Risk science in the 21st century: a data-driven framework for incorporating new technologies into chemical safety assessment

Russell S. Thomas

National Center for Computational Toxicology,
US Environmental Protection Agency, USA
Email: Thomas.Russell@epa.gov

Ricky Cheung*

Public Health Agency of Canada,
785 Carling Avenue, Ottawa,
Ontario K1A 0K9, Canada
Email: ricky.cheung@utoronto.ca
*Corresponding author

Margit Westphal

McLaughlin Centre for Population Health Risk Assessment,
Institute of Population Health,
University of Ottawa,
1 Stewart St., Ottawa, Ontario, K1N 6N5, Canada
Email: Margit.Geisterfer@uottawa.ca

Daniel Krewski

McLaughlin Centre for Population Health Risk Assessment,
Institute of Population Health,
University of Ottawa,
850 Peter Morand, Ottawa, Ontario, K1G 3Z7, Canada
and
Department of Epidemiology and Community Medicine,
Faculty of Medicine,
University of Ottawa,
451 Smyth Road, Ottawa, Ontario, K1H 8M5, Canada
and
Risk Sciences International,
55 Metcalfe Street, Suite 700, Ottawa, ON K1P 6L5
Email: dkrewski@uottawa.ca

Melvin E. Andersen

ScitoVation,
Research Triangle Park,
North Carolina, USA
Email: mandersen@scitovation.com

Abstract: One of the major challenges in chemical safety assessment is prioritisation of the large number of chemicals in commerce. Over the past five years, a new tiered approach to chemical safety assessment that uses margin of exposure (MOE) as the metric for determining the level of testing required, identifies chemicals of greatest concern. This paper evaluates the role of new technologies and novel tools in improving different steps of chemical risk assessment processes such as high throughput screening (HTS) in vitro assay platforms, high content biological omics assays, molecular biomarkers, quantitative structure activity relationship (QSAR) modelling, in vitro to in vivo extrapolation (IVIVE) and physiologically-based pharmacokinetic (PBPK) modelling. Other technologies such as functional genomics, bioinformatics, and computational biology can expedite the analysis. This new approach could potentially be used to prioritise and categorise chemicals on the domestic substance list (DSL) under the Canadian Environmental Protection Act (CEPA).

Keywords: risk assessment; chemical safety evaluation; margin of exposure; MOE; tiered approach; predictive toxicology.

Reference to this paper should be made as follows: Thomas, R.S., Cheung, R., Westphal, M., Krewski, D. and Andersen, M.E. (2017) 'Risk science in the 21st century: a data-driven framework for incorporating new technologies into chemical safety assessment', *Int. J. Risk Assessment and Management*, Vol. 20, Nos. 1/2/3, pp.88-108.

Biographical notes: Russell S. Thomas is the Director of the National Center for Computational Toxicology at the US Environmental Protection Agency. The centre is researching new, more efficient, ways to evaluate the safety of chemicals, particularly in assessing chemicals for human health effects. Prior to coming to the US EPA, he was the Director of the Institute for Chemical Safety Sciences at The Hamner Institutes for Health Sciences and worked in the biotech and biopharmaceutical industry. His academic training includes a BA in Chemistry from Tabor College, an MS in Radiation Ecology and Health Physics from Colorado State University, and a PhD in Toxicology also at Colorado State. Following his doctoral studies, he performed postdoctoral research in molecular biology and genomics at the McArdle Cancer Research Laboratory at the University of Wisconsin.

Ricky Cheung is an Analyst at the Public Health Agency of Canada. His academic background includes a PhD in Pharmacology, Post-Doctoral Training in Drug Discovery Research, and Interdisciplinary Training in Population Health Risk Assessment and Management. His research interests include risk assessment issues relating to pharmaceutical and toxicity testing.

Margit Westphal is a Risk Analyst at the McLaughlin Centre for Population Health Risk Assessment at the University of Ottawa. Her academic background includes a PhD in Medical Sciences from the McMaster University, a Master's in Clinical Biochemistry from the University of Toronto, and a Master's certificate in Population Health Risk Assessment and Management from the University of Ottawa. Her current focus is on health risk issues related to chemical toxicity testing and prion diseases.

Daniel Krewski is a Professor in the School of Epidemiology, Public Health and Preventive Medicine at the University of Ottawa, where he also serves as a Scientific Director of the McLaughlin Centre for Population Health Risk Assessment. His research interests include epidemiology, biostatistics, risk assessment, and risk management. He is a Fellow of the Society for Risk Analysis, the American Statistical Association, and a National Affiliate of the US National Academy of Sciences. He holds the Natural Sciences and Engineering Research Council of Canada Chair in Risk Science at the University of Ottawa.

Melvin E. Andersen is Senior Scientist at ScitoVation in Research Triangle Park, North Carolina. His 45-year career has focused on developing physiologically-based pharmacokinetic and biologically-based pharmacodynamic models and applying these tools in human safety assessments. He was an author of the 2007 NRC report, *Toxicity Testing in the 21st Centurt: A Vision and A Strategy*. His recent research has developed toxicity pathway-based case studies to show how in vitro assays in human cells can provide the primary data for conducting chemical safety assessments in the 21st century.

This paper is a revised and expanded version of a paper entitled 'A data-driven framework for incorporating new technologies into chemical safety assessment' presented at Risk Science in the 21st Century, Ottawa, 4 March 2013.

1 Introduction

New approaches are underway to evaluate the safety of tens of thousands of chemicals in the USA. The catalyst for this change began with a report written in 2007 by the National Research Council (NRC) called *Toxicity Testing in the 21st Century: A Vision and A Strategy* (now known as TT21C). This report recommended the utilisation of in vitro testing strategies and computational technologies with the aim to better understand chemical perturbation of biological systems or pathways (Krewski et al., 2011). A series of case studies have been researched to further exemplify the utility of the original TT21C vision, changing it from a concept to a data-driven toxicity testing framework (Thomas et al., 2013a). This new data-driven framework proposed by Thomas et al. (2013a) is based on the previously developed tiered approach for prioritising a large number of chemicals (Meek et al., 2011), and uses the margin of exposure (MOE) as the primary metric. In Canada, this framework could be used to identify and categorise chemicals that are on the domestic substances list (DSL) as defined under the Canadian Environmental Protection Act (CEPA) (Canadian Environmental Protection Act, 1999) and that have the potential to compromise human health or the environment (Environment Canada, 1999).

1.1 Evaluating the role of new technologies within the traditional steps of chemical risk assessment

The traditional chemical risk assessment process as originally outlined in the Red Book, consisted of three steps:

1 hazard identification

2 dose-response assessment

3 exposure assessment [NRC (US): Committee on the Institutional Means for Assessment of Risks to Public Health, 1983].

The TT21C vision framework (National Research Council, 2007), along with the recent NexGen framework for risk science (Krewski et al., 2014) have maintained these three main steps in their risk assessment process. The question still remains whether in vitro high throughput screening (HTS) assays and in silico modelling can fulfil the criteria required within each step and be used effectively in chemical risk assessment.

1.1.1 Step one: hazard identification

Traditional approaches of hazard identification utilise both epidemiological and animal studies (O'Bryan and Ross, 1988). To date, animal studies have been thought of as well-controlled experiments, requiring uncertainty extrapolations for high chemical doses and inter-species differences. Although the continued reliance on animal studies as a gold standard is under debate (Hartung, 2010), these in vivo models are still believed to be the best method for identifying higher-dosage chemical hazards as the intact animal encompasses the ongoing complex interactions of an intact biological system. The change to a foundation based on data from in vitro assays as proposed by the TT21C vision has yet to prove itself as an effective method for hazard identification to the extent that hazards must associate directly with results from the animal studies. Although the current suite of in vitro assays may not fully capture all potential biological targets, it is believed that as our knowledge-base increases so will the ability to interpret and predict probabilities of hazard or assurance of safety (Andersen and Krewski, 2010) based on in vitro assays and in silico methods. In the interim, HTS assays appear to be useful as an effective first tier screening tool for chemicals (Cote et al., 2012; Thomas et al., 2013a).

The US Environmental Protection Agency (EPA) has launched several programs under the umbrella of Computational Toxicology – CompTox (Dix et al., 2007), including the ToxCast program, to investigate the feasibility of using HTS for hazard identification and determining the mode-of-action. For hazard identification, ToxCast phase 1 evaluated the predictive performance of over 600 assays across dozens of in vivo endpoints (Thomas et al., 2012a). The results of these studies indicated that the HTS assays used in the ToxCast program showed limited capability for predicting traditional in vivo chemical hazards (Thomas et al., 2013a). However, the HTS assay have demonstrated utility for identifying potential molecular initiating events (MIE) associated with specific modes of action (Thomas et al., 2012a) and ability to separate chemicals into two main categories:

1 chemicals that cause toxicity primarily through non-selective interactions with cellular or molecular targets (e.g., through cytotoxicity via cellular stress pathways)

2 those that act through more selective interactions (e.g., receptor-mediated toxic response).

Selective chemicals can be further investigated using assay platforms that test specific modes-of-action at relevant doses whereas non-selective chemicals can be tested in assays evaluating cell stress pathways. Following considerations of the value of various assays and redundancy in the phase 1 assays themselves, the Tox21 assay suite at NCGC (NIH Chemical Genomics Center) has been reduced to approximately 80 HTS assays (in ToxCast phase 1 and phase 2, over 1,000 data-rich chemicals were run through more than 600 HTS assays. NCGC streamlined and optimised this HTS assay set from ToxCast phases 1 and 2 to under 100 HTS assays run with a robotics system) (Tice et al., 2013). The US EPA Tox21 program is now in the process of screening and prioritising 10,000 chemical substances with the reduced set of assays (Huang et al., 2014). This HTS platform will form the basis of in vitro tests for determining potential hazardous exposure dose (as shown in the next section) in calculating MOE.

1.1.2 Step two: dose response assessment

Dose response assessment has traditionally relied on sub-chronic and chronic animal studies. The data are then fitted with mathematical models to identify point of departure (POD), such as the benchmark dose (BMD) (dose that produces a predetermined change in adverse response) (Gephart et al., 2001; Thomas et al., 2012b). Traditional safety standards can be set by deriving reference dose values (RfD) or risk-specific dose (RSD) based on BMD information, with the application of uncertainty factors – a factor of ten fold for species differences and an additional factor of ten fold for high to low dose extrapolations (Walton et al., 2001).

In contrast, the TT21C approach uses pathway perturbations as the basis for determining the POD or the newly defined BPAD – biological pathway altering dose (Judson et al., 2011; Thomas et al., 2012b). One of the many advantages of the quantitative-HTS approaches is that chemicals can be assayed at multiple concentrations generating a concentration-response curve to evaluate the AC_{50} values, i.e., the concentration that causes a 50% change in activity. A wide range of concentrations in a variety of assays, including relatively low concentrations [reverse toxicokinetics (TKs) convert this to dose], are evaluated to determine the nominal in vitro concentration at which a chemical can initiate potential molecular events (Kavlock et al., 2012).

In the pharmaceutical industry, in vitro to in vivo extrapolation (IVIVE) methods have been developed to parameterise simple TK models that relate blood and tissue concentrations to therapeutic doses. The IVIVE methods typically include in vitro measurements of hepatic clearance, plasma protein binding, and potentially other measures such as bioavailability. The IVIVE methods developed for pharmaceuticals have also been used to parameterise TK models for hundreds of environmental and industrial chemicals. The TK models are used to estimate the daily oral dose (mg/kg BW/day) needed to produce steady-state plasma concentrations equivalent to concentrations resulting in biological activity in the ToxCast and Tox21 HTS assays (Rotroff et al., 2010; Wetmore et al., 2012, 2013, 2015).

To assess the applicability of data generated from high-throughput in vitro screening, comparison of the lowest oral equivalent dose from in vitro assays (i.e., most sensitive assays) were compared with the in vivo response at the lowest effect level (LEL) from ToxRef database for each of the 59 ToxCast phase 1 chemicals. The results demonstrated that for over 94% of the chemicals, the lowest oral equivalent dose derived from the most sensitive in vitro endpoint was less than the in vivo response with the LEL, suggesting that information generated from the most sensitive high throughput in vitro screening assays may provide a reasonable, conservative estimate of the POD for a chemical in a dose-response assessment (Wetmore et al., 2013). The combination of HTS assays and IVIVE modelling of TK could provide an efficient and cost-effective first tier dose response assessment in calculating MOE (Thomas et al., 2013a).

1.1.3 Step three: exposure assessment

Exposure assessment is the process of estimating or measuring the level, duration, and frequency of exposure to specific chemical agents in the environment. It can also be a procedure of estimating or predicting potential future exposure to an agent that has not yet been released to the environment. A human exposure assessment should take into account the parameters such as size, type, and nature of populations exposed to the agent of interest, as well as the uncertainties associated with these parameters. Zeise et al. (2013) have reviewed human variability extensively and shown how evidence from different data streams could be incorporated for addressing risks to include all susceptible populations. Exposure can be measured directly from human body, but more commonly is estimated using computer modelling and assumptions about its use and associated human behaviour. To evaluate the utility of incorporating exposure assessment to HTS in vitro assays, the oral equivalent dose ranges derived from IVIVE tools applied to the in vitro assays were compared with human exposure estimates for 239 ToxCast phase 1 chemicals. Eighteen of the 182 chemicals (9.9%) had oral equivalent doses that overlapped with the human exposure estimates, implicating potential human adverse health hazards (Wetmore et al., 2012). Comparison of exposure assessment to high throughput in vitro assay screening results which give a MOE estimate can provide a context for using toxicity data for priority setting for further chemical testing.

1.2 New technologies and tools available for chemical risk assessment

Over the last ten years, many new technologies have appeared that could be incorporated into the chemical risk assessment process. These technologies could enhance chemical risk assessment for three categories:

1 hazard identification and dose response assessment

2 dosimetry and exposure assessment

3 cross-cutting assessment.

Many of these new scientific tools and techniques will form the core of the new NexGen risk assessment framework and are outlined in Table 1 (Krewski et al., 2014).

Table 1 Promising risk assessment tools and methodologies

HTS	Uses high throughput in vitro assays to assess in vitro pathway activity from chemicals using human cell lines. There is some predictive capacity of certain perturbation pathways with in vivo health outcomes.
	HTS has the potential to efficiently and quickly screen thousands of chemicals simultaneously, at multiple doses, measuring changes in a wide range of biological pathways. Assays are done intriplicate on three different days resulting in 12 different doses response curves to determine validation, replication, and standard deviation of the curves.
	HTS can be used to determine margin-of-exposure and can be used as a first tier of analysis for risk assessment.
Computational toxicology	Computational toxicology methods integrates modern computing and information technology with molecular biology to develop biologically based in silico models to predict the toxicity of environmental agents, identify modes of action, and predict potential toxicity pathway perturbations.
	Offers to improve the efficiency and effectiveness of the process of determining the hazards and risks of environmental chemical, increasing the number of chemicals and the types of biological interactions that can be evaluated.
	Allow for examination of toxicity pathways across a range of dose levels, including those that are realistically applicable to exposure levels to the human population.
Toxico informatics	Encompasses activities related to the harness, standardisation, and integration of existing disparate and largely textual toxicological and bioactivity information of chemicals.
	Related predictive toxicity approaches and models enables to efficiently screen and prioritise large lists of chemicals.
Biological pathway altering dose (BPAD)	BPAD combines in vitro dose-response data with analysis of uncertainly and population variability to predict in vivo exposure levels.
	Can be calculated from in vitro derived data and mathematical models.
	Using relatively inexpensive high-throughput in vitro data, BPAD can be determined for thousands of data-poor environmental chemicals and prioritise chemicals for further testing
Computational systems biology pathway modelling (CSBPM)	CSBPM utilises understanding of the structure of pathways and cellular networks to understand the shape of dose response curves below the region of observation in the q-HTS assays. These tools, often reflecting the nonlinear stricture of cellular signalling processes, provides the information for more biologically-based modelling of the shape of curves at low levels of exposure/perturbation.
	By incorporating molecular components that regulate dose response the CSBP models can assist invariability analysis for the pharmacodynamic aspects of cellular dose response as is now done for pharmacokinetics through PBPK modelling.

1.2.1 Technologies available for hazard identification and dose response assessment methods

For hazard identification and dose response assessment, several new scientific tools and techniques are available for these purposes. The accuracy and confidence in using pathway perturbations as the basis of hazard identification will be strengthened as our understanding of the toxicity pathways increases. The human toxome project that proposes to map and identify all toxicity pathways in the human genome will facilitate the full understanding of adverse health outcomes as a result of key pathway perturbations (Hartung and McBride, 2011). Case studies (Andersen et al., 2011; Clewell, 2015) promise to show how these new technologies can be integrated to support risk/safety assessments based entirely on results from in vitro, mode-of-action-based assays.

To accomplish the task of testing hundreds of thousands of chemicals, the in vitro quantitative high throughput screening (q-HTS) assay platform is a powerful method to rapidly generate concentration response curves over a wide range of pathways (Knight et al., 2009). In addition, high content biological omics assays including genomics, epigenomics, transcriptomics, proteomics, metabolomics, and micro-RNA platforms can also be used to assess perturbations in cellular and tissue function (Moore et al., 2013; Sui et al., 2013). Data generated from in vitro transcriptomic studies have shown that pathway-based transcriptional BMD values were highly correlated with BMD values of traditional apical (non-cancer and cancer) responses, and can provide a reasonable POD estimate (Thomas et al., 2013b). It is yet unknown whether there is a good correlation exists between in vivo study results compared to transcriptional BMD values derived from in vitro assays after using IVIVE methods to determine equivalent oral equivalent doses.

Biochemical or molecular biomarkers of effects can be used to measure expected biological responses at the cellular, individual or population level. Biomarkers may also be linked to toxicity pathway perturbations, thereby providing direct evidence of critical perturbations in human populations due to harmful exposure such as pesticides (Anwar, 1997). Furthermore, biomarkers of effect can be incorporated into population-based studies (i.e., molecular and genetic population-based studies), by integrating human genome knowledge into epidemiological studies in order to better understand the roles of genetic susceptibility and gene-environment interactions in disease causation (Erickson, 2012; Garcia-Closas et al., 2011), as biological responses have been suggested to be strongly influenced by the host genome and epigenome (Olden et al., 2011).

Biological activity can also be predicted based on chemical structure using QSAR modelling. In chemical risk assessment, QSAR predicts toxicological responses and metabolic pathways based on the chemical properties of environmental agents and comparison with other active structures. This is one of the many tools of computational models, which could increase the efficiency and the effectiveness of both hazard identification and dose response assessment of environmental chemicals risk determination (Dix et al., 2007; Kavlock et al., 2008).

1.2.2 Technologies available for dosimetry and exposure assessment methods

New scientific technologies and tools are also available for the purposes of dosimetry and exposure assessment. For example, physiologically-based pharmacokinetic (PBPK) modelling is another technique used to understand the absorption, distribution, metabolism, and elimination (ADME) process of environmental agents (Clewell and Andersen, 1985; Poulin and Theil, 2002). Dosimetric methods are used to extrapolate between different exposure routes, as well as characterising inter-individual variability in exposure and dose (Valcke and Krishnan, 2010). PBPK modelling can also be used to predict dose- and species-dependent in vivo response based on in vitro data from pharmacokinetic parameters, as well as extrapolating experimental data from animal studies to predict human response (Rietjens et al., 2010). Moreover, PBPK modelling can account for heterogeneity in exposure of human populations including incorporation of chemical risk impact among the susceptible subpopulations (Clewell and Andersen, 1996).

Advances in computational modelling of human exposure are also occurring rapidly. In order to provide the complementary exposure estimates for thousands of chemicals, the US EPA developed the ExpoCast initiative, which uses mechanistic and heuristic exposure models that can be rapidly parameterised for a broad suite of chemicals (Cohen et al., 2010). Recent studies in the ExpoCast program used Bayesian methods to infer ranges of exposure intakes that are consistent with biomarkers of chemical exposures identified in urine samples from the US population by the National Health and Nutrition Examination Survey (NHANES) (Wambaugh et al., 2014). Linear regression was performed on the inferred exposures using high-throughput chemical descriptors, production volume, and chemical use categories. The study found that five descriptors formed the best model and could be used to predict exposures for thousands of chemicals together with associated estimates of uncertainty. Further refinement of the model will increase its predictive capacity and provide exposure estimates for sensitive demographic groups.

1.2.3 Technologies available for cross-cutting assessment methods

Several new tools and technologies developed over the last decade can facilitate the overall chemical risk assessment process, but cannot be grouped into any of the three main categories (hazard identification, dose response assessment, exposure assessment). Some of the new technologies include functional genomics, bioinformatics and computational biology, which can expedite the analysis and interpretation of large volumes of data, model dynamic responses in organisms, and extrapolate results observed in experimental models to humans. Other tools, such as adverse outcome pathways (AOPs) and systems biology, are novel concepts that help interpret perturbations in signaling pathways and networks for better prediction of adverse health responses.

Functional genomics is a specialised discipline of molecular biology that utilises vast amounts of genomic data to identify gene function. The integration and analysis of diverse data generated from omic technologies (e.g., genomics, epigenomics, transcriptomics, proteomics, metabolomics, and micro-RNA platforms) can help understand the consequences of chemical treatment and predict the outcomes of pathway

perturbation at the levels of cell, tissue, organ, and organism (Caba and Aubrecht, 2006; Krewski et al., 2009). Computational biology involves the application of analytical methods of mathematical modelling and computational simulation to delineate biological systems whereas bioinformatics focus on developing new tools (both software and hardware) to store, retrieve, organise, and analyse biological data. Both computational biology and bioinformatics are useful technologies to analyse and interpret complex multivariable data from in vitro HTS assays, high content imaging (HCI), and high content biological omics assays to identify modes of action and predict adverse health outcomes as a result of toxicity pathway perturbations on organs and tissues using mechanistic models at the cellular and molecular level (Zhao et al., 2014). The US EPA has implemented the use of computational toxicology and bioinformatics to help identify chemical hazards and assess risks to human health and the environment (Kavlock and Dix, 2010), as described by *A Framework for a Computational Toxicology Research Program* (US EPA, 2003).

The AOP describes the sequence of biological events that begins with the MIE and results in the development of adverse health outcome (Ankley et al., 2010). These biological events can take place at the individual or population level. The AOP platform helps provide a conceptual framework for identification of specific toxicity pathway perturbations triggered by environmental toxicants that lead to adverse health outcome. This AOP-based approach to chemical risk assessment has recently been implemented as part of the TT21C paradigm to be used as a practical tool for making safety decisions and prioritising chemicals (Adeleye et al., 2014). Systems biology is an emerging approach to understand the complex interaction between the components of the biological system, and how these interactions lead to functional changes within the system. It usually involves the understanding of biological and toxicity pathways, as well as metabolic and cell signalling networks (Mastellos et al., 2005). Systems biology also utilises mathematical and computational modelling to identity novel biological properties of cells, tissues and organisms that function as a system. For chemical risk assessment, systems biology organises information from multiple cellular pathways in order to understand integrated cellular, tissue and organ responses. Computational systems biology pathway modelling (CSBPM) is a tool to understand dose-response relationships in vivo systems in relation to the structure of cellular signalling networks and their responses to perturbations by environmental toxicants (Zhang et al., 2010, 2013, 2014). Furthermore, systems biology may also provide a platform for integrating and conducting the complex analyses required to determine whether a biological system could maintain homeostasis or trigger AOPs resulting in adverse health outcomes (Krewski et al., 2011; Zhang et al., 2015).

1.3 MOE can be used to identify chemicals of greatest concern

1.3.1 Matching in vitro bioactivity with exposure estimates to calculate MOE

One of the major challenges for chemical risk assessment is the prioritisation of the large number of chemical substances for toxicity testing. To deal with this challenge of sorting through a vast quantity of chemicals, MOE is proposed as the primary metric to identify chemicals of greatest concern (Thomas et al., 2013a). MOE is defined as the ratio of a POD (such as BMD or other indicators of biological response) to the human exposure estimate of the chemical of interest.

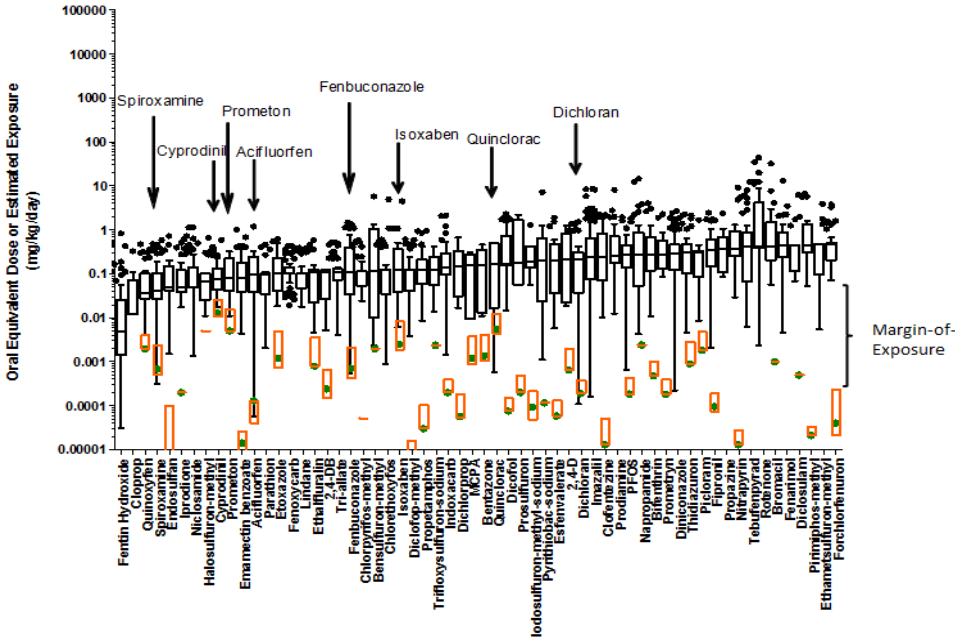
To obtain the MOE using high-throughput *in vitro* approaches, two sets of calculations are estimated. The first, known as oral equivalent dose, is defined as the daily human oral dose required to produce steady-state in vivo blood concentrations equivalent to the in vitro AC50 value (concentration at which 50% of the maximal response was elicited) and is calculated using the IVIVE methods and TK modelling described above (Judson et al., 2011; Rotroff et al., 2010; Wetmore et al., 2012). The IVIVE methods and TK modelling has been demonstrated to provide a conservative estimate of the POD for a chemical (Wetmore et al., 2013, 2015). To account for inter-individual variability of the pharmacokinetic data derived from human samples, Monte calculated is representative of 95% of the population. Ideally, the biokinetic properties of the in vitro system (e.g., evaporation of chemicals or culture medium, binding of chemicals to plastic or proteins, and interaction between the culture medium and the cells) are also measured, modelled, and also incorporated into the IVIVE model in order to improve the ability of the oral equivalent dose derived from in vitro assay data to predict in vivo response (Blaauboer, 2010; Louisse et al., 2010).

The second calculation for MOE, estimated human exposure, can be obtained from various sources, but they are inherently associated with varying degree of uncertainty depending on the sources and the ways data are derived. The use of surrogates such as chemical usage or emission profile while taking into account the physiochemical properties of chemicals may provide a simple and reasonable, though not highly accurate, estimation of potential human exposure (Arnot et al., 2012; Meek et al., 2011). More accurate human exposure estimates can be obtained from biomonitoring data such as the Canadian Health Measures Survey (CHMS) or the US NHANES conducted by Statistics Canada and Centers for Disease Control and Prevention (CDC), respectively. In these cases, human exposure levels can be estimated based on chemical concentrations in blood and urine collected from biomonitoring surveys using reverse dosimetry methods (Tan et al., 2007). If biomonitoring data are not available, high-throughput exposure assessment can be used to predict human exposure potential by integrating chemical use information with production volumes as parameter inputs to the model. When compared with biomonitoring data collected from NHANES, the high-throughput exposure assessment model can estimate human exposure to chemicals with quantifiable uncertainty (Wambaugh et al., 2013). Data generated from the high-throughput exposure assessment models are also useful for chemical prioritisation and decision making.

1.3.2 A specific example looking at utility of the MOE approaches with existing data

An example of the MOE approach is shown in Figure 1. Of the 182 ToxCast chemicals investigated, 18 (9.9%) exhibited oral equivalent doses lower than the estimated human exposure estimates, indicating potential health hazard to the population. When data from general US population were compared against exposure levels, ten of the 18 chemicals had oral equivalent dose ranges that overlapped with human exposure estimates (5.5% of the 182), suggesting that MOE is a useful metric for accurately identifying chemicals of greatest concern (Wetmore et al., 2012). For chemicals with MOE greater than a pre-determined cut off value, no further testing is required, whereas chemicals with MOE below the cut off would proceed to second tier level testing.

Figure 1 MOE can be used as a metric to identify chemicals of greatest concern



Notes: The distribution of the oral equivalent dose ranges required to achieve the upper 95th percentile C_{ss} across all the in vitro ToxCast assays are calculated for a subset of 60 ToxCast phase 1 chemical. The subset of ToxCast phase 1 chemicals are ordered from low to high median oral equivalent dose and depicted in this box-and-whisker plot. Horizontal lines depict the medians oral equivalent dose, the lower and upper edges of the black boxes represent the 25th and 75th percentiles, and the whiskers represent the range of values 1.5 times the interquartile range below or above the 25th and 75th percentiles, respectively. The matching human exposure estimates were obtained with the orange floating boxes representing the range of exposure estimates obtained for various age- and gender-based subpopulations whereas the green circles represent the exposure estimates for the general US population. For some of the chemicals, the exposure estimates fell below the units on the axes and are therefore not shown on the graphs. The MOE is the difference between the oral equivalent dose range and the human exposure estimate as labelled in the graph. Chemicals where any of the human exposure estimates fall within the range of the oral equivalent doses are highlighted with arrows. The graph and associated data are reproduced with permission from Wetmore et al. (2012).

1.3.3 Expanded approaches to account for susceptible populations

The current approach of calculating MOE as a metric to identify chemicals of greatest concern may be underestimated because it is based on healthy adults within a certain age range and does not include susceptible populations. Variability among a population, including difference in exposure pattern and clearance of toxicants, could have a significant impact on the dose range considered acceptable for a given chemical. Individuals of different ethnicity, age, sex, and health status can have very different exposure patterns (due to lifestyle or diet) and clearance rates (due to pharmacogenetic factors) (Ginsberg et al., 2005, 2002), which may contribute to underestimation of

risk to subpopulations. For example, hepatic clearance variability among different subpopulations can be attributed to the difference in expression of metabolising enzymes such as P450s and UGTs (Ginsberg et al., 2004; Punt et al., 2010). To compensate for these differences, recombinant P450s and UGTs isoforms can be used in place of human hepatocytes from healthy donors to determine hepatic metabolic clearance (Wetmore et al., 2014). The use of the expanded dosimetry to include susceptible populations in IVIVE modelling to determine PODs better accounts for population variability in chemical risk assessment process. Zeise et al. (2013) illustrate that population susceptibility comes from variability and that multiple data streams could be used in modelling approaches to address population variability.

1.4 Incorporating the MOE approach into toxicity testing and chemical risk assessment

To demonstrate how the MOE can fit into the current toxicity testing strategy and streamline the overall chemical risk assessment process, a new, data-driven, multi-tiered toxicity testing framework (Figure 2) has been developed (Thomas et al., 2013a). This new framework has evolved from an existing tiered approach formed in response to regulatory policy mandates to assess and prioritise a large number of chemical substances for testing (Meek et al., 2011). Therefore, the adaptation of this new framework into chemical risk assessment process could have broad applications across multiple regulatory agencies in many different countries. This framework can be used to:

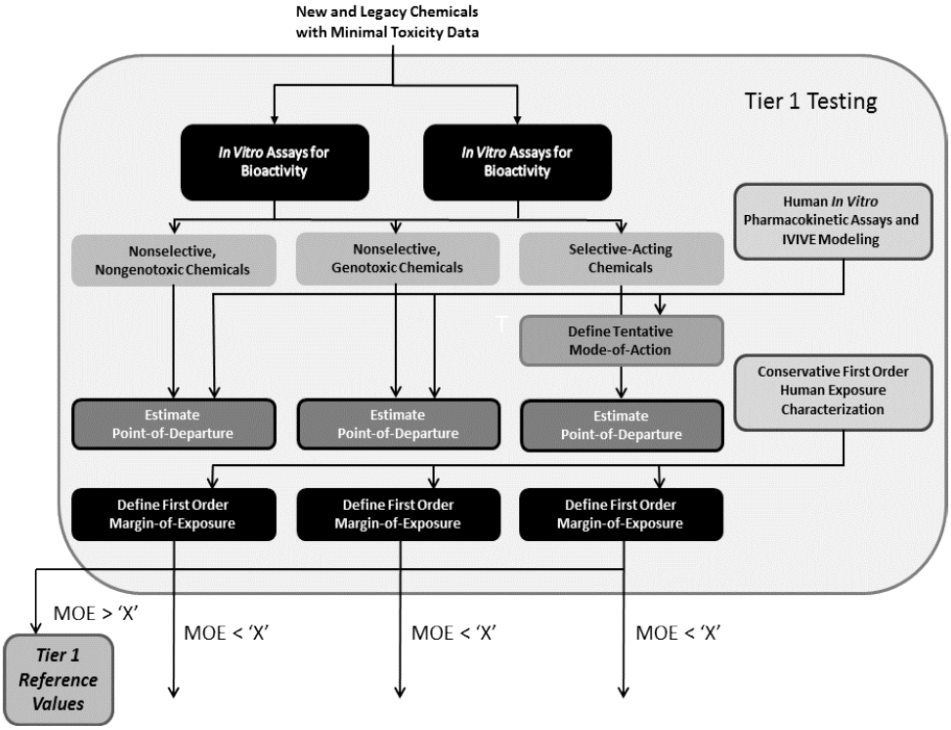
1 categorise and prioritise chemicals on the DSL in Canada, under Canadian Environmental Protection Act, 1999 (Environment Canada, 1999), and chemicals regulated under the Toxic Substances Control Act (TSCA) in the US (US EPA, 1981)

2 identify chemical substances of health and environment concerns under Registration, Evaluation, Authorization and Restriction of Chemicals (REACH) in the European Union (EU) (European Commission, 2006).

The new proposed framework has three tiers to accomplish this task (Thomas et al., 2013a).

The three levels of testing complexity are closely analogous to the NexGen three tiered framework (Cote et al., 2012). The first tier consists of five concise steps and the overall purpose for this tier is to screen and prioritise tens of thousands of chemicals. The first two steps involve the use of HTS assays to sub-categorise chemicals based on their modes-of-action. First, chemicals are subdivided based on whether their MOAs are selective or non-selective. Then they are further subdivided based on whether MOE is genotoxic or not. The remaining three steps involve converting the *in vitro* concentration to an equivalent oral administered dose, determination of chemical exposure and calculation of the MOE. In this framework, the MOE is the primary metric for determining whether a chemical advances to the second tier of testing. Chemicals with MOE greater than some pre-defined cut-off require no further testing, whereas chemicals with MOE below the cut-off would advance on to tier two testing. These triaged chemicals are thus deemed of insufficient concern to justify further testing and become regarded as safe for use in commerce.

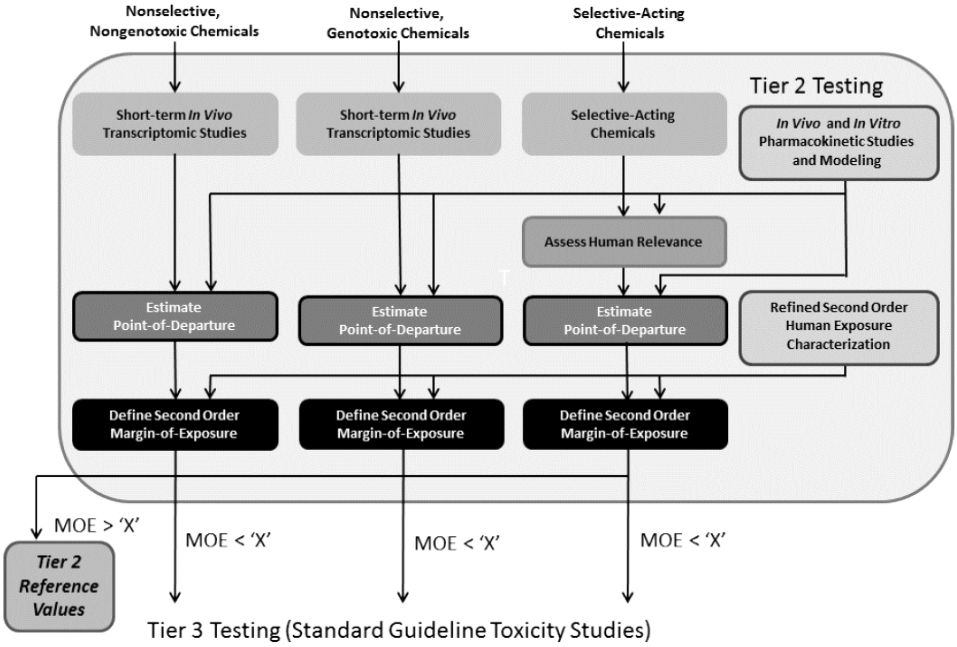
Figure 2 A flowchart outlining tiers 1, 2, and 3 testing in the proposed framework



Notes: New and legacy chemicals with no or minimal toxicity data served as the inputs for the tier 1 testing framework. Chemicals are categorised based on target selectivity and genotoxicity, which are determined by the high-throughput in vitro screening assays and the in vitro genotoxicity assays, respectively. For the selective chemicals, the tentative mode of action is defined based on which high-throughput in vitro assays were selectively activated or inhibited. The points of departure for all chemicals are estimated using in vitro pharmacokinetic assay data and IVIVE modelling. The oral equivalent doses are then compared with human exposure estimates to determine MOE. For chemicals with a MOE greater than a defined cut-off 'X', no further testing would be required and tier 1 reference values would be reported. Chemicals with a MOE less than the cut-off 'X' advance to tier 2 testing. Chemicals categorised in tier 1 testing as selective or non-selective serve as inputs for the tier 2 testing framework and their mode of action are confirmed by the in vivo studies or short term in vivo transcriptomic studies, respectively. PODs for all chemicals are estimated by modelling with data from in vivo and in vitro pharmacokinetic studies, and are compared with refined human exposure characterisation to define MOE. For chemicals with a MOE greater than a defined cut-off 'X', no further testing is performed and tier 2 reference values are published. Chemicals with a MOE less than the cut-off 'X' then move on to tier 3 testing.

Source: Adapted with permission from Thomas et al. (2013a)

Figure 2 A flowchart outlining tiers 1, 2, and 3 testing in the proposed framework (continued)



Notes: New and legacy chemicals with no or minimal toxicity data served as the inputs for the tier 1 testing framework. Chemicals are categorised based on target selectivity and genotoxicity, which are determined by the high-throughput in vitro screening assays and the in vitro genotoxicity assays, respectively. For the selective chemicals, the tentative mode of action is defined based on which high-throughput in vitro assays were selectively activated or inhibited. The points of departure for all chemicals are estimated using in vitro pharmacokinetic assay data and IVIVE modelling. The oral equivalent doses are then compared with human exposure estimates to determine MOE. For chemicals with a MOE greater than a defined cut-off 'X', no further testing would be required and tier 1 reference values would be reported. Chemicals with a MOE less than the cut-off 'X' advance to tier 2 testing. Chemicals categorised in tier 1 testing as selective or non-selective serve as inputs for the tier 2 testing framework and their mode of action are confirmed by the in vivo studies or short term in vivo transcriptomic studies, respectively. PODs for all chemicals are estimated by modelling with data from in vivo and in vitro pharmacokinetic studies, and are compared with refined human exposure characterisation to define MOE. For chemicals with a MOE greater than a defined cut-off 'X', no further testing is performed and tier 2 reference values are published. Chemicals with a MOE less than the cut-off 'X' then move on to tier 3 testing.

Source: Adapted with permission from Thomas et al. (2013a)

The tier 2 testing framework consists of five components, all of which refine the calculations for MOE. For example, more complex in vivo animal systems are used to refine the calculations for POD. These include short-term in vivo transcriptomic studies used to calculate transcriptional POD values for non-selective chemicals and knockout or humanised rodent models to confirm the mode of action of selective chemicals and calculate POD values. Pharmacokinetic studies would be expanded through the collection and chemical analysis of additional samples from the in vivo studies (Saghir et al., 2006)

and the identification of potential metabolites using rodent and human microsomes and S9 (Bonn et al., 2010). The fourth component of tier 2 testing is refining estimates of human exposures where new technologies such as QSARs (Wambaugh et al., 2013), high throughput physicochemical profiling (Kerns, 2001), and in vitro degradation screening method (Hussain et al., 2007) would be utilised to measure both physical-chemical properties and environmental half-lives of all tier 2 chemicals. The end result is a more refined estimation of MOE within tier 2.

Tier 3 testing would be conceptually equivalent to the traditional in vivo studies performed on high-value chemicals with potential for human exposure, or specific in vivo assays based on understanding of the mechanism of action and toxicological profile acquired in tier 1 and 2 testing such as rodent cancer bioassays, developmental, and reproductive toxicity studies. Depending on the MOE cut-off value imposed, it is estimated that only a small portion (3–15 %) of the total chemicals would require these types specialised in vivo testing, whereas majority of the chemicals would be screened out in the preceding tiers. Chemicals that require tier 3 testing would be prioritised based on the results acquired from tier 1 and 2 testing.

2 Conclusions

This paper describes and summarises a novel framework proposed through work conducted by Thomas and various colleagues at the Hamner Institute over a period of about five years for using MOE as a risk-based metric to identify chemicals of greatest concern to human health. This approach incorporates recent new technologies to increase the efficiency and efficacy of the risk assessment process. One of the major challenges in chemical risk assessment is the prioritisation and management of the large number of chemical substances for toxicity testing. One unique approach of this framework is the initial separation of chemicals into specific and non-specific mode of action, which can be efficiently applied to all chemicals. This subsequently allows for determination of PODs by different suites of bioassays based on specificity of the chemicals.

New technologies have been incorporated into all three stages of chemical risk assessment, namely hazard identification, dose-response assessment, and exposure assessment, to increase both the efficiency and accuracy. To compensate for the limited capability for predicting in vivo hazards by in vitro assays as reported previously (Thomas et al., 2012a), new technologies including refined in vitro HTS assays and high content biological omics (e.g., transcriptomic) assays are utilised in the screening process of chemicals for tier 1 and 2 risk assessments, utilising MOE as an indirect measure for potential hazard of a substance. Data derived from transcriptomic assays of the most sensitive pathways have been shown to be highly correlated with the in vivo apical responses (Thomas et al., 2013b).

Information generated from the most sensitive HTS in vitro assays have already been shown to provide a conservative estimate of the dose at which adverse in vivo response may be observed (i.e., POD) for a chemical in a dose-response assessment (Wetmore et al., 2013, 2015). In an effort to further increase the efficiency and effectiveness for dose-response assessment, QSARs may be integrated into HTS in vitro assays as part of the new computational modelling tools to predict toxicological responses and metabolic pathways based on the chemical properties of toxicants and comparison with other active structures (Dix et al., 2007; Kavlock et al., 2008).

Under the proposed framework, IVIVE methods and TK modelling play a critical role in converting in vitro assay concentrations into administered doses to estimate PODs in both tier 1 and 2 testing framework. Furthermore, QSARs and exposomics database are new tools that may be integrated specifically into tier 2 testing to improve the high-throughput exposure modelling method and reduce the uncertainties associated with it for the purpose of improving the overall accuracy of the calculated human exposure estimates. As an alternative to estimate human exposure under the tier 2 testing framework, high throughput biomonitoring studies are proposed to carry out with the aid of new tool like dual-chromatography with Fourier-transform mass spectrometry (DC-FTMS). The increased efficiency and accuracy of exposure assessment by the new technologies could ultimately help provide important context for interpreting toxicity data for prioritising chemicals for further testing (Wetmore et al., 2012).

By integrating new technologies into the framework and incorporating data generated from these technologies into the risk assessment process, the new data-driven framework provides a risk-based, practical, and animal-sparing approach to evaluate chemical risk. Furthermore, the application of MOE as a metric to identify chemicals of greatest concern also offers a near-term solution for making economical, efficient, and health-protective decisions on chemical safety evaluation.

References

Adeleye, Y., Andersen, M., Clewell, R., Davies, M., Dent, M., Edwards, S., Fowler, P., Malcomber, S., Nicol, B., Scott, A., Scott, S., Sun, B., Westmoreland, C., White, A., Zhang, Q. and Carmichael, P.L. (2015) 'Implementing toxicity testing in the 21st century (TT21C): making safety decisions using toxicity pathways, and progress in a prototype risk assessment', *Toxicology*, Vol. 332, pp.102–111.

Andersen, M.E. and Krewski, D. (2010) 'The vision of toxicity testing in the 21st century: moving from discussion to action', *Toxicological Sciences*, Vol. 117, No. 1, pp.17–24.

Andersen, M.E., Clewell, H.J., Carmichael, P.L. and Boekelheide, K. (2011) 'Can case study approaches speed implementation of the NRC report: toxicity testing in the 21st century: a vision and a strategy?', *ALTEX*, Vol. 28, No. 3, pp.175–182.

Ankley, G.T., Bennett, R.S., Erickson, R.J., Hoff, D.J., Hornung, M.W., Johnson, R.D., Mount, D.R., Nichols, J.W., Russom, C.L., Schmieder, P.K., Serrrano, J.A., Tietge, J.E. and Villeneuve, D.L. (2010) 'Adverse outcome pathways: a conceptual framework to support ecotoxicology research and risk assessment', *Environmental Toxicology and Chemistry*, Vol. 29, No. 3, pp.730–741.

Anwar, W.A. (1997) 'Biomarkers of human exposure to pesticides', *Environmental Health Perspectives*, Vol. 105, No. 4, pp.801–806.

Arnot, J.A., Brown, T.N., Wania, F., Breivik, K. and McLachlan, M.S. (2012) 'Prioritizing chemicals and data requirements for screening-level exposure and risk assessment', *Environmental Health Perspectives*, Vol. 120, No. 11, pp.1565–1570.

Blaauboer, B.J. (2010) 'Biokinetic modeling and in vitro-in vivo extrapolations', *J Toxicology and Environmentall Health B Critical Reviews*, Vol. 13, Nos. 2/4, pp.242–252.

Bonn, B., Leandersson, C., Fontaine, F. and Zamora, I. (2010) 'Enhanced metabolite identification with MS(E) and a semi-automated software for structural elucidation', *Rapid Communication Mass Spectrometry*, Vol. 24, No. 21, pp.3127–3138.

Caba, E. and Aubrecht, J. (2006) 'Genomic approaches for investigating mechanisms of genotoxicity', *Toxicology Mechanisms and Methods*, Vol. 16, Nos. 2/3, pp.69–77.

Clewell III, H.J. and Andersen, M.E. (1985) 'Risk assessment extrapolations and physiological modeling', *Toxicology and Industrial Health*, Vol. 1, No. 4, pp.111–131.

Clewell III, H.J. and Andersen, M.E. (1996) 'Use of physiologically based pharmacokinetic modeling to investigate individual versus population risk', *Toxicology*, Vol. 111, Nos. 1/3, pp.315–329.

Clewell, H.J., McMullen, P.D., Adeleye, Y., Carmichael, P.L. and Andersen, M.E. (2015) 'Pathway based toxicology and fit-for-purpose assays', in Eskes, M.C.W. (Ed.): *Validating Alternative Methods for Toxicity Testing*, Springer International Publishing AG, Cha.

Cohen Hubal, E.A., Richard, A.M., Shah, I., Gallagher, J., Kavlock, R., Blancato, J. and Edwards, S.W. (2010) 'Exposure science and the U.S. EPA national center for computational toxicology', *J Exposure Science and Environment Epidemiology*, Vol. 20, No. 3, pp.231–236.

Cote, I., Anastas, P.T., Birnbaum, L.S., Clark, R.M., Dix, D.J., Edwards, S.W. and Preuss, P.W. (2012) 'Advancing the next generation of health risk assessment', *Environmental Health Perspectives*, Vol. 120, No. 11, pp.1499–1502.

Dix, D.J., Houck, K.A., Martin, M.T., Richard, A.M., Setzer, R.W. and Kavlock, R.J. (2007) 'The ToxCast program for prioritizing toxicity testing of environmental chemicals', *Toxicological Sciences*, Vol. 95, No. 1, pp.5–12.

Environment Canada (1999) *Canadian Environmental Protection Act, 1999*, Environment Canada, Ottawa.

Erickson, H.S. (2012) 'Measuring molecular biomarkers in epidemiologic studies: laboratory techniques and biospecimen considerations', *Statistics in Medicine*, Vol. 31, No. 22, pp.2400–2413.

European Commission (2006) 'Proposal concerning the registration, evaluation, authorisation and restriction of chemicals (REACH)', *Official J European Union L396*, Vol. 49, pp.1–849.

Garcia-Closas, M., Vermeulen, R., Cox, D., Lan, Q., Caporaso, N.E. and Rothman, N. (2011) *Population-Based Study Designs in Molecular Epidemiology*, pp.241–259, IARC Scientific Publication.

Gephart, L.A., Salminen, W.F., Nicolich, M.J. and Pelekis, M. (2001) 'Evaluation of subchronic toxicity data using the benchmark dose approach', *Regulatory and Toxicology Pharmacology*, Vol. 33, No. 1, pp.37–59.

Ginsberg, G., Hattis, D., Russ, A. and Sonawane, B. (2004) 'Physiologically based pharmacokinetic (PBPK) modeling of caffeine and theophylline in neonates and adults: implications for assessing children's risks from environmental agents', *J Toxicology and Environmental Health A*, Vol. 67, No. 4, pp.297–329.

Ginsberg, G., Hattis, D., Russ, A. and Sonawane, B. (2005) 'Pharmacokinetic and pharmacodynamic factors that can affect sensitivity to neurotoxic sequelae in elderly individuals', *Environmental Health Perspectives*, Vol. 113, No. 9, pp.1243–1249.

Ginsberg, G., Hattis, D., Sonawane, B., Russ, A., Banati, P., Kozlak, M., Smolenski, S. and Goble, R. (2002) 'Evaluation of child/adult pharmacokinetic differences from a database derived from the therapeutic drug literature', *Toxicological Sciences*, Vol. 66, No. 2, pp.185–200.

Hartung, T. (2010) 'Food for thought … on alternative methods for chemical safety testing', *ALTEX*, Vol. 27, No. 1, pp.3–14.

Hartung, T. and McBride, M. (2011) 'Food for thought … on mapping the human toxome', *ALTEX*, Vol. 28, No. 2, pp.83–93.

Huang, R., Sakamuru, S., Martin, M.T., Reif, D.M., Judson, R.S., Houck, K.A., Casey, W., Hsieh, J.H., Shockley, K.R., Ceger, P., Fostel, J., Witt, K.L., Tong, W., Rotroff, D.M., Zhao, T., Shinn, P., Simeonov, A., Dix, D.J., Austin, C.P., Kavlock, R.J., Tice, R.R. and Xia, M. (2014) 'Profiling of the Tox21 10K compound library for agonists and antagonists of the estrogen receptor alpha signaling pathway', *Scientific Reports*, Vol. 4, pp.5664.

Hussain, S., Arshad, M., Saleem, M. and Zahir, Z. A. (2007) 'Screening of soil fungi for in vitro degradation of endosulfan', *World J Microbiology and Biotechnology*, Vol. 23, No. 7, pp.939–945.

Judson, R.S., Kavlock, R.J., Setzer, R.W., Hubal, E.A., Martin, M.T., Knudsen, T.B., Houck, K.A., Thomas, R.S., Wetmore, B.A. and Dix, D.J. (2011) 'Estimating toxicity-related biological pathway altering doses for high-throughput chemical risk assessment', *Chemical Research in Toxicology*, Vol. 24, No. 4, pp.451–462.

Kavlock, R. and Dix, D. (2010) 'Computational toxicology as implemented by the U.S. EPA: providing high throughput decision support tools for screening and assessing chemical exposure, hazard and risk', *J Toxicology and Environmental Health B Critical Reviews*, Vol. 13, Nos. 2/4, pp.197–217.

Kavlock, R., Chandler, K., Houck, K., Hunter, S., Judson, R., Kleinstreuer, N., Knudsen, T., Martin, M., Padilla, S., Reif, D., Richard, A., Rotroff, D., Sipes, N. and Dix, D. (2012) 'Update on EPA's ToxCast program: providing high throughput decision support tools for chemical risk management', *Chemical Research in Toxicology*, Vol. 25, No. 7, pp.1287–1302.

Kavlock, R.J., Ankley, G., Blancato, J., Breen, M., Conolly, R., Dix, D., Houck, K., Hubal, E., Judson, R., Rabinowitz, J., Richard, A., Setzer, R.W., Shah, I., Villeneuve, D. and Weber, E. (2008) 'Computational toxicology--a state of the science mini review', *Toxicological Sciences*, Vol. 103, No. 1, pp.14–27.

Kerns, E.H. (2001) 'High throughput physicochemical profiling for drug discovery', *J Pharmceutical Sciences*, Vol. 90, No. 11, pp.1838–1858.

Knight, A.W., Little, S., Houck, K., Dix, D., Judson, R., Richard, A., McCarroll, N., Akerman, G., Yang, C., Birrell, L. and Walmsley, R.M. (2009) 'Evaluation of high-throughput genotoxicity assays used in profiling the US EPA ToxCast chemicals', *Regulatory and Toxicology Pharmacology*, Vol. 55, No. 2, pp.188–199.

Krewski, D., Andersen, M.E., Mantus, E. and Zeise, L. (2009) 'Toxicity testing in the 21st century: implications for human health risk assessment', *Risk Analysis*, Vol. 29, No. 4, pp.474–479.

Krewski, D., Westphal, M., Al-Zoughool, M., Croteau, M.C. and Andersen, M.E. (2011) 'New directions in toxicity testing', *Annual Review Public Health*, Vol. 32, pp.161–178.

Krewski, D., Westphal, M., Andersen, M.E., Paoli, G.M., Chiu, W.A., Al-Zoughool, M., Croteau, M.C., Burgoon, L.D. and Cote, I. (2014) 'A framework for the next generation of risk science', *Environtal Health Perspectoves*, Vol. 122, No. 8, pp.796–805.

Louisse, J., de Jong, E., van de Sandt, J.J.M., Blaauboer, B.J., Woutersen, R.A., Piersma, A.H., Rietjens, I.M.C.M. and Verwei, M. (2010) 'The use of in vitro toxicity data and physiologically based kinetic modeling to predict dose-response curves for in vivo developmental toxicity of glycol ethers in rat and man', *Toxicological Sciences*, Vol. 118, No. 2, pp.470–484.

Mastellos, D., Andronis, C., Persidis, A. and Lambris, J. D. (2005) 'Novel biological networks modulated by complement', *Clinical Immunology*, Vol. 115, No. 3, pp.225–235.

Meek, M.E., Boobis, A.R., Crofton, K.M., Heinemeyer, G., Raaij, M.V. and Vickers, C. (2011) 'Risk assessment of combined exposure to multiple chemicals: a WHO/IPCS framework', *Regulatory and Toxicology Pharmacology*, Vol. 60, No. 2, pp.S1–14.

Moore, L.E., Karami, S., Steinmaus, C. and Cantor, K.P. (2013) 'Use of OMIC technologies to study arsenic exposure in human populations', *Environmental and Molecular Mutagensis*, Vol. 54, No. 7, pp.589–595.

National Research Council (NRC) (2007) *Toxicity Testing in the 21st Century: A Vision and a Strategy*, The National Academies Press, Washington, DC.

National Research Council (US): Committee on the Institutional Means for Assessment of Risks to Public Health (1983) *Risk Assessment in the Federal Government: Managing the Process*, National Academy Press, Washington, DC.

O'Bryan, T.R. and Ross, R.H. (1988) 'Chemical scoring system for hazard and exposure identification', *J Toxicology and Environmental Health*, Vol. 25, No. 1, pp.119–134.

Olden, K., Isaac, L. and Roberts, L. (2011) 'Neighborhood-specific epigenome analysis: the pathway forward to understanding gene-environment interactions', *North Carolina Medical Journal*, Vol. 72, No. 2, pp.125–127.

Poulin, P. and Theil, F.P. (2002) 'Prediction of pharmacokinetics prior to in vivo studies. II. Generic physiologically based pharmacokinetic models of drug disposition', *J Pharmceutical Sciences*, Vol. 91, No. 5, pp.1358–1370.

Punt, A., Jeurissen, S.M., Boersma, M.G., Delatour, T., Scholz, G., Schilter, B., van Bladeren, P.J., and Rietjens, I.M. (2010) 'Evaluation of human interindividual variation in bioactivation of estragole using physiologically based biokinetic modeling', *Toxicological Sciences*, Vol. 113, No. 2, pp.337–348.

Rietjens, I.M., Punt, A., Schilter, B., Scholz, G., Delatour, T. and van Bladeren, P.J. (2010) 'In silico methods for physiologically based biokinetic models describing bioactivation and detoxification of coumarin and estragole: implications for risk assessment', *Molecular Nutrition and Food Research*, Vol. 54, No. 2, pp.195–207.

Rotroff, D.M., Wetmore, B.A., Dix, D.J., Ferguson, S.S., Clewell, H.J., Houck, K.A., Lecluyse, E.L., Andersen, M.E., Judson, R.S., Smith, C.M., Sochaski, M.A., Kavlock, R.J., Boellmann, F., Martin, M.T., Reif, D.M., Wambaugh, J.F., Thomas, R.S. (2010) 'Incorporating human dosimetry and exposure into high-throughput in vitro toxicity screening', *Toxicological Sciences*, Vol. 117, No. 2, pp.348–358.

Saghir, S.A., Mendrala, A.L., Bartels, M.J., Day, S.J., Hansen, S.C., Sushynski, J.M. and Bus, J.S. (2006) 'Strategies to assess systemic exposure of chemicals in subchronic/chronic diet and drinking water studies', *Toxicology and Applied Pharmacology*, Vol. 211, No. 3, pp.245–260.

Sui, W., Hou, X., Che, W., Yang, M. and Dai, Y. (2013) 'The applied basic research of systemic lupus erythematosus based on the biological omics', *Genes and Immunity*, Vol. 14, No. 3, pp.133–146.

Tan, Y.M., Liao, K.H. and Clewell 3rd, H.J. (2007) 'Reverse dosimetry: interpreting trihalomethanes biomonitoring data using physiologically based pharmacokinetic modeling', *J Exposure Science and Environment Epidemiology*, Vol. 17, No. 7, pp.591–603.

Thomas, R.S., Black, M.B., Li, L., Healy, E., Chu, T.M., Bao, W., Andersen, M.E. and Wolfinger, R.D. (2012a) 'A comprehensive statistical analysis of predicting in vivo hazard using high-throughput in vitro screening', *Toxicological Sciences*, Vol. 128, No. 2, pp.398–417.

Thomas, R.S., Clewell 3rd, H.J., Allen, B.C., Yang, L., Healy, E. and Andersen, M.E. (2012b) 'Integrating pathway-based transcriptomic data into quantitative chemical risk assessment: a five chemical case study', *Mutation Research*, Vol. 746, No. 2, pp.135–143.

Thomas, R.S., Philbert, M.A., Auerbach, S.S., Wetmore, B.A., Devito, M.J., Cote, I., Rowlands, J.C., Whelan, M.P., Hays, S.M., Andersen, M.E., Meek, M.E., Reiter, L.W., Lambert, J.C., Clewell 3rd, H.J., Stephens, M.L., Zhao, Q.J., Wesselkamper, S.C., Flowers, L., Carney, E.W., Pastoor, T.P., Petersen, D.D., Yauk, C.L. and Nong, A. (2013a) 'Incorporating new technologies into toxicity testing and risk assessment: moving from 21st century vision to a data-driven framework', *Toxicological Sciences*, Vol. 136, No. 1, pp.4–18.

Thomas, R.S., Wesselkamper, S.C., Wang, N.C., Zhao, Q.J., Petersen, D.D., Lambert, J.C., Cote, I., Yang, L., Healy, E., Black, M.B., Clewell 3rd, H.J., Allen, B.C. and Andersen, M.E. (2013b) 'Temporal concordance between apical and transcriptional points of departure for chemical risk assessment', *Toxicological Sciences*, Vol. 134, No. 1, pp.180–194.

Tice, R.R., Austin, C.P., Kavlock, R.J. and Bucher, J.R. (2013) 'Improving the human hazard characterization of chemicals: a Tox21 update', *Environmental Health Perspectives*, Vol. 121, No. 7, pp.756–765.

United States Environmental Protection Agency (US EPA) (1981) *Toxic Substances Control Act (TSCA): Report to Congress for Fiscal Year*, United States Environmental Protection Agency, Washington, DC.

United States Environmental Protection Agency (US EPA) (2003) *A Framework for A Computational Toxicological Research Program in ORD* [online] http://www.epa.gov/comptox/download…/comptoxframework06_02_04.pdf (accessed 25 October 2015).

Valcke, M. and Krishnan, K. (2010) 'An assessment of the interindividual variability of internal dosimetry during multi-route exposure to drinking water contaminants', *Int J Environmental Research and Public Health*, Vol. 7, No. 11, pp.4002–4022.

Walton, K., Dorne, J.L. and Renwick, A.G. (2001) 'Uncertainty factors for chemical risk assessment: interspecies differences in the in vivo pharmacokinetics and metabolism of human CYP1A2 substrates', *Food and Chemical Toxicology*, Vol. 39, No. 7, pp.667–680.

Wambaugh, J.F., Setzer, R.W., Reif, D.M., Gangwal, S., Mitchell-Blackwood, J., Arnot, J.A., Joliet, O., Frame, A., Rabinowitz, J., Knudsen, T.B., Judson, R.S., Egeghy, P., Vallero, D., Cohen Hubal, E.A. (2013) 'High-throughput models for exposure-based chemical prioritization in the ExpoCast project', *Environmental Science and Technology*, Vol. 47, No. 15, pp.8479–8488.

Wambaugh, J.F., Wang, A., Dionisio, K.L., Frame, A., Egeghy, P., Judson, R. and Setzer, R.W. (2014) 'High throughput heuristics for prioritizing human exposure to environmental chemicals', *Environmental Science and Technology*, Vol. 48, No. 21, pp.12760–12767.

Wetmore, B.A., Allen, B., Clewell 3rd, H.J., Parker, T., Wambaugh, J.F., Almond, L.M., Sochaski, M.A. and Thomas, R.S. (2014) 'Incorporating population variability and susceptible subpopulations into dosimetry for high-throughput toxicity testing', *Toxicological Sciences*, Vol. 142, No. 1, pp.210–224.

Wetmore, B.A., Wambaugh, J.F., Allen, B., Ferguson, S.S., Sochaski, M.A., Setzer, R.W., Houck, K.A., Strope, C.L., Cantwell, K. and Judson, R.S. (2015) 'Incorporating high-throughput exposure predictions with dosimetry-adjusted in vitro bioactivity to inform chemical toxicity testing', *Toxicological Sciences*, Vol. 148, No. 1, pp.121–136.

Wetmore, B.A., Wambaugh, J.F., Ferguson, S.S., Li, L., Clewell 3rd, H.J., Judson, R.S., Freeman, K., Bao, W., Sochaski, M.A., Chu, T.M., Black, M.B., Healy, E., Allen, B., Andersen, M.E., Wolfinger, R.D. and Thomas, R.S. (2013) 'Relative impact of incorporating pharmacokinetics on predicting in vivo hazard and mode of action from high-throughput in vitro toxicity assays', *Toxicological Sciences*, Vol. 132, No. 2, pp.327–346.

Wetmore, B.A., Wambaugh, J.F., Ferguson, S.S., Sochaski, M.A., Rotroff, D.M., Freeman, K., Clewell 3rd, H.J., Dix, D.J., Andersen, M.E., Houck, K.A., Allen, B., Judson, R.S., Singh, R., Kavlock, R.J., Richard, A.M. and Thomas, R.S. (2012) 'Integration of dosimetry, exposure, and high-throughput screening data in chemical toxicity assessment', *Toxicological Sciences*, Vol. 125, No. 1, pp.157–174.

Zeise, L., Bois, F.Y., Chiu, W.A., Hattis, D., Rusyn, I. and Guyton, K.Z. (2013) 'Addressing human variability in next-generation human health risk assessments of environmental chemicals', *Environmental Health Perspectives*, Vol. 121, No. 1, pp.23–31.

Zhang, Q., Bhattacharya, S. and Andersen, M.E. (2013) 'Ultrasensitive response motifs: basic amplifiers in molecular signalling networks', *Open Biology*, Vol. 3, No. 4, pp.130031.

Zhang, Q., Bhattacharya, S., Andersen, M.E. and Conolly, R.B. (2010) 'Computational systems biology and dose-response modeling in relation to new directions in toxicity testing', *J Toxicology and Environmental Health B Critical Reviews*, Vol. 13, Nos. 2/4, pp.253–276.

Zhang, Q., Bhattacharya, S., Conolly, R.B., Clewell, H.J., Kaminski, N.E. and Andersen, M.E. (2014) 'Molecular signaling network motifs provide a mechanistic basis for cellular threshold responses', *Environmental Health Perspectives*, Vol. 122, No. 12, pp.1261–1270.

Zhang, Q., Bhattacharya, S., Pi, J., Clewell, R.A., Carmichael, P.L. and Andersen, M.E. (2015) 'Adaptive posttranslational control of cellular stress pathways in relation to toxicity testing and safety assessment', *Toxicological Sciences*, Vol. 147, No. 2, pp.302–316.

Zhao, Z., Zhang, B., Huang, Y., Xu, H. and McDermott, J.E. (2014) 'Computational methods for omics data', *Int J Computational Biology and Drug Design*, Vol. 7, Nos. 2/4, pp.97–101.