Background Paper on Predictive Toxicology

Prepared for the 2014 Advancing Alternatives Analysis Workshop

This paper provides background on predictive toxicological testing approaches that may be useful in alternatives analysis (AA). After an introduction to the challenges of collecting and generating hazard data in the AA context, the paper (1) surveys existing and emerging predictive toxicological approaches and (2) briefly summarizes methods for integrating predictive data with other forms of evidence. (The paper assumes a basic knowledge of AA; for more information on AA see the *Background Paper on Alternatives Analysis*.)

INTRODUCTION

In our case study of marine anti-foul paint, the City of Beachside must identify safer, viable alternatives to copper-based paint for boats. The City seeks to avoid regrettable substitution, a scenario in which the replacement for a toxic product turns out to cause significant health or environmental problems. Alternatives analysis can avoid regrettable substitution through systematic identification, assessment and comparative evaluation of alternatives.

However, as the *Background Paper on Alternatives Analysis* illustrates, AA presents its own set of challenges. Most relevant for our purposes is the fact that the scope and volume of toxicological information needed for a comprehensive alternatives analysis can be formidable. AA calls for information about the relative hazards and exposure potential associated with the baseline product *and* its potential replacements. And the scope of the toxicological assessment can be broad; for example, human health impacts may include numerous endpoints such as acute toxicity, carcinogenicity, endocrine disruption, respiratory sensitization, developmental toxicity, neurotoxicity, and so on. The practical problem here is that existing methodologies for hazard identification, exposure assessment and risk assessment are time-consuming and expensive.[1] Ironically, efforts to avoid regrettable substitution through prevention exacerbate the challenges presented by conventional risk assessment by increasing the focus from a single regulated chemical to that chemical *and* its alternatives.

Predictive toxicology offers a potential solution to this dilemma.[2] Generally speaking, predictive toxicity involves studying a mechanism of injury *in vitro* (*e.g.*, a cell) that also applies to pathophysiology of disease, and is therefore predictive of whether that disease may develop if a substance triggers the particular injury mechanism. It stands in contrast to conventional regulatory toxicology methods which have primarily focused on "*in vivo*" (whole animal)

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[1] *See* Thomas Hartung, *Toxicology Testing for the Twenty-First Century*,460 Nature 208 (2009); National Academy of Science, TOXICITY TESTING IN THE 21ST CENTURY: A VISION AND A STRATEGY (2007).

[2] *See* Daniel Krewski, *et al.*, *New Directions in Toxicity Testing*, 32 Annu. Rev. Public Health 161, 163 (2011).; Douglas W. Bristol, *et al.*, *The NIEHS Predictive-Toxicology Evaluation Project*, 104 Environ. Health Perspect. 1001, 1002 (1996).

approaches, usually relying on testing of mammals such as rodents and rabbits to observe a chemical's adverse effects.[3] Those effects and the doses at which they may occur are then extrapolated to characterize toxicity in humans.

In the last decade, there has been increased interest and activity in the United States and Europe regarding alternative testing approaches grounded in predictive toxicology. Alternative testing approaches" is a broad term used to describe a variety of predictive methods and approaches (such as cell-based assays, biochemical assays, high throughput and high content methods, and computational (*in silico)* methods) as well as integrated frameworks which combine the methods to characterize toxicity.[4] (See Table 1 at the back of this paper for a glossary of terms.) Based on systems biology and leveraging the power of bioinformatics, these methods and approaches focus upon understanding the mechanisms of toxicity by detecting disruptions in human cellular and system functions that lead to disease.[5]

The balance of this paper summarizes a range of alternative testing approaches that may be relevant to alternatives analysis, explores emerging efforts to integrate them with conventional testing data and other evidence, and provides an annotated list of materials for further reading.

ALTERNATIVE TESTING APPROACHES: A SURVEY

This survey offers brief summaries of four types of testing methods and predictive approaches that may be useful in the AA context: grouping, mechanistically-based *in vitro* testing; high throughput and high content screening, and *in silico* methods such as quantitative structure activity relationships (QSARs).

Grouping. Grouping is defined as the arrangement of chemicals or substances (including nanomaterials) into groups based on common attributes.[6] The nature of attributes varies depending upon the type and purpose of the grouping and other factors. In some cases, the attribute may be a human health or environmental endpoint such as carcinogenicity or aquatic toxicity. In others it may be a physicochemical feature of the substance or volumes of material produced or used. Grouping can take a variety of forms, most notably 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.[7] Thus, in a relevant group the data for chemicals and endpoints that have been tested are used to

[3] T.G. Vermeire, *et al.*, *Toxicity Testing for Human Health Risk Assessment*, in C.J. van Leeuwen and T.G. Vermeire (eds.), RISK ASSESSMENT OF CHEMICALS: AN INTRODUCTION 227 (2007).
[4] Andre E. Nel, *et al*, *A Multi-Stakeholder Perspective on the Use of Alternative Test Strategies for Nanomaterial Safety Assessment*, 7 ACS NANO 6422 (2013).
[5] NAS, *supra* n. 1.
[6] Organisation for Economic Co-operation and Development (OECD), GUIDANCE ON GROUPING OF CHEMICALS, ENV/JM/MONO(2007).
[7] J.C. Madden, *Introduction to QSAR and Other In Silico Methods to Predict Toxicity*, in Mark T. D. Cronin and Judith C. Madden (eds.), IN SILICO TOXICOLOGY: PRINCIPLES AND APPLICATIONS 11 (2010).

estimate the outcomes for untested chemicals and endpoints.[8] Carefully and rigorously defining the scope of the relevant group (or in other words operationalizing the concept of "similar") is central to grouping. A variety of methods for grouping chemicals exist, including grouping based on mechanistic knowledge (such as molecular initiating events or adverse outcome pathways), chemoinformatics, or structural similarities (such as functional groups).[9]

The categories may vary in size. The ***analog approach*** refers to the use of groups of ten or fewer chemicals to fill data gaps, with the ***category approach*** referring to the use of larger groups.[10] The "gap filling" methods (that is, methods for estimating properties or activity for a "data poor" chemical based on one or more similar "data rich" chemicals) also vary.

Gap filling may involve read across methods or trend analysis. "***Read across***" is a qualitative assessment of toxicity based upon expert judgment regarding the similarity with the other chemical or chemicals and likely activity. Examples 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.

Grouping has not played a significant role in quantitative risk assessment to date, although EPA and others have efforts underway to incorporate it in some aspects of risk assessment.[11] However, grouping has been used in the alternatives analysis setting to a limited degree. Grouping's value in AA is particularly enhanced by the comparative orientation of alternatives analysis. AA is aimed at identifying and evaluating relative performance of the baseline chemical and its alternatives rather than identifying a single acceptable exposure level. Consequently finely grained,

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[8] (OECD), GUIDANCE ON GROUPING OF CHEMICALS, *supra* n. 6; J. Jaworska and N. Nikolova-Jeliazkova, *How Can Structural Similarity Analysis Help in Category Formation*, 18 SAR AND QSAR IN ENVIRONMENTAL RESEARCH 195 (2007).

[9] S. J. Enoch and D. W. Roberts, *Approaches for Grouping Chemicals into Categories*, in Mark T. D. Cronin, *et al* (eds.), Chemical Toxicity Prediction: Category Formation and Read-Across 30 (2013); (OECD), Guidance on Grouping of Chemicals, *supra* n. 6.

[10] *Id.*; Grace Patlewicz, *Use of Category Approaches, Read-Across and (Q)SAR: General Considerations*, 67 REGULATORY TOXICOLOGY AND PHARMACOLOGY 1 (2013).

[11] EPA, Next Generation Risk Assessment: Incorporation of Recent Advances in Molecular, Computational, and Systems Biology, EPA/600/R-13/214A (2013)(External Review Draft); *see* Kenneth S. Crump, *et al.*, *The Future Use of in vitro Data in Risk Assessment to Set Human Exposure Standards: Challenging Problems and Familiar Solutions*, 118 ENVTL. HEALTH PERSP. 1350 (2010). Grouping has been used in qualitative and streamlined risk assessment for some time in a variety of settings. For example, its New Chemical Review program under the Toxic Substance Control Act (TSCA) EPA generates new chemical exposure limits (NCELs) for acceptable air-borne exposure concentrations in the workplace. Due to the paucity of data typically available for new chemicals, NCELs are often produced through risk assessments utilizing toxicity data from analog chemicals. EPA, QUESTIONS & ANSWERS FOR THE NEW CHEMICALS PROGRAM (Undated Draft) (http://www.epa.gov/opptintr/newchems/pubs/qanda-newchems.pdf accessed April 18, 2014); EPA, RESPONSE TO EXTERNAL COMMENTS ON NEW CHEMICAL EXPOSURE LIMITS IN TOXIC SUBSTANCES CONTROL ACT SECTION 5(E) ORDERS, ATTACHMENT I- EPA'S METHOD FOR DETERMINING NCELS (1995).

quantitative results are less critical than in quantitative risk assessment.[12] EPA has relied upon chemical grouping in alternatives analyses performed in its Design for the Environment (DfE) program, using the analog approach and SARs for hazard identification, including carcinogenicity and endocrine activity. In the European Union's REACH chemical program, which requires alternatives analysis for certain substances of very high concern,[13] The REACH AA guidance emphasizes the use of analogs and categories.[14]

Mechanistic In Vitro Assays. *In vivo* studies (or whole animal studies) are based on the assumption that chemicals that cause injury to animals may have similar impacts on humans. Accordingly, data from animal studies can be extrapolated to humans, taking into account interspecies differences. While knowledge regarding a chemical's mechanism of toxicity is certainly relevant, *in vivo* testing and its use in hazard and risk assessment does not depend upon mechanistic understanding of toxicity. The heavy reliance upon animal testing has come under increasing fire over the last decades. Some challenges are grounded in scientific concerns regarding the efficacy of such testing in predicting human outcomes. Others are based upon its high cost and time consuming nature; still others focus upon protection of the animals subjected to the testing.[15]

In vitro testing does not involve use of whole animals; rather it focuses upon the effects of chemicals upon cells, cell lines or biological molecules (such as proteins), preferably but not always of human origin. Importantly *in vitro* testing of the sort envisioned by recent reports by the National Academy of Sciences and by European authorities is mechanistically based. Chemicals of interest are introduced into the testing medium, and observations are made regarding changes in biologic processes that may lead to toxicity. In this way, researchers can evaluate whether the tested material is implicated in the initiation or progression of a pathway of toxicity. *In vitro* assays can also provide information regarding the relative potency of materials as compared to other reference materials or alternatives.

There are a variety of *in vitro* assays using different types of cells or molecules, and focusing upon different mechanisms of injury such as enzyme inhibition, cell membrane injury, and oxidative stress (injury caused by chemically reactive molecules containing oxygen.) For example, in one *in vitro* study researchers exposed white blood cells and bronchial epithelial cells to nano-sized metal oxides, using assays to measure signs of oxidative stress in the cell functions.[16] Another study used *in vitro* assays to evaluate whether antimicrobials such as triclosan may affect endocrine and non–endocrine-signaling systems. The mechanistically

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[12] *See* H. Niskaa, *et al.*, *Characterisation of the Chemical and Biological Properties of Molecules with QSAR/QSPR and Chemical Grouping, and its Application to a Group of Alkyl Ethers*, 19 SAR AND QSAR IN ENVIRONMENTAL RESEARCH 263 (2008).
[13] Timothy F. Malloy, *Principled Prevention*, 46 ARIZ. ST. L. J.105 (2014).
[14] ECHA, GUIDANCE ON THE PREPARATION OF AN APPLICATION FOR AUTHORISATION (ECHA-11-G-01, January 2011).
[15] Shahnaz Bakand, *et al.*, *Toxicity Assessment of Industrial Chemicals and Airborne Contaminants: Transition from In Vivo to In Vitro Test Methods: A Review*, 17 INHALATION TOXICOLOGY 775 (2005).
[16] Tian Xia, *et al.*, *Comparison of the Mechanism of Toxicity of Zinc Oxide and Cerium Oxide Nanoparticles Based on Dissolution and Oxidative Stress Properties*, 2 ACS NANO 2121 (2008); *see also* Andre Nel, *et al.*, *Toxic Potential of Materials at the Nanolevel*, 311 SCIENCE 622 (2006).

derived assays measured the effect of the antimicrobials on nuclear receptors, proteins found within cells that activate in the presence of certain hormones to regulate gene expression.[17]

Data from *in vitro* testing has been used in alternatives analysis for a range of hazard endpoints. EPA's Design for the Environment program relies upon *in vitro* data in assessing mutagenicity and endocrine activity.[18] The EU's REACH program does not limit *in vitro* data to particular endpoints. Instead it 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.[19]

High Throughput Screening. The value of mechanistically-based *in vitro* assays can be vastly enhanced through the use of high throughput screening (HTS). Taking advantage of advanced robotics and automation of the testing, HTS allows researchers to test hundreds or even thousands of materials at once across a range of concentrations for a variety of parameters.[20] The cells or molecules of interest are placed in small wells on plates; HTS plates typically have 384, 1536, or 3456 wells. The materials to be tested are added to the wells, and relevant readings are automatically made at pre-determined intervals. Readings are made through such techniques as epifluorescense microscopy, luminescence-based reporter gene activity, multiplex cytokine assays and brightfield microscopy.[21] In addition to human and other mammalian cells, HTS has also been used with bacteria, yeasts and small whole organisms such as zebrafish embryos. However, not every *in vitro* assay is amenable to HTS. Researchers must choose assays that are relevant to the type of toxicity and mechanism of concern, and which can be successfully miniaturized and automated.[22]

High Content Screening (HCS) is a variant of HTS in which the readout of the assay captures images. 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 material on zebrafish embryos would generate quantitative data regarding hatching, developmental abnormalities and mortality using high content imaging software.

HTS and HCS generate a wealth of data, so much so that specialized tools and strategies are needed to sort through it, separate relevant information from noise, and organize it to facilitate

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[17] Ki Chang Ahn, *et al.*, *In Vitro Biologic Activities of the Antimicrobials Triclocarban, Its Analogs, and Triclosan in Bioassay Screens: Receptor-Based Bioassay Screens*, 116 ENVT'L HEALTH PERSP. 1203 (2008).
[18] EPA, DESIGN FOR THE ENVIRONMENT PROGRAM ALTERNATIVES ASSESSMENT CRITERIA FOR HAZARD EVALUATION (2011).
[19] ECHA, GUIDANCE ON THE PREPARATION OF AN APPLICATION FOR AUTHORISATION (ECHA-11-G-01, January 2011); ECHA, GUIDANCE ON INFORMATION REQUIREMENTS AND CHEMICAL SAFETY ASSESSMENT PART B: HAZARD ASSESSMENT (ECHA-11-G-16, DECEMBER 2011).
[20] National Academy of Science, TOXICITY TESTING IN THE 21ST CENTURY: A VISION AND A STRATEGY (2007).
[21] Andre Nel, *et al.*, *Nanomaterial Toxicity Testing in the 21st Century: Use of a Predictive Toxicological Approach and High-Throughput Screening*, 46 ACCOUNTS OF CHEM. RES. 607 (2013).
[22] Hemant Varma, *et al.*, *High-Throughput and High-Content Screening for Huntington's Disease Therapeutics*, Donald C. Lo and Robert E. Hughes (eds.), NEUROBIOLOGY OF HUNTINGTON'S DISEASE (2011).

analysis.[23] Typical steps include (1) initial inspection and visualization of the results of each plate to guide subsequent analysis, (2) summarizing and prioritizing results for further testing, and (3) data mining (*e.g*. use of self-organizing maps to cluster together materials that exhibit similar behavior across multiple *in vitro* assays).[24]

Analysis of HTS data may be helpful in AA in at least two ways in the near term. First, concentration-response curves for the tested materials can be generated based on high throughput screening at various concentrations. This allows for comparison of the relative potency of the materials.[25] In 2010, EPA's Office of Research and Development (ORD) used HTS data in that way in a comparative evaluation of eight oil spill dispersants performed in connection with the Deepwater Horizon oil spill. ORD generated concentration-response curves for HTS data based on *in vitro* assays for endocrine and other biological activity.[26] Second, HTS data can be used to generate quantitative structure activity relationships, discussed in detail below.

Quantitative Structure Activity Relationships. Quantitative structure activity relationship (QSAR) analysis uses a mathematical model 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.[27] Development of a robust QSAR depends heavily upon the existence of rich physico-chemical and toxicological data for a large enough set of chemicals. For some endpoints, such as aquatic toxicity, and skin/eye irritation, sufficient information exists and QSARs are well established.[28] For example, ECOSAR is a library of 704 class-based QSARs used by EPA for predicting aquatic toxicity of chemicals, coupled with an expert decision tree for identifying the appropriate chemical class.[29] It provides estimates for six endpoints related to aquatic toxicity. Well accepted QSARs are lacking for many other endpoints such as carcinogenicity, repeated dose toxicity, and developmental toxicity.[30]

QSARs are used somewhat in existing AA frameworks. For example, EPA's DfE program relies upon ECOSAR to predict aquatic toxicity in the absence of data on the chemical. That program also provides for consideration of QSARs with respect to endocrine activity. [31]

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[23] Rong Lui, *et al.*, *HDAT: Web-Based High-Throughput Screening Data Analysis Tools*, 6 COMP. SCI. & DISCOVERY 1 (2103).
[24] Yoram Cohen, *et al.*, *In Silico Analysis of Nanomaterials Hazard and Risk*, 46 ACCOUNTS OF CHEM. RES. 802 (2013).
[25] Fred Parham, *et al.*, *Dose-Response Modeling of High-Throughput Screening Data*, 14 J BIOMOL SCREEN. 1216 (2009)
[26] Richard S. Judson, *Analysis of Eight Oil Spill Dispersants Using Rapid, In Vitro Tests for Endocrine and Other Biological Activity*, 44 Envt'l Sci. & Tech. 5979 (2010).
[27] J.C. Madden, *Introduction to QSAR and Other In Silico Methods to Predict Toxicity*, in Mark T. D. Cronin and Judith C. Madden (eds.), IN SILICO TOXICOLOGY: PRINCIPLES AND APPLICATIONS 11 (2010).
[28] Grace Patlewicz, *Use of Category Approaches, Read-Across and (Q)SAR: General Considerations*, 67 REGULATORY TOXICOLOGY AND PHARMACOLOGY 1 (2013).
[29]Kelly Mayo-Bean, *et al.*, Methodology Document for the ECOlogical Structure-Activity Relationship Model (ECOSAR) Class Program (May 2012).
[30] Patewicz, *supra* n. 29; Mark T. D. Cronin, PREDICTION OF HARMFUL HUMAN HEALTH EFFECTS OF CHEMICALS FROM STRUCTURE in Tomasz Puzyn, Jerzy Leszczynski, and Mark T.D. Cronin (eds.), RECENT ADVANCES IN QSAR STUDIES 305(2010).
[31] EPA, DESIGN FOR THE ENVIRONMENT PROGRAM ALTERNATIVES ASSESSMENT CRITERIA FOR HAZARD EVALUATION (2011).

The REACH program likewise allows for the use of QSARs in lieu of testing data in AA where certain conditions are met. The user must demonstrate compliance with the conditions by using a (Q)SAR Model Reporting Format.[32]

Integration of Alternative Testing Approaches. The growing availability of alternative testing approaches gives rise to two questions relating to integration: (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. The first question regarding the integration of conventional data and alternative testing data is a new twist on an old problem in AA and in environmental health sciences generally. Analysts are often faced with a range of data, including epidemiological data, human studies, animal studies and conventional *in vitro* data. In a given case 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 where emerging testing approaches providing mechanistic information about impacts at the cellular level enter the fray with conventional animal studies. A variety of tools and methods are available to integrate multiple streams of data, ranging from qualitative weight of evidence approaches, to structured forms of systematic review, to quantitative, probabilistic methods.[33]

Now we turn to the question of melding together the four types of alternative testing methods and approaches described above—grouping, mechanistic *in vitro* assays, high throughput screening, and QSAR. It is worth noting at the outset that individually each of them can enhance toxicological assessment in AA. Indeed to varying degrees, they are already in use in regulatory settings generally and in AA in particular. But their greatest value may flow from integrated use. Integration takes a variety of forms and goes by a number of names, including "tiered approaches," integrated testing strategies," and "intelligent testing strategies."[34] There are important differences among the various approaches to integration that are beyond the scope of this background paper. Yet each of them aim to meld together the various methods in an efficient, well-grounded manner. For example, the UC Center for Environmental Implications of Nanotechnology (CEIN) implemented a "bottom up" hypothesis-driven predictive toxicology paradigm. In this paradigm, *in vitro* assays are selected based on hypothesized pathways of toxicity. High throughput *in vitro* screening of well characterized libraries of materials of interest is performed to test these hypotheses, generate hazard ranking, and ultimately elucidate

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[32] ECHA, GUIDANCE ON THE PREPARATION OF AN APPLICATION FOR AUTHORISATION (ECHA-11-G-01, January 2011); ECHA, GUIDANCE ON INFORMATION REQUIREMENTS AND CHEMICAL SAFETY ASSESSMENT PART B: HAZARD ASSESSMENT (ECHA-11-G-16, DECEMBER 2011).

[33] Sung Jin Park, *et al.*, *Dempster-Shafer Theory Applied to a Regulatory Decision Process for Selecting Safer Alternatives to Toxic Chemicals in Consumer Products*, 10 INTEGRATED ENVIRONMENTAL ASSESSMENT AND MANAGEMENT 12 (2013); Andrew A. Rooney, et al., Systematic Review and Evidence Integration for Literature-Based Environmental Health Science Assessments, 122 ENVT'L HEALTH PERSP. 711 (2014); Tracey J. Woodruff, *et al.*, *Environmental Health Sciences: An Evidence-Based Medicine Methodology To Bridge The Gap Between Clinical and Environmental Health Sciences*, 30 Health Affairs 931 (2011); NANOEIA, http://nanoinfo.org/#!/nanoeia/ (last visited September 18, 2014).

[34] Andre E. Nel, *et al*, *A Multi-Stakeholder Perspective on the Use of Alternative Test Strategies for Nanomaterial Safety Assessment*, 7 ACS NANO 6422 (2013); Steven P. Bradbury, Tom C. J. Feijtel, Cornelis J. Van Leeuewn, *Meeting the Scientific Needs of Ecological Risk Assessment in a Regulatory Context*, 38 ENVL'T. SCI. TECH. 463a (2004).

quantitative structure activity relationships that inform assessments of hazard. Limited but essential animal or whole organism studies are performed to validate predictive testing.[35]

CONCLUSION

The comparative perspective of AA creates demand for more toxicological data at lower cost and in shorter time frames than conventional toxicological methods can manage. Alternative testing approaches—some new and some well-established—have the potential to meet this demand. Yet many questions remain regarding which predictive methods or approaches are appropriate for AA and under what conditions. Practical concerns regarding how this data can be integrated with other conventional data in evaluating hazard also must be addressed. During the course of the workshop we will take on these and other related questions.

ANNOTATED LIST OF ADDITIONAL RESOURCES

Steven P. Bradbury, Tom C. J. Feijtel, Cornelis J. Van Leeuewn, *Meeting the Scientific Needs of Ecological Risk Assessment in a Regulatory Context*, 38 ENVL'T. SCI. TECH. 463a (2004): **An overview of integrated strategies for using alternative testing in the ecological setting.**

Yoram Cohen, *et al.*, *In Silico Analysis of Nanomaterials Hazard and Risk*, 46 ACCOUNTS OF CHEM. RES. 802 (2013): **Describing a process for the integration of *in silico* nanomaterial toxicity analysis and fate and transport analysis for environmental impact assessment**.

S. Dimitrov and O. Mekenyan, *An Introduction to Read-Across for the Prediction of the Effects of Chemicals*, in Mark T. D. Cronin and Judith C. Madden (eds.), IN SILICO TOXICOLOGY: PRINCIPLES AND APPLICATIONS 372 (2010): **An introduction to the use of "read across" as a gap filling technique for missing data.**

EPA, ToxCast™: Advancing the Next Generation of Chemical Safety Evaluation (http://www.epa.gov/ncct/toxcast/ last visited Sep. 18, 2014): **A summary of the predictive toxicology paradigm used in the EPA ToxCast program.**

Richard S. Judson, *Analysis of Eight Oil Spill Dispersants Using Rapid, In Vitro Tests for Endocrine and Other Biological Activity*, 44 ENVT'L SCI. & TECH. 5979 (2010): **Application of high throughput screening to the comparative analysis to oil dispersants considered for responding to the Deepwater Horizon disaster.**

R. L. Lipnick, *Correlative and Mechanistic QSAR Models in Toxicology*, 10 SAR AND QSAR IN ENVIRONMENTAL RESEARCH 239 (1999): **An overview of the historical and contemporary use of different types of QSARs.**

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[35] Andre Nel, *et al.*, *Nanomaterial Toxicity Testing in the 21st Century: Use of a Predictive Toxicological Approach and High-Throughput Screening*, 46 ACCOUNTS OF CHEM. RES. 607 (2013).

Rong Lui, *et al.*, *HDAT: Web-Based High-Throughput Screening Data Analysis Tools*, 6 COMP. SCI. & DISCOVERY 1 (2103): **A discussion of web-based data analysis tools for *in vitro* HTS developed by the UC CEIN.**

National Academy of Science, TOXICITY TESTING IN THE 21ST CENTURY: A VISION AND A STRATEGY (2007): **A report making the case for a shift in toxicological paradigm at EPA towards emerging "predictive toxicology" tools.**

Andre E. Nel, *et al*, *A Multi-Stakeholder Perspective on the Use of Alternative Test Strategies for Nanomaterial Safety Assessment*, 7 ACS NANO 6422 (2013): **A discussion of the opportunities, limitations and barriers to the use of alternative testing approaches in toxicological assessment of nanomaterials.**

Andre Nel, *et al.*, *Nanomaterial Toxicity Testing in the 21st Century: Use of a Predictive Toxicological Approach and High-Throughput Screening*, 46 ACCOUNTS OF CHEM. RES. 607 (2013): **A description of the predictive toxicology paradigm used by the UC Center for Environmental Implications of Nanotechnology.**

Organisation for Economic Co-operation and Development (OECD), GUIDANCE ON GROUPING OF CHEMICALS, env/jm/mono (2007): **Guidance developed by OECD on the use grouping as an alternative to animal testing.**

Andrew A. Rooney, et al., Systematic Review and Evidence Integration for Literature-Based Environmental Health Science Assessments, 122 ENVT'L HEALTH PERSP. 711 (2014): **A description and evaluation of the use of systematic review with respect to toxicological assessment.**

Raymond R. Tice, *et al.*, *Improving the Human Hazard Characterization of Chemicals: A Tox21 Update*, 121 ENVT'L HEALTH PERSP. 756 (2013): **A summary of the predictive toxicology paradigm used in the federal government's Tox21 program.**

Tracey J. Woodruff, *et al.*, *Environmental Health Sciences: An Evidence-Based Medicine Methodology To Bridge The Gap Between Clinical and Environmental Health Sciences*, 30 HEALTH AFFAIRS 931 (2011): **A description and evaluation of the use of systematic review with respect to toxicological assessment.**

Table 1
Glossary of Terms

Terms	Description
Adverse Outcome Pathway	An Adverse Outcome Pathway (AOP) is "an analytical construct that describes a sequential chain of causally linked events at different levels of biological organisation that lead to an adverse health or ecotoxicological effect."[36]
Cellular response pathway	A cellular response pathway is an "interconnected pathway composed of complex biochemical interactions of genes, proteins, and small molecules that maintain normal cellular function, control communication between cells, and allow cells to adapt to changes in their environment."[37]
Grouping	Grouping is the arrangement of chemicals or substances (including nanomaterials) into groups based on common attributes.[38]
High throughput screening (HTS)	HTS is "an approach that uses automated tools to facilitate the rapid execution of a large number of assays (that may include 100s to 1000s of substances in each assay) per day to identify and hazard rank substances of concern for subsequent testing." [39]
High content screening (HCS)	HCS is a variant of HTS in which the readout of the assay captures more complex data than in an HTS screen. 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.
***In silico* testing**	*In silico* testing is analysis of toxicity based upon computer simulation.
***In vitro* testing**	*In vitro* testing refers to studies performed with cells or biological molecules.
***In vivo* testing**	*In vivo* testing means studies performed with whole living organisms.
Molecular Initiating Event (MIE)	The MIE is the first point of chemical-biological interaction within an organism which starts the adverse outcome pathway.
Pathway of toxicity (or toxicity pathway)	A pathway of toxicity is a cellular response pathway that, when sufficiently disrupted, is expected to result in adverse health effects.[40]
Systems Biology	"Study of the mechanisms underlying complex biological processes as integrated systems of many diverse, interacting components." [41]
Quantitative/Qualitative Structure-activity relationship	"Structure-Activity Relationship (SAR) is an approach designed to find relationships between chemical structure (or structural-related

[36] OECD, Adverse Outcome Pathways, Molecular Screening and Toxicogenomics (last visited Aug. 8, 2013), http://www.oecd.org/chemicalsafety/testing/adverse-outcome-pathways-molecular-screening-and-toxicogenomics.htm.
[37] National Academy of Science, TOXICITY TESTING IN THE 21ST CENTURY: A VISION AND A STRATEGY (2007).
[38] Organisation for Economic Co-operation and Development (OECD), GUIDANCE ON GROUPING OF CHEMICALS, ENV/JM/MONO(2007).
[39] Andre E. Nel, et al., A Multi-Stakeholder Perspective on the Use of Alternative Test Strategies for Nanomaterial Safety Assessment, ACS Nano 2013 7 (8), 6422-6433;
[40] National Academy of Science, TOXICITY TESTING IN THE 21ST CENTURY: A VISION AND A STRATEGY (2007).
[41] OECD, ENV/JM/MONO(2013)6, Series on Testing and Assessment No. 184, GUIDANCE DOCUMENT ON DEVELOPING AND ASSESSING ADVERSE OUTCOME PATHWAYS (April 17, 2013), http://www.oecd.org/officialdocuments/publicdisplaydocumentpdf/?cote=env/jm/mono(2013)6&doclanguage=en.

Terms	Description
[(Q)SAR]	properties) and biological activity (or target property) of studied compounds. As such it is the concept of linking chemical structure to a chemical property (e.g., water solubility) or biological activity including toxicity (e.g., fish acute mortality). Qualitative SARs and quantitative SARs, collectively are referred to as (Q)SARs. Qualitative relationships are derived from non-continuous data (e.g., yes or no data), while quantitative relationships are derived for continuous data (e.g., toxic potency data).”[42]

[42] OECD, Introduction to (Quantitative) Structure Activity Relationships (last visited Aug. 13, 2014), http://www.oecd.org/chemicalsafety/risk-assessment/introductiontoquantitativestructureactivityrelationships.htm