Integrated Environmental Assessment and Management — Volume 13, Number 5—pp. 915–925
Received: 20 July 2016 | Returned for Revision: 26 September 2016 | Accepted: 7 February 2017

Environmental Policy & Regulation

Advancing Alternatives Analysis: The Role of Predictive Toxicology in Selecting Safer Chemical Products and Processes

Timothy Malloy,†‡§ Virginia Zaunbrecher,†‡ Elizabeth Beryt,§ Richard Judson,‖ Raymond Tice,# Patrick Allard,‡†† Ann Blake,‡‡ Ila Cote,§§ Hilary Godwin,‡§ Lauren Heine,‖‖ Patrick Kerzic,## Jakub Kostal,††† Gary Marchant,‡‡‡ Jennifer McPartland,§§§ Kelly Moran,‖‖‖ Andre Nel,§ Oladele Ogunseitan,### Mark Rossi,†††† Kristina Thayer,# Joel Tickner,‡‡‡‡ Margaret Whittaker,§§§§ and Ken Zarker‖‖‖‖‖‖‖

†School of Law, University of California Los Angeles (UCLA), Los Angeles, California, USA
‡Fielding School of Public Health, UCLA, Los Angeles, California, USA
§UC Center for the Environmental Implications of Nanotechnology, UCLA, Los Angeles, California, USA
‖National Center for Computational Toxicology, Research Triangle Park, North Carolina, USA
#National Toxicology Program, Durham, North Carolina, USA
††Institute for Society & Genetics, UCLA, Los Angeles, California, USA
‡‡Environmental and Public Health Consulting, Alameda, California, USA
§§US Environmental Protection Agency, Washington, DC
‖‖‖Northwest Green Chemistry, Juneau, Alaska, USA
##California Department of Toxic Substances Control, Chatsworth, California, USA
†††Computational Biology Institute at the George Washington University, Ashburn, Virginia, USA
‡‡‡Sandra Day O'Connor School of Law, Arizona State University, Tempe, Arizona, USA
§§§Environmental Defense Fund, Washington, DC, USA
‖‖‖‖TDC Environmental, San Mateo, California, USA
###School of Public Health, University of California Irvine (UCI), Irvine, California, USA
††††Clean Production Action, Somerville, Massachusetts, USA
‡‡‡‡University of Massachusetts, Lowell, Massachusetts, USA
§§§§ToxServices, Washington, DC, USA
‖‖‖‖‖‖‖Washington State Department of Ecology, Olympia, Washington, USA

ABSTRACT

Alternatives analysis (AA) is a method used in regulation and product design to identify, assess, and evaluate the safety and viability of potential substitutes for hazardous chemicals. It requires toxicological data for the existing chemical and potential alternatives. Predictive toxicology uses in silico and in vitro approaches, computational models, and other tools to expedite toxicological data generation in a more cost-effective manner than traditional approaches. The present article briefly reviews the challenges associated with using predictive toxicology in regulatory AA, then presents 4 recommendations for its advancement. It recommends using case studies to advance the integration of predictive toxicology into AA, adopting a stepwise process to employing predictive toxicology in AA beginning with prioritization of chemicals of concern, leveraging existing resources to advance the integration of predictive toxicology into the practice of AA, and supporting transdisciplinary efforts. The further incorporation of predictive toxicology into AA would advance the ability of companies and regulators to select alternatives to harmful ingredients, and potentially increase the use of predictive toxicology in regulation more broadly. *Integr Environ Assess Manag* 2017;13:915–925. © 2017 SETAC

Keywords: Alternatives analysis Alternatives assessment Predictive toxicology Regulation Alternative testing strategies

INTRODUCTION

Chemical regulation generally focuses on risk assessment and risk management. It assumes toxic chemicals will be used and seeks to mitigate their harmful impact through controls (Malloy 2014). The last few years have brought increasingly insistent calls for a "prevention-based" approach to addressing environmental and human exposures to toxic chemicals (DHHS 2010; NCCELC 2011). Unlike risk management, the prevention-based approach seeks to minimize the use of toxic chemicals through adoption of safer, viable alternative chemicals or processes (Cummings and Kuzma 2017). With limited exceptions, the preventive approach has lingered in the periphery of regulatory programs and private environmental management for decades. The US Environmental

* Address correspondence to malloy@law.ucla.edu
Published 1 March 2017 on wileyonlinelibrary.com/journal/ieam.

Protection Agency (USEPA) pioneered early efforts in its rulemaking under Section 6 of the Toxic Substances Control Act (US Congress 1976) and in its Design for the Environment (DfE) program (Whittaker 2015). More recently, policymakers in Europe and individual American states have embraced mandatory and voluntary prevention-based approaches to chemical policy. The European Union (EU) adopted this approach through its Registration, Evaluation, Authorisation, and Restriction of Chemicals (REACH) regulation (OJEU 2007). California, USA, is employing the approach in its Safer Consumer Products (SCP) regulations (CCR 2013).

Prevention-based regulations such as REACH and California's SCP program require manufacturers seeking to use certain chemicals of concern to evaluate safer, viable alternatives by performing "alternatives analyses." In chemical regulation, alternatives analysis (AA), also known as "alternatives assessment," is a method used to determine the relative safety and viability of potential alternatives to existing products or processes that use hazardous chemicals (Malloy et al. 2013; NRC 2014). Alternatives may include chemical substitutes, changes to manufacturing operations, and/or changes to product design (Sinsheimer et al. 2007). Alternatives analysis is a 2-part process. In the first part, the methodology identifies potential alternatives and assembles information regarding the performance of the regulated chemical and those alternatives across relevant attributes, typically including public health impacts, environmental effects, technical performance, and economic impacts on the manufacturer and the consumer. The second step identifies and evaluates trade-offs between the original product and its alternatives (Malloy et al. 2013).

Government agencies, businesses, nongovernmental organizations, and academics have used various AA frameworks and tools in voluntary initiatives for some time (Edwards et al. 2011; Rossi et al. 2012; ICC 2013), although their use in mandatory chemical regulation is new. Recent evaluations of these tools identified gaps in the existing approaches, including absence of a decision-making framework, lack of sufficient toxicology data regarding alternatives, and failure of certain approaches to assess exposure (NRC 2014). Although many of these challenges and considerations are also present in conventional risk assessment, they arise in AA in ways that are unique to the prevention-based setting.

Alternatives assessment involves assessment of a variety of hazards associated with human and environmental exposures to chemicals. There are significant gaps in toxicity information for the vast majority of chemicals or potential exposure pathways. Traditional toxicological testing relies heavily upon in vivo (whole-animal) studies mostly in mammals, particularly with respect to carcinogenicity, reproductive toxicity, and other complex endpoints (Ellinger-Ziegelbauer et al. 2008; Ferreira et al. 2014). It assumes chemicals that injure animals may have similar impacts on humans. Heavy reliance upon animal testing has come under increasing scrutiny. There are scientific concerns regarding the accuracy of such testing in predicting human outcomes (NRC 2007). Other concerns are based upon its high cost and time-consuming nature (NRC 2007) and animal welfare issues (Bakand et al. 2005).

The problem of data gaps is exacerbated in the AA context in which multiple chemicals contained in various alternatives must be characterized and compared. Predictive toxicological methods offer the potential for obtaining the necessary toxicity and exposure estimates in substantially less time and at significantly less cost conventional methods. Rather than relying upon conventional in vitro assays and whole-animal studies, the emerging field of predictive toxicology uses high-throughput screening (HTS) in vitro and in vivo assays, knowledge of the mechanisms of toxicity, and advanced computational methods ("in silico" toxicology) to evaluate toxicity. This natural complementarity led a recent report on AA by the National Academy of Sciences to suggest that incorporating predictive toxicology into future AA frameworks is critical (NRC 2014). Figure 1 illustrates the ways in which predictive toxicology may be useful in various steps of the assessment process in a typical AA.

The present article suggests the next steps for integrating predictive toxicology and AA. First, it provides general background on the types of predictive toxicological approaches relevant to AA; this is not intended as a thorough review of predictive toxicology. Second, it identifies the challenges and benefits presented by predictive toxicological tests in AA. Third, it offers recommendations for next steps.

OUTLINING PREDICTIVE TOXICOLOGICAL TECHNOLOGIES

Predictive toxicology encompasses a broad range of methods that can generate differing levels of information. Many toxicological methods are predictive. But there have been recent developments in toxicology, specifically with regard to in vitro assays and computer models that offer the opportunity to provide more efficient and less expensive

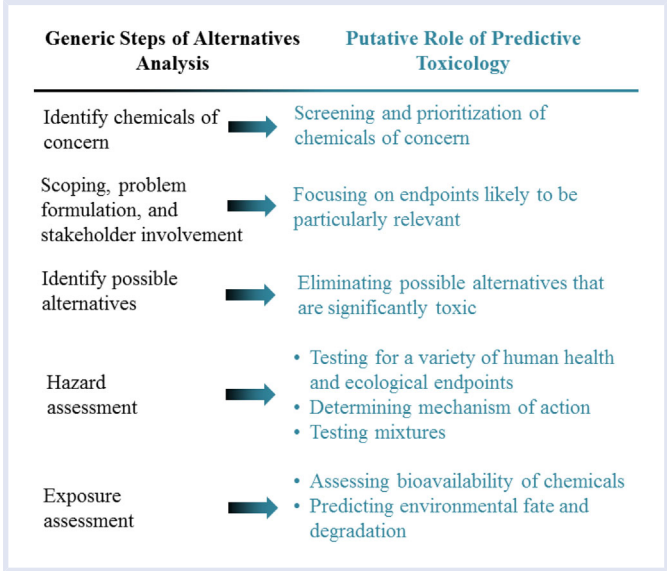

Figure 1. Uses of predictive toxicology in alternatives analysis.

ways to evaluate various chemicals or products than do conventional testing techniques. Generally speaking, predictive toxicology focuses upon the toxicity mechanism, following it from the initial interaction between a chemical with a biological target and ultimately leading to a whole-organism adverse outcome (AO).

There are several conceptual frameworks for defining the path from the so-called "molecular initiating event" (MIE) to the AO, of which the one most widely used is the adverse outcome pathway (AOP) approach (Ankley et al. 2010; Villaneuve et al. 2014a, 2014b). An AOP always includes an MIE and an AO, plus one or more intermediate key events (KEs). The identified MIE and KEs are probed using in vitro or model organism assays or chemical structure-based computer models. The results of these assays or models can therefore be predictive of whether that outcome or disease may develop via the particular injury mechanism defined by the AOP. Predictive toxicology tools exist for both human health and environmental endpoints. They stand in contrast to conventional toxicology methods, which have primarily focused on detecting the apical AO in in vivo (whole-animal) studies. Those effects and the doses at which the AOs occur are then extrapolated to characterize toxicity in humans or wildlife.

During the last decade, many predictive toxicology tools were developed for use in traditional risk assessment in the United States, Canada, and Europe. However, predictive toxicology tools can also be applied in the AA setting and can provide hazard or risk information with the possibility of comparative analysis across materials. There are 4 general types of predictive technology approaches that could be used in an AA:

1. grouping,
2. high-throughput in vitro assays,
3. in silico modeling, and
4. nontraditional in vivo testing.

Grouping

Grouping is the arrangement of chemicals or substances into groups on the basis of common attributes, such as human health or ecological endpoints (e.g., carcinogenicity, aquatic toxicity) or physicochemical features (e.g., the shape of nanoparticles, existence of a particular functional group) (OECD 2014). Grouping can include identifying individual chemical analogues or creating larger chemical categories. The underlying principle is relatively straightforward: "Similar" chemicals will exhibit "similar" activity such that the activity of one or more members of a group is predictive of the activity of other members of the group (Jaworska and Nikolova-Jeliazkova 2007; Enoch and Roberts 2013). The USEPA has relied upon chemical grouping in AAs performed in its DfE program (USEPA 2014). The EU's REACH program provides for the use of grouping in its guidance on AA (ECHA 2011a).

The "gap-filling" methods for estimating properties or activity for a "data-poor" chemical based on one or more similar "data-rich" chemicals vary (Cronin 2013). "Read across" is a qualitative assessment of toxicity that is based upon expert judgment regarding the similarity with the other chemical or chemicals and likely activity (OECD 2014). Examples of read-across approaches include qualitative structure activity relationships (SARs) analysis, structural alerts, and expert systems. Trend analysis is a statistical technique used to determine whether a series of observations (in this case, toxicity or some other activity) form some pattern such as an upward trend across the group of chemicals (Dimitrov and Mekenyan 2010).

High-throughput in vitro assays

In vitro focuses on the interactions and effects of chemicals upon cells, cell lines, or biological molecules (such as proteins), preferably but not always of human origin, rather than using whole animals. Recent reports by the National Academy of Sciences and European authorities envision increased reliance on mechanistically based in vitro testing (NRC 2007). Researchers introduce chemicals of interest into the testing medium and observe changes in biologic processes that may lead to toxicity to evaluate whether the tested material is implicated in the initiation or progression of an AOP. One can design in vitro tests that probe molecular events in an AOP. This provides the scientific support linking the results of a simple in vitro test to a potential AO. In vitro assays can also provide information regarding the relative potency of materials compared to reference materials or alternatives. In vivo potency is modulated by toxicokinetics and/or pharmacokinetics or by administration, distribution, metabolism, excretion (ADME) processes, which can enhance or even reverse the relative potency observed in vitro (Rotroff et al. 2010).

Data from in vitro testing has been used in AA for a range of hazard endpoints. The USEPA's DfE program relies upon in vitro data in assessing mutagenicity and endocrine activity (USEPA 2011). The REACH program provides that "scientifically validated in vitro tests" may fully or partly replace animal testing where the information generated in the in vitro assay is adequate for the regulatory use in question (ECHA 2011a, 2011b).

The value of mechanistically based in vitro assays can be vastly expanded through HTS; HTS allows researchers to use advanced robotics and automation to simultaneously test thousands of materials across a range of concentrations for a variety of parameters (NRC 2007). The HTS methods generate a wealth of data that requires specialized tools and strategies for sorting it, separating relevant information from noise and artifact, and organizing it to facilitate analysis (Cohen et al. 2013; Liu et al. 2013). Concentration–response curves for the tested materials allow for comparison of the relative potency of the materials in AA (Parham et al. 2009). High-throughput screening data can be employed for rapid response assessment. For example, in 2010, USEPA's Office of Research and Development used HTS data regarding

 DOI: 10.1002/ieam.1923

endocrine disruption and other biological activity to compare 8 oil spill dispersants in connection with the Deepwater Horizon oil spill (Judson, Martin et al. 2010). High-throughput screening data can be used to generate quantitative structure-activity relationship (QSAR) models, discussed immediately below.

In silico modeling

In silico alternatives rely upon computational techniques that use the structure or other features of a chemical to assess its toxicity or fate (Madden 2010). These approaches include computer-assisted expert systems, modeling approaches such as QSAR, toxicokinetic models, fate and transport models, and the creation of virtual cells, tissues, and organs (Hartung and Hoffman 2009; Shah and Wambaugh 2010). The most prominent in silico approach is QSAR analysis, which uses mathematical models to relate the activity or potency of a set of chemicals to their physicochemical properties or other descriptors so as to generate predictions of toxicological data for a target chemical (Shah and Wambaugh 2010; Patlewicz et al. 2013). Development of a robust QSAR requires rich physicochemical and toxicological data for a large enough set of chemicals. For some endpoints, such as bacterial mutagenicity, aquatic toxicity, and skin or eye irritation, sufficient information exists, and QSARs are well established for many chemical classes (Patlewicz et al. 2013). Well-accepted QSARs are lacking for many other endpoints such as carcinogenicity, repeated dose toxicity, and developmental toxicity (Cronin 2010).

Quantitative structure-activity relationships are used in existing AA frameworks. For example, USEPA's DfE program relies upon USEPA's ECOlogical Structure–Activity Relationship Model (ECOSAR) model to predict aquatic toxicity in the absence of data on the chemical (Mayo-Bean et al. 2012). That program also contemplates the use of QSARs with respect to predicting endocrine activity (ECHA 2011b). The REACH program allows for the use of QSARs in lieu of testing data in AA where certain conditions are met (ECHA 2011a; Liu et al. 2013).

Nontraditional in vivo testing

Unlike in vitro and in silico approaches, in vivo testing provides an understanding of how the organism as a whole will respond to a chemical and its metabolites over time. Nontraditional in vivo approaches can combine the integrative benefits of whole-animal testing with the speed and reduced costs of in vitro and in silico approaches (Mesens 2014). Some types of short-term rodent studies fall within the nontraditional category, for example, short-term or accelerated cancer bioassays using rodents that have been genetically modified to exhibit high sensitivity to chemically induced cancers (Eastmond et al. 2013). Other approaches involve the use of smaller animals as surrogates for higher, more complex ones. Model organisms such as the vertebrate zebrafish (*Danio rerio*) and invertebrate nematode (*Caenorhabditis elegans*) have been used in assessing complex endpoints such as reproductive and developmental toxicity (Balls 1995; Ferreira et al. 2014).

Given the size and availability of zebrafish embryos and *C. elegans*, these animals can be used in medium-throughput high-content screening (HCS), a variant of HTS in which the readout of the assay captures more complex data than in an HTS screen (Taylor 2006). A typical HCS readout may be a microscopic image from which quantitative information may be drawn regarding observable physical or biochemical characteristics of the cell or organism. For example, HCS of the effects of a material on zebrafish embryos would generate quantitative data regarding hatching, developmental abnormalities, and mortality using high-content imaging software.

ASSESSING THE CHALLENGES AND BENEFITS OF APPLYING PREDICTIVE TOXICOLOGY TO AA

Using these predictive toxicology methodologies in AA has advantages and limitations, which are summarized in Tables 1 to 4. The tables are not exhaustive. They capture the most salient advantages and limitations when comparing the potential use of these technologies in AA.

While each of the approaches raises its own benefits and limitations, there are 5 common challenges, which are enumerated in the following sections.

Validating methods and achieving acceptance by all stakeholders

Validation requires an objective demonstration that a test method measures the attribute that it is intended to detect in a reliable manner. Publications on formal validation outline the following major criteria: 1) reliability of the assay, 2) relevance to the scientific question being addressed, 3) fitness for purpose, 4) providing an adequate definition of the test, 5) demonstration of within-laboratory reproducibility, 6) demonstration of the ability to transfer the test to other laboratories, and 7) demonstration of the accuracy of the test (Balls 1995; ICH 1996; DHHS 1997; Hartung et al. 2004; Stokes et al. 2006; Hartung 2007; Stokes and Schechtman 2008; Stokes and Wind 2010; Judson et al. 2013). Formal validation, such as that performed by the Organisation for Economic Co-operation and Development (OECD) or other governmental bodies, is generally required for acceptance of data from predictive toxicology tests in a regulatory context. The validation required to use toxicology data in the AA context varies depending on the purpose of the AA. If a test is being used to guide development of a new chemical or product, then the developers will need scientific confidence in the results of the test but will not require full regulatory approval. If data are being used to make a safety case to regulators as part of a required AA, then more formal validation may be needed. A higher level of validation is desired for a method that provides the only data around a particular aspect of safety (e.g., skin sensitization). With different levels of validation available for different tests, AA practitioners need a clearer understanding of what level of validation is required for regulatory AA.

Table 1. Major advantages and limitations of grouping in AA

Advantages	Limitations
Fast, even in comparison to other alternative testing strategies	Requires particular data and analysis capacity
Least expensive	Chemical similarity sometimes does not translate into mechanistic similarity
Can rely on established categories or groupings	Acceptance by regulators as basis for regulatory response may be low
Does not require a chemical sample for testing	Potential legal challenges

AA = alternatives analysis.

Properly interpreting the results from predictive toxicology tests

Predictive toxicology tests produce data that are used to predict the potential for an AO. Though potential of adversity is not the same as an observed adverse effect, such information is increasingly being used to make regulatory decisions. Similar to any in vivo test method, properly interpreting predictive toxicology tests requires a comprehensive understanding of the validation status and limitations of each assay, which requires the interpreter to answer such questions as these:

- Is the test method reliable and relevant?
- Does the assay test the parent compound only?
- What is the chemical domain of applicability?
- How are the data analyzed statistically, and is there potential bias?
- Does the test battery exclude key AOPs?
- Can the data be extrapolated to in vivo doses?
- Are the methods properly described?

There are no appropriate in vitro model systems for a number of priority areas in toxicology, including hepatotoxicity, cancer prediction, and developmental or reproductive toxicity (Knudsen et al. 2015), although efforts in those areas are ongoing (Liu et al. 2015).

Integrating data from different methods

Integrating toxicological data obtained using different experimental platforms is a major challenge for the following reasons:

- The types of endpoints evaluated, whether in vitro or in vivo, can vary greatly, ranging from mechanistic ones (e.g., changes in gene expression in target cell populations) to apical ones (e.g., cell death, developmental deficits).
- Even when there is a common endpoint (e.g., alteration of the function of the estrogen receptor using a reporter gene assay), different data analysis methods are often used, which can result in different conclusions about the activity or potency of the same compound tested under seemingly identical experimental conditions (Shockley 2015).

Table 2. Major advantages and limitations of high-throughput in vitro assays in AA

Advantages	Limitations
Provides fast screening, particularly for potential alternatives for which there often are no data. Screening can also address large numbers of chemicals and help target additional research.	Concerns regarding the relevance of results to effects that may occur in the whole organism
Can easily test a range of concentrations	For some assays, higher likelihood of false positives and false negatives
More quantitative than descriptive methods	Can be difficult to link to exposure data
Less expensive than traditional approaches	The data generated can be difficult to interpret and communicate, making it difficult for the public and third parties to understand how conclusions regarding toxicity were developed
Often provides mechanistic information that can facilitate translation to predictions of human health impacts	Some assays are proprietary, limiting access
Can compare active ingredient to complete product formulation	Mechanistic basis results in selection bias for known mechanisms and against unknown or difficult-to-predict pathways
Opportunity to evaluate mixtures	Investment in robotics can be expensive
Opportunity to examine genetic variability	Difficult to assess temporal patterns and longer-term effects
Ability to simulate different windows of exposure	May be difficult to assess important ecological effects such as persistence and bioaccumulation

AA = alternatives analysis.

 DOI: 10.1002/ieam.1923

Table 3. Major advantages and limitations of in silico modeling in AA

Advantages	Limitations
Offers a cost-effective way either to prioritize chemicals of concern or to assess potential alternatives for which there often are no data	Variable predictive ability of models
Allows for modeling of initiating events	No institution to monitor validation
Can inform the design of safer chemicals	Some models are proprietary, which limits access and impairs transparency
Fast	Because many models are based upon experimental results regarding a set of structurally similar data-rich chemicals, model applicability is limited to data-poor chemicals with similar structure
Does not require chemical sample for testing	Can be more difficult to develop if the mechanistic basis is unknown
Computational predictions of environmental fate and degradation allow exposure to be estimated without expensive monitoring and can predict bio-persistence and concentrations in the environment	Model quality varies considerably and model performance can be manipulated by choosing particular chemicals
—	For some chemicals there may be a lack of quality data to develop and use models

AA = alternatives analysis.

- Generally, only study summary data are available, whereas the more useful, detailed experimental data are maintained in unconnected data silos, making it difficult if not impossible to evaluate all data using a standardized data analysis method.

In addition to developing databases in which all relevant publications and electronic data can be archived, there is a critical need for a set of commonly accepted methods for comparing results across different test method platforms. Efforts at systematic review may fill this gap, for example, federal frameworks for assessing human and experimental animal data are currently available, and a similar approach for mechanistic data is under development (Judson et al. 2012).

Assessing and addressing data quality issues

A decision is only as good as the data it is based on. Addressing data quality issues is ultimately critical and unfortunately in toxicology has been given little attention until recently (Przybylak et al. 2012; Matevia 2015). Recent implementation of systematic review methods in

Table 4. Major advantages and limitations of nontraditional in vivo testing in AA

Advantages	Limitations
Tissue and organ specific	Increased difficultly in determining mechanism by which the chemical may be causing toxicity
Response more accurately represents how the organism will respond	Does not necessarily translate across species
Integrative, can show systematic impact including impacts on behavior	Longer and more expensive than grouping, HTS, and in silico
Provides confidence that you are testing the metabolites, although not necessarily the human metabolites	In some jurisdictions, it is legally limited or prohibited
Faster than traditional full-rodent studies	In some cases, better at assessing short-term impacts than long-term impacts
Need for fewer animals	—
Can be paired with mechanistic information to shorten study length	—
Can study exposure-specific impacts	—
Easier to integrate into AA	—
Applicable to mixtures	—

AA = alternatives analysis; HTS = high-throughput screening.

environmental health should help. Although systematic review methods are most developed for epidemiological and traditional in vivo evidence, there is great interest in extending the methods to the type of evidence used in alternative testing strategies (Murray and Thayer 2014; Thayer et al. 2014; DHHS 2015; Mandrioli and Silbergeld 2016). Increased concern about the quality of shared data should also lead to increased attention to the development of standardized tools and approaches, which in turn can be incorporated to compare alternatives as part of the AA process.

Standardizing and sharing data

In order to perform the hazard assessment of an AA, one needs either data on the chemicals of interest or models that can be used to predict the chemicals' toxicology profiles. To build such models requires relatively large databases of chemical toxicology data. Several efforts have been gathering such data and publishing it online to make direct evaluation or model building possible, including the following:

- the USEPA's ACToR system, a data warehouse that aggregates toxicology and other relevant data from thousands of sources, including ToxCast (Judson et al. 2008, 2012; USEPA 2015);
- the National Institutes of Health's PubChem (NIH 2016) that collects data on chemical bioactivity (mainly from in vitro experiments);
- ChEMBL similar to PubChem, but with a focus on extracting data from the open literature (Gaulton et al. 2012; EBI 2016);
- the National Toxicology Program's Chemical Effects in Biological Systems (CEBS) project (NIEHS 2016); and
- the National Library of Medicine's toxicology data network (TOXNET) (USNLM 2016).

All of these systems allow browsing by chemical and large-scale downloads for modeling efforts (Judson et al. 2005; Richard et al. 2008; Judson 2010). The advent of this kind of database advances access to the information needed for AA, but publicly available information is still lacking for many chemical–endpoint combinations and transformation products.

Despite these challenges, the possible benefits that the methods could have if applied in regulatory AA are significant. The present paper now turns to developing a road map for assimilating predictive toxicology methods into AA.

A roadmap for incorporating predictive toxicology into AA

The speed and low expense of many of these methods make them particularly attractive for use in AA. However, predictive toxicology is still an emerging field, as is regulatory AA. In the short term, a measured introduction of predictive toxicology into AA is appropriate, at first focusing on more established methodologies for limited applications. For example, existing and emerging QSARs and nontraditional in vivo testing could be used in the near term to fill data gaps for certain endpoints. Likewise, mechanistically based in vitro approaches may be used for screening alternatives, or for identifying endpoints for which additional testing may be needed.

These recommendations mirror suggestions made in a recent report by the National Academy of Sciences on selecting chemical alternatives (NRC 2014). Given AA's more expansive need for toxicology data on multiple chemicals compared to traditional one-at-a-time chemical risk assessment, AA could act as a test case to drive the use of predictive toxicology in traditional regulatory decision making. The application of predictive toxicology in these situations should be transparent, allowing for ex post assessment that could inform and refine the use of predictive toxicology in AA and in other regulatory applications, allowing it to increase its robustness and reliability over time.

Such limited uses would not capture the full potential value of predictive toxicology. In the long term, the discipline should move toward broader use of these approaches. Systematic and broader application of individual approaches will be important to the growth of effective AA methods, and will help build confidence in the use of these approaches for other applications. Such application presents 2 challenges: 1) how to combine data from alternative testing approaches with conventional data and 2) how to meld together the different types of alternative testing approaches.

Analysts are often faced with a range of data, including epidemiological data, human studies, animal studies, and conventional in vitro data. Much of the data may relate to a common issue of concern (such as acute toxicity or developmental toxicity), but the form, nature, and quality of the data can vary. This issue can be exacerbated when emerging testing approaches providing mechanistic information about impacts at the cellular level are included with conventional animal studies, or when data from different studies or assays give incongruent results. Tools and methods available to integrate multiple streams of data include qualitative weight-of-evidence approaches, structured forms of systematic review, and quantitative, probabilistic methods (Woodruff and Sutton 2011; Park et al. 2013; Rooney et al. 2014; NanoInfo 2016).

Integration of alternative testing strategies includes the development of a cohesive suite of mechanistically based assays that could provide an extensive data set for chemical formulations and mixtures. Alternatively, integration could be the systematic use of several predictive approaches, including "tiered approaches," "integrated testing strategies," and "intelligent testing strategies" (Bradbury et al. 2004; Nel et al. 2013). Each of these integration approaches aims to meld the various methods in an efficient, well-grounded manner and are beyond the scope of the present paper.

To successfully deploy predictive toxicology into AA, resources will need to be invested in research, policy development, capacity building, and education. This includes short-, medium-, and long-term steps that could be taken to develop the field of predictive toxicology and to

 DOI: 10.1002/ieam.1923

encourage its acceptance and use in AA. To do that, the present paper offers the 4 recommendations, spelled out in the sections that follow.

Recommendation 1: Use case studies to advance the integration of predictive toxicology in AA. Systematic case studies can answer specific questions about how to integrate predictive toxicology into AA, including these:

- How well do HTS approaches work for screening potential alternatives about which there is little to no available conventional toxicological data?
- How should predictive toxicology data be normalized and weighted in AA?
- How should entities conducting AA determine what predictive toxicology data should be included or excluded?
- How can predictive toxicology advance the consideration of ecotoxicity in AA?
- How can predictive toxicology advance the consideration of interactive effects in AA?

Case studies should be specifically designed to address these questions, possibly running multiple scenarios with different approaches to data inclusion and weighting to assess the impact of various uses of toxicological data. Case study topics that have the potential to address one or more of the specific questions listed above include marine antifouling paint, chemicals used in fracking, flame retardant alternatives, C nanotubes, and bisphenol A (BPA) alternatives. It is important to follow up these case studies over time as new data become available to evaluate how well the AA methods worked in improving overall safety.

Recommendation 2: Employ predictive toxicology approaches to screen chemicals of concern for AA. A large number of chemicals used in commerce have been identified as chemicals of concern by entities such as the USEPA, the National Toxicology Program, the European Commission, and the International Agency for Research on Cancer. Regulators and companies face the task of prioritizing chemicals for AA and subsequent substitution or regulation. Predictive toxicology approaches—including in silico modeling and creation of new data through HTS—could be used to prioritize these chemicals of concern along with other relevant toxicological data. For example, prioritization of the approximately 300 chemicals on Washington State's list of Chemicals of High Concern to Children presents a useful opportunity to use predictive toxicology methods (WAC 2017).

Various government entities are already testing a number of compounds using predictive methods (Judson, Houck et al. 2010) and are making much of the data public. Most recently, the USEPA's Endocrine Disruptor Screening Program identified an integrated testing approach using predictive methods as an acceptable alternative to several Tier 1 assays used to screen for estrogen activity (Browne et al. 2015). However, such efforts can be complicated by difficulty acquiring a reasonable amount of a compound for testing, and securing resources to support the efforts, particularly when undertaken by state agencies or private entities. Academics often are willing to run some tests at cost and share cell lines and protocols.

Recommendation 3: Use existing resources to advance the integration of predictive toxicology in AA . A number of existing predictive toxicology and AA resources could be leveraged or modified to advance the integration of predictive toxicology into AA, including these:

- Having existing AA frameworks agree that predictive toxicology has a place in AA and should be used. The frameworks could uniformly accept certain tests and tools. Ideally, this would include a tools clearinghouse to assist decision makers in the selection and use of predictive toxicology methods that recommended methods by chemical class and endpoint.
- Assessing the toxicology data available on PubChem from different endpoints generated using a common set of chemicals in grants. This would allow the comparison of the same chemical sets across different assays and approaches, which would facilitate validation.
- Looking at nonregulatory validation options or validation from other jurisdictions. This includes investigating whether existing OECD-approved tests for alternatives to animal testing or a forthcoming EU ranking system for data quality could facilitate the use of predictive toxicology in AA.

New predictive toxicology methods should be considered for integration into AA as they are developed. Funding and interdisciplinary education and training for students and professionals are important to increasing the capacity to perform AAs.

Recommendation 4: Support trans-sector and transdisciplinary efforts to integrate predictive toxicology in AA. Though there is interest in incorporating predictive toxicology methods into regulatory AA, the relevant disciplines (toxicologists, decision analysts, regulatory and legal experts, and policy makers) and sectors (government, industry, civil society, and academia) often work in silos, inhibiting the integration of predictive toxicology into AA. Specific suggestions to expand transdisciplinary work include these:

- Develop a research coordination network to provide the necessary vehicle for systematic collaboration across disciplines and institutions.
- Use existing efforts to bring together regulators, industry, civil society, and academics to agree on testing protocols for nanotechnologies as a model.
- Facilitate the creation of a safe harbor from litigation or regulatory action for data provided to regulators during an AA to encourage the sharing of industry data as part of a collaborative learning process.

- Encourage acceptance of data across and within sectors. For instance, state government agencies could use HTS screening data generated by the federal government in their prioritization processes.

Although some of these actions are partially underway, there is still much to be gained from interaction between professionals from diverse sectors and industries.

CONCLUSION

Predictive toxicology has an important role to play in AA. Although many of the challenges associated with using predictive toxicology in other realms will also exist in AA, it also offers some promising opportunities to advance the use of predictive toxicology for regulatory purposes. Continued collaboration among toxicologists, decision analysts, regulators, and engineers on case studies and other projects is the next step to advancing AA and predictive toxicology.

Acknowledgment—This manuscript is the product of a workshop hosted by the UCLA Sustainable Technology and Policy Program, a joint collaboration of the UCLA School of Law and the Center for Occupational and Environmental Health in the UCLA Fielding School of Public Health in partnership with the UC Center for Environmental Implications of Nanotechnology (UC CEIN). UC CEIN is funded by a cooperative agreement from the National Science Foundation and the EPA (NSF DBI-0830117; NSF DBI-1266377). The Institute of the Environmental and Sustainability and the Emmett Institute on Climate Change and the Environment, both at UCLA, also provided support.

Data Accessibility—No data were generated for this article. All data used are publicly available through the references cited.

REFERENCES

Ankley GT, Bennett RS, Erickson RJ, Hoff DJ, Hornung MW, Johnson RD, Mount DR, Nichols JW, Russom CL, Schmeider PK et al. 2010. Adverse outcome pathways: A conceptual framework to support ecotoxicology research and risk assessment. *Environ Toxicol Chem* 29:730–741.

Bakand S, Winder C, Khalil C, Hayes A. 2005. Toxicity assessment of industrial chemicals and airborne contaminants: Transition from in vivo to in vitro test methods: a review. *Inhal Toxicol* 17:775–787.

Balls M. 1995. Defining the role of ECVAM in the development, validation, and acceptance of alternative tests and testing strategies. *Toxicol In Vitro* 9:863–869.

Bradbury SP, Feijtel TC, Van Leeuewn CJ. 2004. Meeting the scientific needs of ecological risk assessment in a regulatory context. *Environ Sci Technol* 38:463a–470a.

Browne P, Judson RS, Casey WM, Kleinstreuer NC, Thomas RS. 2015. Screening chemicals for estrogen receptor bioactivity using a computational model. *Environ Sci Technol* 49:8804–8814.

[CCR] California Code of Regulations. 2013. Safer consumer products. Division 4.5, Title 22, Chapter 55. Sacramento (CA). [cited 2016 June 21]. https://perma.cc/C7ZY-SL73

Cohen Y, Rallo R, Liu R, Liu HH. 2013. In silico analysis of nanomaterials hazard and risk. *Acc Chem Res* 46:802–812.

Cronin MTD. 2010. Prediction of harmful human health effects of chemicals from structure. In: Puzyn T, Leszczynski J, Cronin MTD, editors. Recent advances in QSAR studies. New York (NY): Springer. p 305–325.

Cronin MTD. 2013. An introduction to chemical grouping, categories and read-across to predict toxicity. In: Cronin MTD, Madden JC, Enoch SJ, Roberts DW, editors. Chemical toxicity prediction: Category formation and read-across. Cambridge (UK): Royal Soc Chemistry. p 1–29.

Cummings CL, Kuzma J. 2017. Societal risk evaluation scheme (SRES): Scenario-based multi-criteria evaluation of synthetic biology applications. *PLoS One*. 12:e0168564. DOI:10.1371/journal.pone.0168564

[DHHS] US Department of Health and Human Services. 1997. Validation and regulatory acceptance of toxicological test methods: A report of the Ad Hoc Interagency Coordinating Committee on the Validation of Alternative Methods. Washington (DC). [cited 2016 June 21]. https://perma.cc/K9R7-DCV9

[DHHS] US Department of Health and Human Services. 2010. Reducing environmental cancer risk: What we can do now. Washington (DC). [cited 2016 June 21]. https://perma.cc/Z8WL-BLZZ

[DHHS] US Department of Health and Human Services. 2015. Handbook for conducting a literature-based health assessment using OHAT approach for systematic review and evidence integration. Washington (DC). [cited 2016 June 21]. https://perma.cc/G3XS-L9WN

Dimitrov S, Mekenyan O. 2010. An introduction to read-across for the prediction of the effects of chemicals. In: Cronin MTD, Madden JC, editors. In silico toxicology: Principles and applications. Cambridge (UK): Royal Soc Chemistry. p 372–384.

Eastmond DA, Vulimiri SV, French JE, Sonawane B. 2013. The use of genetically modified mice in cancer risk assessment: Challenges and limitations. *Crit Rev Toxicol* 43:611–631.

[EBI] The European Bioinformatics Institute. 2016. ChEMBL. Cambridge (UK). [cited 2017 May 8]. https://www.ebi.ac.uk/chembl/

[ECHA] European Chemicals Agency. 2011a. Guidance on the preparation of an application for authorisation. Ver. 1. Helsinki (FI). [cited 2016 June 22]. https://perma.cc/EH24-HC7E

[ECHA] European Chemicals Agency. 2011b. Guidance on information requirements and chemical safety assessment. Part B: Hazard Assessment. Helsinki (FI). [cited 2016 June 22]. https://perma.cc/WUS5-EZRP

Edwards S, Tickner J, Torrie Y, Coffin M, Kernan, L. 2011. A compendium of methods and tools for chemical hazard assessment. Lowell (MA): Lowell Center for Sustainable Production, Univ Massachusetts. [cited 2017 May 8]. https://perma.cc/H3VE-Y8V5

Ellinger-Ziegelbauer H, Gmuender H, Banderburg A, Ahr HJ. 2008. Prediction of a carcinogenic potential of rat hepatocarcinogens using toxicogenomics analysis of short-term in vivo studies. *Mutat Res* 637:23–29.

Enoch SJ, Roberts DW. 2013. Approaches for grouping chemicals into categories. In: Cronin MTD, Madden JC, Enoch SJ, Roberts DW, editors. Chemical toxicity prediction: Category formation and read-across. Cambridge (UK): Royal Soc Chemistry. p 30–43.

Ferreira DW, Chen Y, Allard P. 2014. Using alternative model *C. elegans* in reproductive and developmental toxicology studies. In: Kang YJ, editor. Methods in pharmacology and toxicology. New York (NY): Springer.

Gaulton A, Bellis LJ, Bento AP, Chambers J, Davies M, Hersey A, Light Y, McGlinchey S, Michalovich D, Al-Lazikani B et al. 2012. ChEMBL: A large-scale bioactivity database for drug discovery. *Nucleic Acids Res* 40: D1100–D1107.

Hartung T. 2007. Food for thought. . .On validation. *ALTEX* 24:67–73.

Hartung T, Bremer S, Casati S, Coecke S, Corvi R, Fortaner S, Gribaldo L, Halder M, Hoffman S, Roi AJ et al. 2004. A modular approach to the ECVAM principles on test validity. *Altern Lab Anim* 32:467–472.

Hartung T, Hoffman S. 2009. Food for thought. . .On in silico methods in toxicology. *ALTEX* 26:155–166.

[ICC] Interstate Chemicals Clearinghouse. 2013. Alternatives assessment guide. Ver. 1.0. Boston (MA). [cited 2016 June 22]. https://perma.cc/5X7T-XQNC

[ICH] International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use. 1996. ICH harmonized tripartite guideline validation of analytical procedures: Text and methodology Q2(R1). Geneva (CH). [cited 2016 June 22]. https://perma.cc/VY6Z-5RRN

Jaworska J, Nikolova-Jeliazkova N. 2007. How can structural similarity analysis help in category formation? *SAR QSAR Environ Res* 18:195–207.

Judson PN, Cooke PA, Doerrer NG, Greene N, Hanzlik RP, Hardy C, Hartmann A, Hinchliffe D, Holder J, Muller L et al. 2005. Towards the creation of an international toxicology information centre. *Toxicol* 213:117–128.

Judson R. 2010. Public databases supporting computational toxicology. *J Toxicol Environ Health B Crit Rev* 13:218–231.

Judson R, Kavlock R, Martin M, Reif D, Houck K, Knudsen T, Richard A, Tice RR, Whelan M, Xia M et al. 2013. Perspectives on validation of high-throughput assays supporting 21st century toxicity testing. *ALTEX* 30:51–56.

Judson R, Richard A, Dix D, Houck K, Elloumi F, Martin M, Cathey T, Transue TR, Spencer R, Wolf M. 2008. ACToR-aggregated computational toxicology resource. *Toxicol Appl Pharmacol* 233:7–13.

Judson RS, Houck KA, Kavlock RJ, Knudsen TB, Martin MT, Mortensen HM, Reif DM, Rotroff DM, Shah I, Richard AM et al. 2010. In vitro screening of environmental chemicals for targeted testing prioritization: The ToxCast project. *Environ Health Perspect* 118:485–492.

Judson RS, Martin MT, Egeghy P, Gangwal S, Reif DM, Kothiya P, Wolf M, Cathey M, Transue T, Smith D. 2012. Aggregating data for computational toxicology applications: U.S. Environmental Protection Agency (EPA) aggregated computational toxicology resource (ACTOR) system. *Int J Mol Sci* 13:1805–1831.

Judson RS, Martin MT, Reif DM, Houck KA, Knudsen TB, Rotroff DM, Xia M, Sakamuru S, Huang R, Shinn P et al. 2010. Analysis of eight oil spill dispersants using rapid, in vitro tests for endocrine and other biological activity. *Environ Sci Technol* 44:5979–5985.

Knudsen TB, Keller DA, Sander M, Carney EW, Doerrer NG, Eaton DL, Fitzpatrick SC, Hastings KL, Mendrick DL, Tice RR et al. 2015. FutureTox II: In vitro data and in silico models for predictive toxicology. *Toxicol Sci* 143:256–267.

Liu J, Mansouri K, Judson RS, Martin MT, Hong H, Chen M, Xu X, Thomas RS, Shah I. 2015. Predicting hepatotoxicity using ToxCast in vitro bioactivity and chemical structure. *Chem Res Toxicol* 28:738–751.

Liu R, Hassan T, Rallo R, Cohen Y. 2013. HDAT: Web-based high-throughput screening data analysis tools. *Comp Sci Discovery* 6:1–11.

Madden JC. 2010. Introduction to QSAR and other in silico methods to predict toxicity. In: Cronin MTD, Madden JC, editors. In silico toxicology: Principles and applications. Cambridge (UK): Royal Soc Chemistry. p 11–30.

Malloy TF. 2014. Principled prevention. *Arizona State L Rev* 46:105–190.

Malloy TF, Sinsheimer PJ, Blake A, Linkov I. 2013. Use of multi-criteria decision analysis in regulatory alternatives analysis: A case study of lead free solder. *Integr Environ Assess Manag* 9:652–664.

Mandrioli D, Silbergeld EK. 2016. Evidence from toxicology: The most essential science for prevention. *Environ Health Perspect* 124:6–11.

Matevia M. 2015. A brief introduction to systematic review. AltTox. [cited 2016 June 22]. https://perma.cc/6QZN-DXCZ

Mayo-Bean K, Moran K, Meylan B, Ranslow P. 2012. Methodology Document for the ECOlogical Structure–Activity Relationship Model (ECOSAR) Class Program.

Mesens N. 2014. The zebrafish model in toxicology. In: Pfannkuch F, Suter-Dick L, editors. Predictive toxicology: From vision to reality. Weinheim (DE): Wiley-VCH. p 217–240.

Murray HE, Thayer KA. 2014. Implementing systematic review in toxicological profiles: ATSDR and NIEHS/NTP collaboration. *J Environ Health* 76:34–35.

NanoInfo. 2016. NanoEIA. Los Angeles (CA). [cited 2016 June 22]. http://nanoinfo.org/#!/nanoeia/

[NCCELC] National Conversation on Chemical Exposures Leadership Council (US). 2011. Addressing public health and chemical exposures: An action agenda. Washington (DC). [cited 2016 June 21]. https://perma.cc/SR8T-8T8X, http://www.nationalconversation.us/

Nel AE, Nasser E, Godwin H, Avery D, Bahadori T, Bergeson L, Beryt E, Bonner JC, Boverhof D, Carter J et al. 2013. A multi-stakeholder perspective on the use of alternative test strategies for nanomaterial safety assessment, *ACS Nano* 7:6422–6433.

[NIEHS] National Institute of Environmental Health Sciences (US). 2016. chemical effects in biological systems (CEBS). Research Triangle Park (NC). [cited 2016 June 22]. http://www.niehs.nih.gov/research/resources/databases/cebs/

[NIH] National Institutes of Health (US). 2016. PubChem. Bethesda (MD). [cited 2016 June 22]. https://pubchem.ncbi.nlm.nih.gov/

[NRC] National Research Council (US). 2007. Toxicity testing in the 21st century: A vision and a strategy. Washington (DC): National Academies.

[NRC] National Research Council (US). 2014. A framework to guide the selection of chemical alternatives. Washington (DC): National Academies.

[OECD] Organisation for Economic Co-operation and Development, Environment, Health, and Safety Publications. 2014. Guidance on the grouping of chemicals. 2nd ed. Paris (FR). [cited 2016 June 22]. https://perma.cc/7EK2-6HKL

[OJEU] Official Journal of the European Union. 2007. Corrigendum to Regulation (EC) No 1907/2006 of the European Parliament and of the Council. Brussels (BE). [cited 2016 June 21]. https://perma.cc/5ZV9-WSKB

Parham F, Austin C, Southall N, Huang R, Tice R, Portier C. 2009. Dose-response modeling of high-throughput screening data. *J Biomol Screen* 14:1216–1227.

Park SJ, Ogunseitan OA, Lejano RP. 2013. Dempster-Shafer theory applied to a regulatory decision process for selecting safer alternatives to toxic chemicals in consumer products. *Integr Environ Assess Manag* 10:12–21.

Patlewicz G, Ball N, Booth ED, Hulzebos E, Zvinavashe E, Hennes C. 2013. Use of category approaches, read-across and (Q)SAR: General considerations. *Regul Toxicol Pharmacol* 67:1–12.

Przybylak KR, Madden JC, Cronin MTD, Hewitt M. 2012. Assessing toxicological data quality: Basic principles, existing schemes and current limitations. *SAR QSAR Environ Res* 23:435–459.

Richard AM, Yang C, Judson RS. 2008. Toxicity data informatics: Supporting a new paradigm for toxicity prediction. *Toxicol Mech Meth* 18:103–118.

Rooney AA, Boyles AL, Wolfe MS, Bucher JR, Thayer KA. 2014. Systematic review and evidence integration for literature-based environmental health science assessments. *Environ Health Perspect* 122:711–718.

Rossi M, Peele C, Thorpe B. 2012. BizNGO chemical alternatives assessment protocol: How to select safer alternatives to chemicals of concern to human health or the environment. Somerville (MA). [cited 2016 June 22]. https://perma.cc/LU4U-B3HR

Rotroff DM, Wetmore BA, Dix DJ, Ferguson SS, Clewell HJ, Houck KA, Lecluyse EL, Andersen ME, Judson RS, Smith CM et al. 2010. Incorporating human dosimetry and exposure into high-throughput in vitro toxicity screening. *Toxicol Sci* 117:348–358.

Shah I, Wambaugh J. 2010. Virtual tissues in toxicology. *J Toxicol Environ Health B Crit Rev* 13:314–328.

Shockley KR. 2015. Quantitative high-throughput screening data analysis: Challenges and recent advances. *Drug Discovery Today* 20:296–300.

Sinsheimer P, Grout C, Namkoong A, Gottlieb R, Latif A. 2007. The viability of professional wetcleaning as a pollution prevention alternative to perchloroethylene dry cleaning. *J Air Waste Manage Assoc* 57:172–178.

Stokes WS, Schechtman LM. 2008. Validation and regulatory acceptance of new, revised, and alternative toxicological methods. In: Hayes AW, editor. Principles and methods of toxicology. 5th ed. Boca Raton (FL): CRC. p 1103–1130.

Stokes WS, Schechtman LM, Rispin A, Gupta K, Hamernik K, Tice R, Wind M. 2006. The use of test method performance standards to streamline the validation process. *ALTEX* 23:342–345.

Stokes WS, Wind M. 2010. Recent progress and future directions at NICEATM-ICCVAM: Validation and regulatory acceptance of alternative test methods that reduce, refine, and replace animal use. *ALTEX* 27:221–232.

Taylor DL. 2006. Past, present, and future of high content screening in the field of cellomics. In: Taylor DL, Haskins JR, Guiliano K, editors. High content screening. Totowa (NJ): Humana. p 3–18.

Thayer KA, Wolfe MS, Rooney AA, Boyles AL, Bucher JR, Birnbaum LS. 2014. Intersection of systematic review methodology with the NIH reproducibility initiative. *Environ Health Perspect* 122:A176–177.

U.S. Congress. 1976. Toxic Substances Control Act, Pub. L. No. 94-469, 90 Stat. 2003.

[USEPA] US Environmental Protection Agency, Office of Pollution Prevention and Toxics. 2011. Design for the Environment program alternatives assessment criteria for hazard evaluation. Ver. 2. Washington (DC). [cited 2016 June 22]. https://perma.cc/226V-NPN8

[USEPA] US Environmental Protection Agency. 2014. Flame retardant alternatives for hexabromocyclododecane (HBCD). Washington (DC). [cited 2016 June 22]. https://perma.cc/Q5RD-GUB9

[USEPA] US Environmental Protection Agency. 2015. Aggregated computational toxicology resource. Washington (DC). [cited 2016 June 22]. https://perma.cc/E7BG-D42J

[USNLM] US National Library of Medicine. 2016. TOXNET database. Bethesda (MD). [cited 2016 June 22]. https://toxnet.nlm.nih.gov/

Villaneuve DL, Crump D, Garcia-Reyero N, Hecker M, Hutchinson TH, LaLone CA, Landesmann B, Lettieri T, Munn S, Nepelska M et al. 2014a. Adverse

outcome pathway (AOP) development I: Strategies and principles. *Toxicol Sci* 142:312–320.

Villaneuve DL, Crump D, Garcia-Reyero N, Hecker M, Hutchinson TH, LaLone CA, Landesmann B, Lettieri T, Munn S, Nepelska M et al. 2014b. Adverse outcome pathway (AOP) development II: Best practices. *Toxicol Sci* 142:321–330.

[WAC] Washington Administrative Code. 2017. The reporting list of chemicals of high concern to children (CHCC list). Title 173, Chapter 334, Section 130. [cited 2017 May 8]. http://apps.leg.wa.gov/WAC/default.aspx?cite=173-334-130

Whittaker MH. 2015. Risk assessment and alternatives assessment: Comparing two methodologies. *Risk Analysis* 35:2129–2136.

Woodruff TJ, Sutton P. 2011. Environmental health sciences: An evidence-based medicine methodology to bridge the gap between clinical and environmental health sciences. *Health Aff* 30:931–937.

 DOI: 10.1002/ieam.1923