# Session I-13: Toxicity testing in the 21st century

## Session I-13: Oral presentations

I-13-679

### Tox21 Special Session at the 8th World Congress

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The Toxicology in the 21st Century (Tox21) program is an ongoing collaborative effort among four U.S. Government agencies: the National Toxicology Program (NTP) of the National Institute of Environmental Health Sciences, the Environmental Protection Agency (EPA), and Food and Drug Administration (FDA), and the National Institutes of Health Chemical Genomics Center (NCGC). Tox21 is developing and deploying a wide range of high-throughput *in vitro* biological testing and computational technologies to identify the activities and mechanisms of action of thousands of chemicals, with the goal of providing a science- and data-driven basis for *in vivo* chemical testing prioritization and risk assessment. After a pilot phase that began in 2005 and Tox21 Phase I that began in 2008, the Tox21 program entered Phase II in March 2011 with the completion of a testing library of 11,000 environmental and pharmaceutical chemicals, a dedicated robotics system capable of testing the entire Tox21 library in triplicate 15-concentration quantitative high-throughput screening (qHTS) format across a different *in vitro* assay every week, informatics databases and algorithms to analyze, visualize, and model the data, and targeted testing paradigms to examine the predictive and *in vivo* relevance of the models created. Tox21 also has a robust technology development component focused on the enumeration of all potential toxicity pathways, incorporation of metabolism and cell-cell interactions into *in vitro* assays, and the incorporation of exposure information into the models developed. Strategies and progress in all of these areas will be presented.

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# Tox21: Activities of the U.S. National Toxicology Program (NTP)

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In 2008, the National Institute of Environmental Health Sciences/NTP entered into a Memorandum of Understanding with the NIH Chemical Genomics Center and the Environmental Protection Agency's National Center for Computational Toxicology on the research, development, validation, and translation of new and innovative *in vitro* and lower organism test methods that characterize key steps in toxicity pathways. This collaborative effort, known informally as Tox21, was expanded in 2010 with the addition of the U.S. Food and Drug Administration. In support of Tox21, the NTP has (1) produced a large library of environmentally relevant compounds for screening across toxicity pathways; (2) identified and/or supported the development of assays suitable for use in quantitative high throughput and high content screens (qHTS, qHCS); (3) established a WormTox Screening Facility with the goal of developing toxicological assays using the nematode *Caenorhabditis elegans*; (4) developed statistically-based approaches for distinguishing between active, inactive, and inconclusive responses in these screens and informatic tools for identifying predictive toxicity patterns; (5) expanded the NTP's publicly accessible Chemical Effects in Biological Systems (CEBS) database to contain all Tox21-related data as well as the NTP historical data; (6) conducted qHTS studies to probe mechanisms of inter-individual susceptibility to toxicants; (7) evaluated next generation molecular tools for mining the formalin fixed, paraffin embedded animal tissues in the NTP Tissue Archives for predictive gene signatures; and (8) supported assay and informatic developments through the NIEHS Small Business Innovative Research contract award process. Advantages and limitations of these activities will be presented.

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# Development of an integrative approach for the prediction of systemic toxicity: Combination of cell toxicity, pharmacological and physical chemical properties

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Ethical, scientific and economic constraints have motivated the scientific community to develop alternatives to animal testing. Developing alternatives for acute/chronic systemic toxicity testing represents a challenge because of the complex biological processes implied. A realistic approach could rely on the combination of data generated for multiple endpoints. The Ctox panel®, which is a multiparameter cell-based *in vitro* system for predicting rat acute systemic toxicity, is a typical example. Preliminary studies conducted in a blinded manner showed a good sensitivity and specificity (91% and 78%) while defining a $LD_{50}$ threshold at 2000 mg/kg. However, the model failed to accurately predict very toxic chemicals displaying ($LD_{50}$ below 300 mg/kg). Further to an in-depth analysis of the misclassified chemicals, we concluded that both pharmacological data (for the reduction of false negatives) and physical-chemical properties (for the reduction of false positives) had to be considered. The modified approach was applied to 76 non-proprietary compounds previously tested with the standard method. A significant improvement in the prediction of the GHS categories was observed. Indeed, 75% of the chemicals pertaining to GHS 1, 2 and 3 were correctly classified, compared to 50% with the standard model. In addition, at an arbitrarily defined $LD_{50}$ threshold of 500 mg/kg, the sensitivity and specificity were 85% and 89% with the new model against 71% and 83% with the standard model. Future directions will consist of challenging the newly built model with a new set of chemicals and foreseeing the application of such a strategy for repeated dose toxicity.

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# AXLR8 strategic directions for development of alternatives in the EU

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Conventional approaches to toxicity testing and risk assessment are often decades old, costly and low-throughput, and of dubious relevance to humans. The call for a transition to a 21st century toxicity testing paradigm will require a robust understanding of the cellular response/toxicity pathways which that can lead to adverse effects when perturbed; appropriate *in vitro* systems to study chemical interactions at key targets along a pathway; and computational systems biology models to describe the underlying pathways as a basis for creating biologically realistic dose-response models.

The EU FP7 coordination support action project AXLR8 (=accelerate) aims to support the transition to a toxicity pathway-based paradigm for quantitative risk assessment and will: 1) organize a series of annual workshops to map research progress, gaps and needs in the FP6/FP7 program on alternative testing strategies. 2) Provide a range of tools and opportunities for enhanced interdisciplinary and international communication, coordination and collaboration in order to maximise the impact of available resources. 3) Work to streamline regulatory acceptance procedures to provide for the uptake of validated 3Rs methods, including a smooth transition to 21st century systems as they become available. 4) Produce annual progress reports on the state of the science, including recommendations on priority research and funding targets, in order to ensure a prominent role for European science in this rapidly developing global research area.

In 2010 and 2011 the first AXLR8 workshops (AXLR8-1 & AXLR8-2) have focused on progress made in the EU FP6/FP7 projects funded by the health theme of the DG RTD "*Alternative Testing Strategies: Replacing, reducing and refining use of animals in research*". The results of the discussions and recommendations of the AXLR8 Scientific Panel at the AXLR8-1 2010 workshop have been published in the AXLR8 Progress Report 2010. These results and the recommendations of the AXLR8-2 2011 workshop on a "Roadmap to innovative toxicity testing (ITT)" will be presented.

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# The OECD QSAR toolbox

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The OECD QSAR Toolbox is a software application intended to be used by governments, industry and other stakeholders to fill gaps in (eco)toxicity data needed for assessing the hazards of chemicals. The two main pillars of the system are (i) the knowledge-base for grouping chemicals into toxicologically meaningful categories and (ii) databases with measured physchem, fate, and toxicity data. The grouping engines allow selection of analogues accounting for underlying interaction mechanisms and metabolism. Read-across and trend analysis are used to predict the endpoint values for a target chemical. The data gap filling methods include also a library of QSAR models to estimate missing experimental values. Each estimated value can be individually justified based on category hypothesis, quality of measured data and computation method used for categorization and data prediction. As the rationales for analogues selection are often based on common mechanisms of action, good regulatory acceptance is expected for predictions provided by the Toolbox. Since October 2010 the OECD QSAR Toolbox version 2.0 is available for free and can be downloaded from the OECD website. Version 2 is available both as a distributed version and as a stand-alone version. This release is part of a four-year collaborative project between OECD, ECHA, LMC and other partners. The aim of this presentation is to elucidate the improvements of the main functionalities as well as the new features introduced in version 2 of the Toolbox.

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# ToxCast Update – predictive signatures and phase II

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Chemical toxicity testing is being transformed by advances in biology and computer modeling, and driven by the thousands of environmental chemicals lacking toxicity data and concern over animal use. The U.S. Environmental Protection Agency's ToxCast program aims to address these concerns by screening and prioritizing chemicals for potential toxicity using *in vitro* assays and *in silico* approaches. This project has evaluated the use of *in vitro* assays for understanding the types of molecular and pathway perturbations caused by environmental chemicals and to build predictive and systems models of *in vivo* toxicity. To date we have tested close to 1000 chemicals in over 500 high throughput screening (HTS) assays across multiple technologies utilizing human and other species genes, proteins, primary and cell lines. Chemicals displayed a broad spectrum of activity at the molecular and pathway levels. We saw many expected interactions, including endocrine and xenobiotic metabolism enzyme activity. Chemical bioactivity ranged across pathways, from no activity to affecting dozens of pathways. We found statistically significant associations between numerous pathways perturbed by chemicals at measured *in vitro* concentrations, and with *in vivo* doses resulting in chemical toxicity. Useful predictive and systems models for reproductive, developmental, and cancer pathways and endpoints have been developed. ToxCast and the Tox21 programs are providing HTS screening and prioritization based on predictive and systems models of toxicity, and meaningful data on thousands of environmental chemicals for guiding targeted testing of chemicals.

*This abstract does not necessarily reflect Agency policy.*

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# Taking a mode-of-action approach to designing a hepatotoxicity screening strategy using the HepaRG cell model and high content imaging

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The liver is central to the metabolism of xenobiotics and faced with harmful effects of toxic substances. Evaluating the risk of liver toxicity is a major issue and there is still no established *in vitro* screening strategy to reliably identify potentially hepatotoxic chemicals. In the approach described here, a mode-of-action targeted analysis of the literature has been used to identify toxicity pathways and the key biological events associated with them. This knowledge has then been used to design a multiparametric HTS experiment to classify chemicals based on their likely association with a specific mode-of-action.

We used a metabolically competent cellular model, HepaRG, and high content imaging implemented on a HTS platform. The HepaRG cell line expresses the major liver functions, including P450s, phase II enzymes, transporters and nuclear receptors at levels comparable to those found in primary hepatocytes.

The high content screening approach we adopted is based on automatic analysis of image-sets acquired with an epifluorescent microscope for the quantification of immuno-fluorescently stained biomarkers expressed by treated HepaRG cells. A quantitative high throughput screening format was employed using a 96-well plate format, which facilitated the testing of a set of 92 reference chemicals and drugs with known hepatotoxic activity. Multiple cellular phenotypic changes were analysed by staining with fluorescent dyes for identification and quantification of response parameters. A biostatistical model was then developed to associate the test chemicals with different mode-of-action based categories. A systematic comparison of the classification results with literature findings allowed a preliminary validation of the approach.

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# Virtual Embryo: Systems modeling in developmental toxicity

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High-throughput screening (HTS) studies are providing a rich source of data that can be applied to chemical profiling to address sensitivity and specificity of molecular targets, biological pathways, cellular and developmental processes. EPA's ToxCast project is testing 960 unique chemicals (drugs, pesticides, etc.) in over 500 distinct assays, testing for diverse biochemical activities, receptor binding activities, reporter gene activation and gene expression profiles, stress-response indicators, and perturbation in cell state and cellular function. Also included are assays to monitor effects in zebrafish embryos and pathways of differentiation in mouse embryonic stem cells. *In vitro* profiles ($AC_{50}$ in $\mu$M) are compared using machine-learning algorithms to identify patterns of biological activity and optimal feature selection for predictive modeling. Early findings suggest that developmental toxicity does not emerge from a simple molecular stream. Because many cells in a system interact to generate emergent properties (growth, patterning, homeostasis, robustness), computer models are needed to capture the complexity of multicellular networks and the key events leading to dysmorphogenesis. A predictive Virtual Embryo framework utilizes detailed knowledge to build computational models that run a morphogenetic series of events and can analyze the complexity of developmental processes. Potential regulatory applications are to inform and guide application QSAR models for predicting developmental effects; extract and organize literature for information relevant to developmental processes and defects; standardize *in vitro* and HTS data for predictive modeling of the disturbances to developmental processes; prioritize environmental chemicals for targeted testing; and systems modeling to analyze key pathways and mechanisms.

*This abstract does not reflect EPA policy.*

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# Integrated approaches to testing and assessment: The expert panel on the integrated testing of pesticides

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Canada relies on well-established approaches to assess the safety and efficacy of chemicals and pharmaceuticals, including pesticides. Alternative testing strategies represent an exciting opportunity for the development of tests that can evaluate a larger number of compounds, in systems that may more reliably predict potential adverse effects in humans. This approach also has the potential to identify the cellular mechanisms that may be the root cause of adverse effects. These new approaches may reduce the reliance on animal-based test systems and increase the reliability and efficiency of testing, while maintaining the highest levels of scientific rigor.

The Pest Management Regulatory Agency of Health Canada requested that the Council of Canadian Academies convene an expert panel to evaluate the use of integrated approaches to testing and assessment for the regulatory risk assessment of pesticides. Specifically, the 15-member panel was asked to address the following questions:

– What is the current status of the use of integrated testing strategies by regulatory agencies around the world?
– What is the state of the science of integrated testing strategies?
– What are the potential impacts on the public's perception and confidence of IATA for pesticides?

Pesticide formulations represent, on the one hand, one of the most data-rich chemical groups in the field of regulatory toxicology while, on the other hand, one of the most data-poor. To this end, they make an excellent model, both for the development and evaluation of new testing protocols and as a validation tool against which alternative testing strategies can be assessed.