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Contemporary Issues in Toxicology

Understanding mechanisms of toxicity: Insights from drug discovery research

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Abstract

Toxicology continues to rely heavily on use of animal testing for prediction of potential for toxicity in humans. Where mechanisms of toxicity have been elucidated, for example endocrine disruption by xenoestrogens binding to the estrogen receptor, *in vitro* assays have been developed as surrogate assays for toxicity prediction. This mechanistic information can be combined with other data such as exposure levels to inform a risk assessment for the chemical. However, there remains a paucity of such mechanistic assays due at least in part to lack of methods to determine specific mechanisms of toxicity for many toxicants. A means to address this deficiency lies in utilization of a vast repertoire of tools developed by the drug discovery industry for interrogating the bioactivity of chemicals. This review describes the application of high-throughput screening assays as experimental tools for profiling chemicals for potential for toxicity and understanding underlying mechanisms. The accessibility of broad panels of assays covering an array of protein families permits evaluation of chemicals for their ability to directly modulate many potential targets of toxicity. In addition, advances in cell-based screening have yielded tools capable of reporting the effects of chemicals on numerous critical cell signaling pathways and cell health parameters. Novel, more complex cellular systems are being used to model mammalian tissues and the consequences of compound treatment. Finally, high-throughput technology is being applied to model organism screens to understand mechanisms of toxicity. However, a number of formidable challenges to these methods remain to be overcome before they are widely applicable. Integration of successful approaches will contribute towards building a systems approach to toxicology that will provide mechanistic understanding of the effects of chemicals on biological systems and aid in rationale risk assessments. Published by Elsevier Inc.

Keywords: High-throughput screening; High-content screening; *In vitro* toxicology; Mechanism of toxicity; Systems toxicology; Predictive toxicology

Contents

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Introduction

Toxicology has traditionally focused on the effects of exogenous chemicals on living organisms through intensive studies done one chemical at a time. Such approaches have served to illuminate the modes of action of many classes of chemicals and provided detailed mechanistic understanding of the molecular targets of toxicity for some. However the costs of this approach have been high and mechanistic understanding remains limited. Toxicology studies rely heavily on use of vertebrate animals, an expensive undertaking in both time and money with debatable predictive power for human safety. For example, carcinogenicity studies are conducted using a 40-year-old model requiring 400 or more study animals and two years of exposure at a cost of millions of dollars (Bucher, 2002). Despite the investment of resources, debate continues on the utility of these data in predicting carcinogenicity potential in humans (Ennever and Lave, 2003). The problem lies chiefly with an inability to discern mechanisms of toxicity for the majority of toxicants tested using the "black box" whole animal assays; hence, cross-species extrapolation and low-dose, real-life exposure effects become very difficult to appropriately assess. With increasing public concern over the minimal toxicity information available for thousands of large volume-production chemicals produced and used in commerce, the inadequacy of existing methods presents a sizeable quandary (De Rosa et al., 2003; National Research Council, 2006). REACH legislation in the European Union covers approximately 30,000 chemicals and would require millions of animals and billions of Euros to conduct safety assessment on all of these using traditional methods (Van der Jagt et al., 2004). Such an enormously expensive undertaking would likely provide useful data in defining the toxicity (or lack thereof) for many chemicals; however, without mechanistic understanding, debate over risk of a subset of these chemicals would most assuredly ensue. What is needed are cost-effective screening assays that would not only identify chemicals of safety concern, but provide quantitative and mechanistic information to inform rationale risk assessment.

In the pharmaceutical and biotechnology industries over the past 15 years, enormous resources have been invested in developing efficient means to screen compounds against large numbers of potential therapeutic targets. The dramatic advances made in high-throughput screening (HTS) technologies now permit ready profiling of biological activity of large chemical libraries using multiwell plates and automated liquid handling equipment. Although developed to support drug discovery, toxicologists have begun applying these batch-testing methodologies to large numbers of chemicals using *in vitro* bioassays and model organism screens to characterize potential for toxicity and understand mechanisms of action. This review will look at applications of HTS techniques to toxicology and their potential impact on shifting testing paradigms for evaluating chemicals for risk.

High-throughput screening

HTS techniques are used primarily in the pharmaceutical industry in support of lead generation projects whose goal is to efficiently sort through enormous numbers of compounds for leads, the starting chemical structures for the drug development process. Large libraries of organic small molecules or natural products are batch-tested against biological targets in industry-standard 96- and 384-well plates, occasionally using even higher density 1536- or 3456-well plates. Relying heavily on automation and robotics, throughput ranges from thousands to 1,000,000 samples tested per day depending on the specific assay format (Table 1). Rate-limiting steps often lie in the type of signal detection required for the assay; simple fluorescence signals on the high end of the throughput range and cellular imaging assays at the lower end. Costs range from well under $1 per well for reagents and consumables to $10–75 per well for work performed at contract research laboratories.

Although the numbers of chemicals requiring toxicity screening is not in the range of the numbers of chemicals typically screened for lead generation for drug discovery, the opportunity to broadly profile compounds for toxicity with a variety of assays efficiently and at low cost makes HTS an attractive approach. This strategy, i.e. few chemicals tested against a large number of assays, is the converse of the drug discovery paradigm where many compounds are tested against one biological target, but makes use of the same efficiency infrastructure used for HTS assays. Initial work on adopting HTS techniques for toxicity testing occurred in the pharmaceutical industry and centered largely on the cytochrome P450 monooxygenase (CYPs) drug metabolizing enzymes since interference with these enzymes was a commonly encountered problem during development of new drug candidates (Crespi and Stresser, 2000). This area continues to receive much focus as new technologies are brought to bear on inadequacies of existing approaches. Beyond drug metabolizing enzymes, *in vitro* assays for specific targets associated with toxicity, e.g. ion channel assays, have been developed and are now routinely run. In addition, pharmacology profiling panels targeting representative members of many protein families are used to understand

Table 1
Definitions of screening modes

Screening mode	Abbreviation	Samples tested per day	Example
Low-throughput	LTS	1–500	Animal models
Medium-throughput	MTS	500–10,000	Fluorescent cellular microscopic imaging assay
High-throughput	HTS	10,000–100,000	Fluorescent enzymatic inhibition assay
Ultra-high-throughput	uHTS	>100,000	Beta-lactamase cell reporter assay