. Cell morphology, protein synthesis and release, energy metabolism, receptor interaction, neurotransmitter uptake and release, as well as electrolyte and non-electrolyte uptake and release can be easily studied. Dispersion of cells in culture permits access to clean membrane surfaces for electrophysiological studies utilizing patch clamping and allows for rapid and reliable exchange of solutions. Furthermore, direct effects of chemicals on a relatively homogeneous population allows for study of specific aspects of the growth and differentiation of cells, as well as the kinetics of uptake and metabolism of the parent compound. The culture

model also makes it possible to study regional specialization, and can be extended to study cellular interactions by co-culturing various cell types.

However, limitations of the culture systems should also be considered. For example, cells can undergo varying degrees of differentiation. In addition, a number of different, sometimes competing, processes can influence the ability of a toxin to damage specific cells. The reductionist approach where one removes many cell types and barriers, focusing on a single cell type can facilitate diffusion or even active transport of a given toxic compound or its metabolite, limiting or enhancing toxicity. The ability of a cell to repair or replace damaged organelles or enzymes can also be critical in determining cell survival. This effect may also be dependent on neighboring cells and physical barriers, which may altogether be absent in a culture system.

The purpose of this special issue of Neurotoxicology and Teratology *is* to provide state-of-the-art review of recent developments of non-mammalian experimental models and their utility in addressing issues pertinent to both neurotoxicity testing as well as addressing mechanisms specific to neurodegeneration. The issue provides concrete examples on the power inherent to these systems, and addresses both the strengths and weaknesses of these models. The editors are hopeful that the reviews of complementary experimental platforms will enable the reader to become acquainted with the latest information and scientific breakthroughs in this fast-paced research area.

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References

NAS. National Academy of Sciences, National Research Council, National Academy Press, Washington DC. Washington, DC: National Academy Press; 1984. Toxicity Testing: Strategies to Determine Needs and Priorities.

Muir DC, Howard PH. Are there other persistent organic pollutants? A challenge for environmental chemists. Environ Sci Technol 2006;40(23):7157–7166. [PubMed: 17180962]

Collins FS, Gray GM, Bucher JR. Toxicology. Transforming environmental health protection. Science 2008;319(5865):906–907. [PubMed: 18276874]