Future Directions Workshop: Advancing the Next Scientific Revolution in Toxicology

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Thomas Hartung, *Johns Hopkins University, University of Konstanz, and Georgetown University*

Ana Navas-Acien, *Columbia University*

Weihsueh A. Chiu, *Texas A&M University*

Prepared by:
Kate Klemic, *Virginia Tech Applied Research Corporation*
Matthew Peters, *Virginia Tech Applied Research Corporation*
Shanni Silberberg, *Office of the Under Secretary of Defense (Research & Engineering), Basic Research Office*

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Innovation is the key to the future, but basic research is the key to future innovation.

–Jerome Isaac Friedman,
Nobel Prize Recipient (1990)

Preface

Over the past century, science and technology has brought remarkable new capabilities to all sectors of the economy, from telecommunications, energy, and electronics to medicine, transportation and defense. Technologies that were fantasy decades ago, such as the internet and mobile devices, now inform the way we live, work, and interact with our environment. Key to this technological progress is the capacity of the global basic research community to create new knowledge and to develop new insights in science, technology, and engineering. Understanding the trajectories of this fundamental research, within the context of global challenges, empowers stakeholders to identify and seize potential opportunities.

The Future Directions Workshop series, sponsored by the Basic Research Directorate of the Office of the Under Secretary of Defense for Research and Engineering, seeks to examine emerging research and engineering areas that are most likely to transform future technology capabilities. These workshops gather distinguished academic researchers from around the globe to engage in an interactive dialogue about the promises and challenges of each emerging basic research area and how they could impact future capabilities. Chaired by leaders in the field, these workshops encourage unfettered considerations of the prospects of fundamental science areas from the most talented minds in the research community.

Reports from the Future Direction Workshop series capture these discussions and therefore play a vital role in the discussion of basic research priorities. In each report, participants are challenged to address the following important questions:

- How will the research impact science and technology capabilities of the future?
- What is the trajectory of scientific achievement over the next few decades?
- What are the most fundamental challenges to progress?

This report is the product of a workshop held April 28-29, 2022, at the Basic Research Innovation Collaboration Center in Arlington, VA on the future of toxicology research. It is intended as a resource for the S&T community including the broader federal funding community, federal laboratories, domestic industrial base, and academia.

Executive Summary

In the nearly two decades since the human genome was sequenced, the field of toxicology has undergone a transformation, taking advantage of the explosion in biomedical knowledge and technologies to move from a largely empirical science aimed at ensuring the absence of harmful effects to a mechanistic endeavor aimed at elucidating disease etiology based on an understanding of the biological responses to chemicals (including biochemistry) and the impact on organ systems. However, a substantial gap remains between the promise of mechanistic toxicology and its actual impacts on improving human health. Toxicology continues to work in a largely reductionist paradigm of single endpoints, chemicals, and biological targets, whereas it is known that biology and pathobiology involve complex interactions across each of these, with the additional recognition that social stressors also have biological consequences. At the same time, the pace of scientific and technical advances has resulted in a deluge of models and data for understanding toxicological exposure, hazard, and risk that is increasingly challenging to evaluate, integrate and interpret. A critical need, therefore, exists to understand how to leverage these new frontiers in toxicology to achieve the desired long-term impact of improving human health. This fundamental problem addresses the question of what exposures, now or in the future, can contribute to disease and calls for a Human Exposome Project.

The 2007 National Research Council report on *Toxicity Testing for the 21st Century—a Vision and a Strategy*[1] (Tox-21c) was a watershed moment for US toxicology, changing the discussion from whether to change to when and how to change. With knowledge in the life sciences doubling every seven years since 1980 and every 3.5 years since 2010, as well as publications doubling every fifteen years (Bornmann, 2021; Densen, 2011), we now have about 16 times as much knowledge and twice as many publications as in 2007.

The Future Directions in Toxicology Workshop convened on April 28-29, 2022, in Arlington, VA, to examine research challenges and opportunities to usher toxicology into a new paradigm as a predictive science. Hosted by the Basic Research Office in the Office of the Under Secretary of Defense for Research and Engineering, this workshop gathered 20 distinguished researchers from across academia, industry, and government to discuss how basic research can advance the science of toxicology. The workshop aimed at the next generation of a vision for toxicology, **"Toxicity Testing for the 21st Century 2.0—Implementation"** that extends the vision of the 2007 report and adapts it to scientific and technological progress. This report is the product of those discussions, summarizing current research challenges, opportunities, and the trajectory of toxicological science for the next twenty years.

The **vision** developed at the workshop foresees toxicology developing into a **Human Exposome Project** that better integrates the exposure side of disease, focusing on real-world exposures affecting diverse populations over time. Thus, changing the principal approach from a hazard-driven to an exposure-driven paradigm. This new paradigm identifies the relevant human or ecological exposures and then bases the risk assessment process on **exposomics**, forming an exposure/mechanism hypothesis from the multi-omics imprint in biofluids and tissues, and **biomonitoring**, the large-scale sampling and measurement of biospecimen. This new paradigm also incorporates negligible exposures and is focused on ensuring safety instead of predicting toxicity. Another critical aspect of this paradigm is the inclusion of disruptive research technologies, such as **microphysiological systems**, the bioengineering of organ architecture and functionality to model (patho-)physiology, and **artificial intelligence/machine learning** to process the complex data generated for informed decisions. Ultimately, we need to **integrate the evidence** provided by these technologies, especially through **probabilistic risk assessment**. An **evidence-based toxicology** approach ensures confidence and trust in the process by which scientific evidence is assessed for the safety of chemicals for human health and the environment.

The workshop was organized around three key areas that are likely to transform toxicology: 1) employing an exposure-driven approach, 2) utilizing technology-enabled techniques, and 3) embracing broad-scale evidence integration. These three key areas are expected to have a huge impact on the development of three key long-term public health goals: 1) Precision Health, 2) Targeted Public Health Interventions and Environmental Regulations and 3) Safer Drugs and Chemicals, through their distinct perspectives and long-term goals.

Exposure-driven Toxicology

Exposure-driven assessments were not covered in the 2007 Tox-21c report and were only the subject of a parallel NRC report, but the needs for integration into toxicology, for example through exposomics, are increasingly evident. Exposure-driven toxicology, focused on real-world exposures and gene-environment interactions that affect diverse populations can contribute to addressing the three aims identified during the workshop: 1) precision health through the identification of environmental exposures for improved health outcomes in specific populations, 2) targeted public health interventions and environmental regulations to address those environmentally-driven health outcomes and 3) the identification of safer drugs and chemicals. Precision health aims for individual, personalized preventive interventions, and pharmaceutical and non pharmaceutical therapies. Targeted public health Interventions and environmental regulations must address population and spatial-temporal variability in genome and epigenome, as well as exposome. Safer drugs and chemicals shall be attained through in vitro/in silico chemical screening, in vitro/in silico clinical trials and identifying intrinsic and extrinsic susceptibilities.

1 https://pubmed.ncbi.nlm.nih.gov/20574894/

Workshop participants envision a Tox-21c 2.0 that reflects real-world based exposure designs (*in silico*, cellular, organoids, models, organisms, longitudinal epidemiological studies). It will include population-scale measurements that are based on readily available biobanks and ecobanks that inform on the distribution of thousands of chemical and non-chemical stressors in relevant populations (general population, relevant subgroups, disease cohorts). Noteworthy, the exposomics approach potentially can interrogate all types of stressors, not just chemicals, that actually perturb biology and change biomarkers in body fluids. Study designs and computational approaches will be aligned to provide interpretable and actionable results. Ethical issues, policy implications, community engagement, and citizen participation will keep pace with and inform the technology, rather than being exclusively reactive to the technology. In the near-term, a critical first implementation step for exposure-driven toxicology and precision health is to scale-up mass spectrometry technology for high-quality inexpensive assessment of thousands of chemicals that can be tagged to exogenous exposures including non-chemical stressors. Libraries that tag key information for those chemicals (metadata layering) will need to be expanded and developed to facilitate interpretation, and to guide preventive strategies, interventions, and policy recommendations. In the mid-term, technologies will be required that link the exposome with health outcomes, and leverage longitudinal studies and biobanks retrospectively and prospectively, ensuring "FAIR"-ness (Findability, Accessibility, Interoperability, and Reuse of digital assets)[2] . In the long-term (20 years), we envision that exposome-disease predictions and exposome-targeted prevention, and treatment solutions will become part of the toxicology and public health practice landscape, leveraging also other ~omics technologies, genomic information, and clinical characteristics.

Technology-enabled Toxicology

The workshop participants discussed technological advances over the last 10-15 years of great relevance to toxicology in three key areas: cell and tissue biology, bioengineering, and computational methods. Workshop participants noted that while biological technologies, such as stem cell engineering, have emerged as routine, commercial enterprises for biomedical research, their potential in toxicology could be further expanded through a) reliable and genetically-diverse cell sourcing, b) improved protocols to differentiate patient-derived stem cells into adult cell phenotypes across essential tissues, c) integrative and non-invasive biomarkers, d) integration of dynamic physiology and pathophysiology outcomes, e) population heterogeneity and susceptibility through life-courses, and f) biological surrogates for non-chemical stressors. On the bioengineering side, workshop participants noted that technological capabilities, such as microphysiological systems (MPS), have shown many successes in the laboratory but need to be further developed to 1) include a variety of models of increasing architectural complexity (monolayer/suspension cultures, organoids and multi-organoid systems) for different stages of drug/chemical development, 2) better represent healthy and diseased populations by a personalized multiverse of possible futures, 3) codify platform standardization, 4) increase throughput, 5) demonstrate validation against *in vivo* outcomes, 6) incorporate perfusion and biosensors with near real-time outputs, and 7) develop automated fabrication. In addition, the workshop participants noted that the emergence of "big data" and "big compute" has revolutionized much of biology, through the ability to analyze and interpret complex and multi-dimensional information. Computational capabilities and models are of utmost importance for toxicology, serving as the key enabling technology. For instance, AI/Machine Learning has emerged as a key technology to support data mining, predictive modeling, hypothesis generation, and evidence interpretation (e.g., explainable AI). Data acquisition and data-sharing following the FAIR principles is key to unleashing these opportunities. The emergence of these needs in toxicology necessitates widespread use and understanding of these technologies combining them with expert knowledge to yield augmented intelligence workflows. Moreover, given the quantity of information generated and consumed by these new technologies, the workshop participants agreed that there is a need for comparable, compatible, integrable multi-omic databases, quantitative *in vitro* to *in vivo* extrapolation, and the development of *in silico* "digital twins" of *in vitro* and *in vivo* systems.

Evidence-integrated Toxicology

Workshop participants discussed the key challenge of integrating data and methods (evidence streams) in test strategies, systematic reviews, and risk assessments. They agree that evidence-based toxicology and probabilistic risk assessments are emerging solutions to this challenge. Evidence integration across evidence streams (epidemiological, animal toxicology, *in vitro*, *in silico*, non-chemical stressors, etc.) is expected to play a key role in translating evidence into knowledge that can inform decision-making. The group developed a vision to conduct complex rapid/real-time evidence integration by combining advancements made in data-sharing, and application of artificial intelligence (e.g., natural language processing), with the transparency and rigor of systematic reviews. To implement this vision, the workshop participants identified a need for collaborative, open platform(s) to transparently collect, process, share, and interpret data, information, and knowledge on chemical and non-chemical stressors. Creating these platforms is foundational for rapid and real-time evidence integration and will empower all steps of protection of human health and the environment. Several needs were identified to create this platform: 1) software development to create dynamic and accessible interfaces, 2) definitive standards and key data elements to facilitate analysis of meta-data and automated annotation, and 3) consideration for quality control.

In conclusion, the workshop advocates a paradigm shift to "Toxicology 2.0" based on the evidence integration of emerging disruptive technologies, especially exposomics, microphysiological systems, and machine learning. To date, exposure considerations typically follow the identification of a hazard. Future Tox-21c 2.0 must be guided by the identification

2 https://www.go-fair.org/fair-principles/

of relevant exposures through exposomics. The adaptation to technical progress, especially microphysiological systems and AI, requires harmonization of reporting and quality assurance. The key challenge lies in the integration of these different evidence streams. evidence-based medicine can serve as a role model with systematic reviews, defined data search strategies, inclusion and exclusion criteria, risk-of-bias analysis, meta-analysis, and other evidence synthesis approaches. While this is mostly applicable to existing data and studies, a new challenge is the prospective application for the composition of test strategies (Integrated Test Strategies—ITS, Integrated Approached to Testing and Assessment—IATA, and Defined Approaches—DA). A key role for Probabilistic Risk Assessment was also identified. The participants also emphasized the need to ensure validation of these new approaches, as well as expand training, communication, and outreach. Ultimately, it calls for expanding the approach to a **Human Exposome Project**.

Introduction

In 1983, the US National Research Council (NRC) Committee on the Institutional Means for Assessment of Risks to Public Health published a foundational study titled "Risk Assessment in the Federal Government: Managing the Process" (NRC, 1983), commonly referred to as the Redbook. As Lynn Goldman put it, the Redbook "has created a framework for incorporation of toxicology into environmental decision-making that has withstood the test of time" (Goldman, 2003). It was complemented by the 2009 report "Science and Decisions: Advancing Risk Assessment," aka the Silverbook (NASEM, 2009), which highlighted some challenges in the process. Parallel work by another NRC committee resulted in the 2007 NRC report on "Toxicity Testing in the 21st Century" (NRC, 2007), or Tox 21c, which developed "a vision and a strategy" to transform toxicological sciences. The gap analysis of the report has not really changed, as toxicology is still *"time-consuming and resource-intensive, it has had difficulty in meeting many challenges encountered today, such as evaluating various life stages, numerous health outcomes, and large numbers of untested chemicals" and needs "*to use fewer animals and cause minimal suffering in the animals used". The 2007 report was complemented by the NRC reports "Exposure Science in the Twenty-first Century: A Vision and a Strategy" (NRC, 2012) and NASEM (2017a) "Using 21st Century Science to Improve Risk-Related Evaluations." The Tox-21c and subsequent reports have changed the debate about safety and risk assessment of substances in the US and beyond, and led to a remarkable number of initiatives and programs (Krewski 2020). The resulting diversity in approaches combined with an ever-accelerating availability of disruptive technologies calls for a re-conceptualization of the future of toxicology. The way forward is to dissolve the dichotomy of hazard and exposure sciences, embrace the disruptive technological advances, and foster evidence integration from these evidence streams.

In the nearly two decades since the human genome was sequenced, the field of toxicology has undergone a transformation, taking advantage of the explosion in biomedical knowledge and technologies to move from a largely empirical science aimed at ensuring the absence of harmful effects to a mechanistic endeavor aimed at elucidating disease etiology and biological response pathways induced by exposures. However, a substantial gap remains between the promise of mechanistic toxicology and the actualization of the field as a predictive science. For instance, high-throughput *in vitro* and *in silico* toxicity testing remains largely focused on prioritization of individual chemicals for future investigation allowing to focus limited resources on the one hand, but which may on the other hand provide a false sense of safety for "de-prioritized" chemicals. Specifically, these efforts, as well as those aimed at translating such data into hazard or risk have been hampered by inadequate coverage of important biological targets given the limitations of current *in vitro* methods to simulate *in vivo* metabolism or predict effects in different tissues and across different life stages (Ginsberg 2019), inadequate consideration of population heterogeneity, and aiming still to provide assurances of safety rather than quantification of effects across the population. Furthermore, there has been little progress in understanding the complex interactions among chemicals and between chemicals and other intrinsic and extrinsic factors that affect population health, such as genetics and non-chemical stressors, including marginalization and other social determinants of health.

In practice, toxicology largely remains a process based on **reductionist paradigm** (Figure 1, left side), classifying individual chemicals for individual hazards, and investigating simplistically "linear" mechanistic pathways based on individual biological targets. Although significant research and development investment has been made in improving the throughput of toxicology through the advent of *in vitro* and *in silico* technologies, the vast majority of these efforts to make toxicity testing faster, cheaper, and perhaps more relevant are still fundamentally "one at a time" approaches that feed into "one at a time" risk assessments and ultimately "one at a time" decisions. Thus, they ultimately only address a narrow slice of the human-relevant experiences of toxicity, where 1) all exposures are time-dependent mixtures of chemical and non-chemical stressors, 2) every individual has unique susceptibilities and baseline conditions, and 3) multi-factoral, multi-causal outcomes are the norm (Figure 1).

A Proposed Paradigm Shift:
Embracing the Multi-Factorial, Multi-Casual Nature of Toxicity

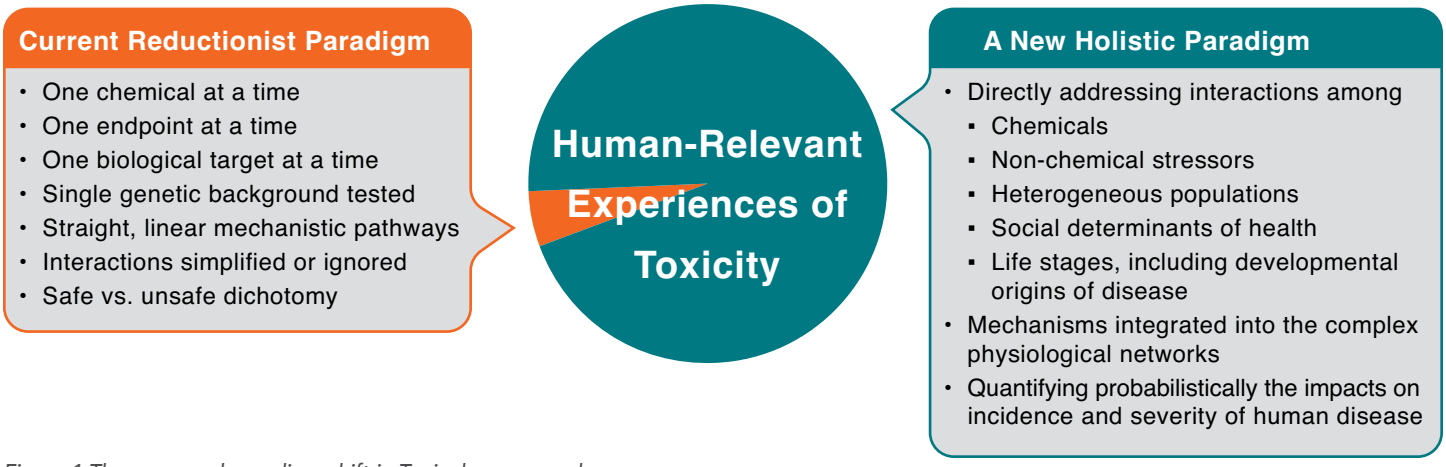

Figure 1 The proposed paradigm shift in Toxicology research.

This report advocates for a fundamental shift to a holistic paradigm where toxicology embraces complexity rather than sweeping it under the rug. Against this backdrop, the workshop was organized around three main research areas (Figure 2) that are key to enabling this paradigm shift.

First, whereas both traditional mammalian toxicity testing, and high-throughput screening assays largely focus on one chemical/mechanism/outcome at a time, this paradigm shift envisions toxicology to be exposure-driven, addressing real-life exposure scenarios in which multiple agents, including social determinants of health, work together to affect multiple mechanistic pathways and health outcomes. Additionally, this paradigm shift requires replacing individual assays that are genetically/epigenetically/exposomically homogeneous with multiplexed systems that incorporate inter-cell/tissue interactions on a backdrop of population variability. Thus, toxicology will become Technology-enabled, leveraging technological advances from genetics to bioengineering to enable the characterization of toxicity in integrated *in vitro/in silico* platforms across the landscape of genomics, epigenomics, life-stage, and non-chemical stressors. Finally, with respect to risk, this paradigm shift requires moving away from single study-based binary (safe/unsafe) decision-making to integrating diverse data across multiple data streams to reach a probabilistic assessment (Maertens, 2022) across multiple outcomes across the population. Thus, especially with the emergence of "big data" along with "big compute," toxicity will be evidence-integrated, combining multiple evidence streams across diverse sources of structured and unstructured information.

The rest of this report summarizes the discussion from the workshop relating to research challenges, research opportunities, and the ultimate trajectory to achieve the vision of a holistic, predictive toxicology.

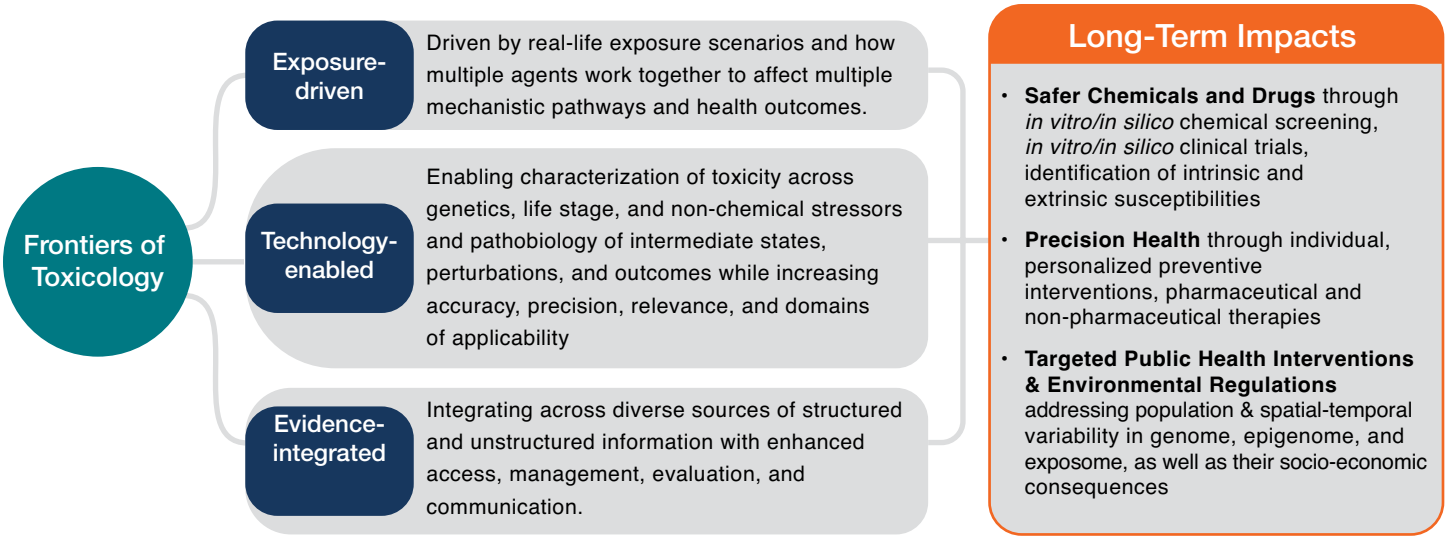

Figure 2 The three workshop topics and the expected long-term impacts.

Toxicology Research Challenges

For many decades the discussion of changing toxicological processes was driven by ethical issues of animal use and the desire to develop and validate so-called "Alternative Methods." In the last two decades, it has become increasingly clear that there are many more reasons to rethink the toolbox of risk sciences (Hartung, 2017a), namely:

- Long duration and low throughput do not match testing or public health needs (Hartung and Rovida, 2009; Meigs, 2018)
- Uncertainty in extrapolating results to humans (NASEM, 1983 [the "Red Book"], 1994, 2009 [Science and Decisions])
- Only single chemical/endpoint at a time; does not account for multiple exposures and non-chemical exposures, including social determinants (Jerez and Tsatsakis, 2016; Bopp, 2019; NASEM, 2009 [Science and Decisions])
- Does not account for inter-individual variability (NAS, 2016)
- Does not incorporate associated socio-economic costs and benefits (Chiu, 2017; Meigs, 2018; NASEM, 2009 [Science and Decisions])

The ongoing transition in terminology in the field from "Alternative Methods" to "New Approach Methods" reflects this broader motivation for change. Tox-21c embraced these challenges and developed a framework of an essentially mechanistic toxicology of perturbed pathways combined with quantitative *in vitro*-to-*in vivo* extrapolation to human exposure (Hartung, 2018). A roadmap of consequential steps was suggested (Hartung, 2009a; Hartung, 2009b).

Workshop participants discussed these overarching challenges to predictive toxicology and defined the key challenges for each area as:

Exposure-driven Toxicology

Populations are exposed to multiple environmental agents, including chemical agents through air, water, food, soil, and non-chemical agents such as noise, light, and social stressors (e.g., racism, socioeconomic deprivation, climate). Therefore, toxicological research that embraces an exposure-driven approach, characterizing real-life exposure scenarios, including exposure mixtures and how these agents work together affecting multiple mechanistic pathways and health outcomes, is needed. A key opportunity is the expansion of exposomic approaches (Sille, 2020; Huang, 2018; Escher, 2020) to include this broader landscape of exposures. The workshop participants highlighted three primary challenges to achieving a more exposure-driven approach:

- **Real-world exposures:** understanding the interplay of environmental and social stressors with genetic and molecular variants
- **Predictive intervention:** understanding the contributions of this research toward the identification and evaluation of effective interventions
- **Targeted populations:** the inclusion of the affected communities through participatory research efforts

There are several reasons why an exposure-driven approach has not yet been embraced. First, many relevant exposures are not yet fully characterized as we lack the tools and technologies needed to characterize these exposures, as well as to understand the health implications. In addition, there has not yet been a successful engagement of the key stakeholders, foremost the populations that are directly affected by these exposures, that is needed for the success of preventive interventions. However, there are currently substantial advances coming in these areas and we can easily anticipate substantial progress in the years to come.

Technology-enabled Toxicology

Predictive toxicology requires expanding the "toolbox" in several directions. The workshop participants identified the key challenges to developing the toolbox as:

- **Broader model systems:** As adverse outcomes involve interactions of the environment (see above), genes, and life stage, we need our "model systems" to cover "gene" and "life stage" more broadly than currently possible using traditional animal studies (e.g., typically inbred strains) or even most current high-throughput testing assays (e.g., typically based on genetically homogeneous immortalized cell lines). Example technologies include genetically diverse population-based *in vitro* and *in vivo* resources, and expansion of experimental designs to cover different stages of development, as well as developmental origins of health and disease.
- **Access to the intermediate state:** Additionally, our approaches currently cluster at the beginning (e.g., high-throughput assays) and the end (e.g., *in vivo* apical endpoints) of the pathophysiological process, neglecting the modulating and stochastic factors that influence outcomes that lie between. Thus, approaches that provide access to intermediate states, perturbations, and outcomes are needed to better understand the progression to disease. Example technologies include novel biomarkers, microphysiological systems (MPS, encompassing organoid and organ-on-chip technologies), and *in silico* models (e.g., systems toxicology/virtual experiments, AI/ML).
- **Assessment tools:** We lack the ability to characterize the predictive accuracy, precision, and relevance of new approaches or to understand their domains of applicability.

Evidence-integrated Toxicology

Toxicology is currently transitioning from a data-poor to a data-rich science with the curation of legacy databases, "grey" information on the internet, mining of scientific literature, sensor technologies, -omics, robotized testing, high-content imaging, and others. The workshop participants identified the key challenges to evidence-integrated toxicology as:

- **Information sources:** There are no established methods or consensus on how to handle new types of information sources (which may be incomplete) or how to weigh evidence strength, risk of bias, quality scoring, etc., or how to integrate the evidence streams.
- **Validation/Verification:** In the case of probabilistic risk assessment, sources of evidence are already integrated, resulting in a more holistic probability of risk/hazard, so the challenge is to determine how to validate real-life, fit for purpose, ground-truthing, qualification, and triangulation, and communicate these probabilities.
- **Data Science:** We have not yet adopted best practices for data curation and storage, data mining, analysis, and visualization.

Toxicology Research Advances and Opportunities

The workshop participants anticipate exciting new research advances on the path to achieving the vision of a holistic, predictive toxicology that addresses real-life exposure scenarios, leverages technological advances, and integrates multiple evidence streams across diverse sources. This section presents those advances and opportunities according to the three workshop themes.

Exposure-driven Toxicology

The risks of developing chronic diseases are attributed to both genetic and environmental factors, e.g., 40% of 560 diseases studied had a genetic component (Lakhani, 2019) while 70 to 90% of disease risks are probably due to differences in environments (Rappaport and Smith, 2010). The Human Genome has been at the center of medical research for the last forty years, but not many major diseases can be explained or treated as a result.

Figure 3 The exposome concept. [Adapted from Vermeulen, 2020].

Understanding exposure effects and genome x exposure (GxE) interactions are thus central to the future of medicine. The original concept of the exposome (Wild, 2016; Vermeulen, 2020), encompassing all exposures of an individual over time, seems to be impractical and unfeasible as a goal (Figure 3). The National Academies of Sciences report (NRC, 2010) has even elaborated on this concept. The 180 million synthesized chemicals, 350,000 of which are registered for marketing in the 19 most developed countries (Wang, 2020) and myriad natural and breakdown products seem to make it impossible to measure and study their effects on humans and the environment. Current approaches in cells or animals can cost from several thousand to a million dollars per substance and health effect (Meigs, 2018).

Worldwide toxicity testing covers only a few hundred substances comprehensively and costs about $20 billion per year. In addition, human and ecological exposure to substances does not occur in isolation of single substances or in any constant exposure scheme. To understand it all or at least a lot of it seems like an impossible mission.

This has led to a hazard-driven approach to toxicology, i.e., an established hazard is followed up with exposure considerations to assess risk. The thresholds of toxicological concern (TTC) (Hartung, 2017b) concept has been introduced to make pragmatic use of this by establishing the fifth percentile of lowest-observed (LOEL) or no-observed effect levels (NOEL) and adding a safety factor of 100. This essentially sets a limit of possible toxicity at one-hundredth of the point of departure below 95% of relevant chemicals. For instance, there is a potential role for TTC to abrogate risk assessment where exposure and/or bioavailability (internal TTC) (Hartung and Leist, 2008; Partosch, 2015) are negligible (Wambaugh, 2015), thus showing a path for how substances could be triaged according to their negligible exposure. However, TTC-type approaches are still a "one chemical at a time" paradigm, may not account for exposures varying temporally or across the population, and do not address potential interactions among the thousands of substances to which people are constantly exposed.

In recent years, the concept of the exposome has been proposed to capture the diversity and range of environmental exposures (e.g., inorganic and organic chemicals, dietary constituents, psychosocial stressors, physical factors), as well as their corresponding biological responses (Vermeulen, 2020). While measuring those exposures throughout the lifespan is challenging, technology-enabled advances, such as high-resolution mass spectrometry, network science, and numerous other tools provide promise that great advances in the characterization of the exposome are possible. Indeed, the exposome approach has achieved traction in recent times because of the availability of -omics technologies (Sille, 2020) as discussed below. A NIEHS workshop (Dennis, 2017) saw the following advantages of an exposome approach:

- Agnostic approaches are encouraged for detection of emerging exposures of concern
- Techniques, and development of techniques promote identification of unknown/emerging exposures of concern
- Links exogenous exposures to internal biochemical perturbations
- Many features can be detected (> 10,000) for the cost of a single traditional biomonitoring analysis.
- Includes biomolecular reaction products (e.g., protein adducts, DNA adducts) for which traditional biomonitoring measurements are often lacking or cumbersome
- Requires a small amount of biological specimen (~100 µL or less) for full-suite analysis

- Enables detection of "features" that are linked to exposure or disease for further confirmation
- Encourages techniques to capture short-lived chemicals
- Aims to measure biologically meaningful lifetime exposures, both exogenous and endogenous, of health relevance

A number of research studies have started to apply these targeted and untargeted technologies to characterize those complex exposures and how they impact health and disease, and which relevant pathways are affected. For instance, birth cohort studies are attempting to characterize those complex and cumulative exposures during critical windows that are of increased importance for long-term human health (Figure 4). Those exposures are not limited to chemical exposures and consider non-chemical stressors throughout the lifespan. The concept of cumulative exposures is critical, as some communities are disproportionally exposed to a cumulation of chemical and non-chemical exposures which over time can result in adverse health outcomes.

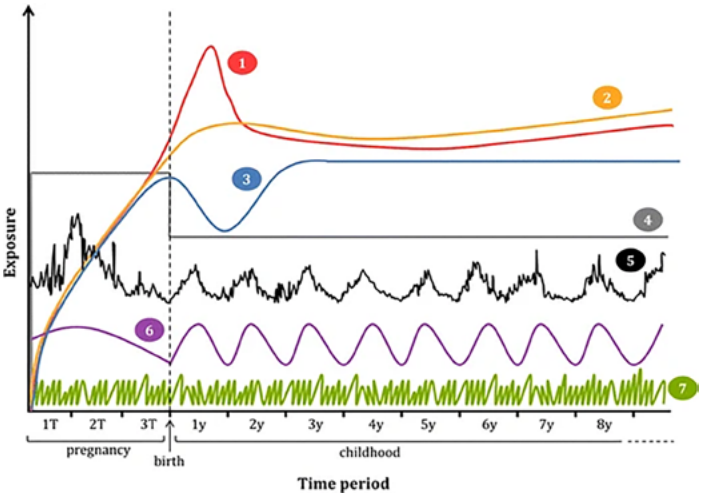

Figure 4 The early life exposome. Examples of relevant exposures and their exposure patterns during pregnancy and childhood, including 1) persistent organic pollutants (POPs), 2) mercury, lead, 3) arsenic, 4) secondhand smoke, 5) air pollution, noise, 6) UV radiation, seasonal exposure to chemicals, 7) non-persistent pollutants. Other exposures such as psychosocial stressors could follow different exposure patterns. [Adapted from Robinson, 2015].

These laboratory and exposure sciences advances also require, in parallel, advancement in biostatistics and data science, to maximize the information that can be obtained from those high-dimensional data. For instance, elastic-net regularization regression is becoming a popular machine learning tool that can be used to identify the relevant predictors from these complex sets of exposure data. For instance, these high-dimensional models were of great relevance to identifying key factors associated with endogenous intermediate pathways (e.g., inflammation, protein damage, oxidative stress, and others) in a pregnancy cohort from Massachusetts called the LIFECODES cohort (Aung, 2021). These types of cohort studies with complex exposure data, in diverse populations, prospective follow-up, and high-quality health outcome data will continue to grow and will become key tools to advance exposure-driven toxicology.

Technology-enabled Toxicology

The near exponential growth in biotechnology and bioengineering over the last few decades has created numerous technologies that could be applied or leveraged in toxicology. Here we highlight three complementary technology areas that have the potential to vastly increase the coverage, biological relevance, and depth of data available for assessing the human health effects of chemical exposures.

Induced Pluripotent Stem Cell Technologies

The discovery that somatic cells can be reprogrammed to become pluripotent, recognized by the Nobel Prize in Physiology or Medicine in 2012, has led to a vast array of advances in biomedical science, from basic cell biology to regenerative medicine. Thus, induced pluripotent stem cells (iPSCs) are among the most substantial research advances of the 21st century, on par with the sequencing of the human genome, with thousands of publications per year utilizing this technology (Figure 5).

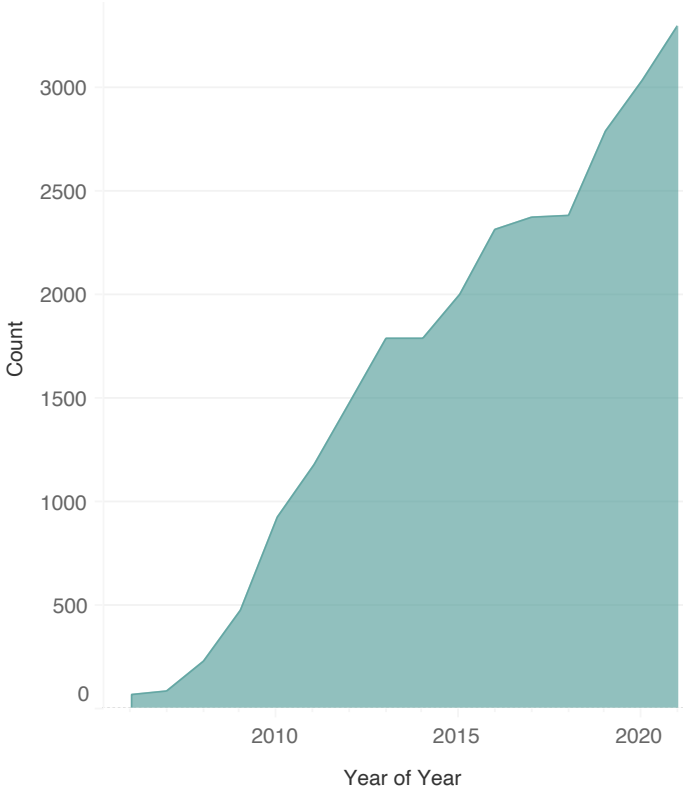

Figure 5 Annual growth in publications for "induced pluripotent stem cells" query in PUBMED, as of July 2022.

In principle, such technologies would enable one to generate unlimited cells and tissues that retain the genetic information of the original donor. Cardiomyocytes were one of the first functional cell types to be successfully differentiated from iPSCs and have gone from research lab to commercial application preclinical safety evaluation of xenobiotics in less than a decade (Burnett, 2021). They have been found to be useful in identifying cardiotoxicity hazards for both drugs and environmental chemicals and are key components of a broad FDA-led initiative

(CiPA)[3] to address drug-induced arrhythmias, see Figure 6. However, even for this relatively "mature" technology of iPSC-derived cardiomyocytes, a number of limitations remain, including their expressing a more fetal-like phenotype and challenges in routine and reproducible differentiation from individual patients. These challenges are even more pronounced for other cell types, as discussed below.

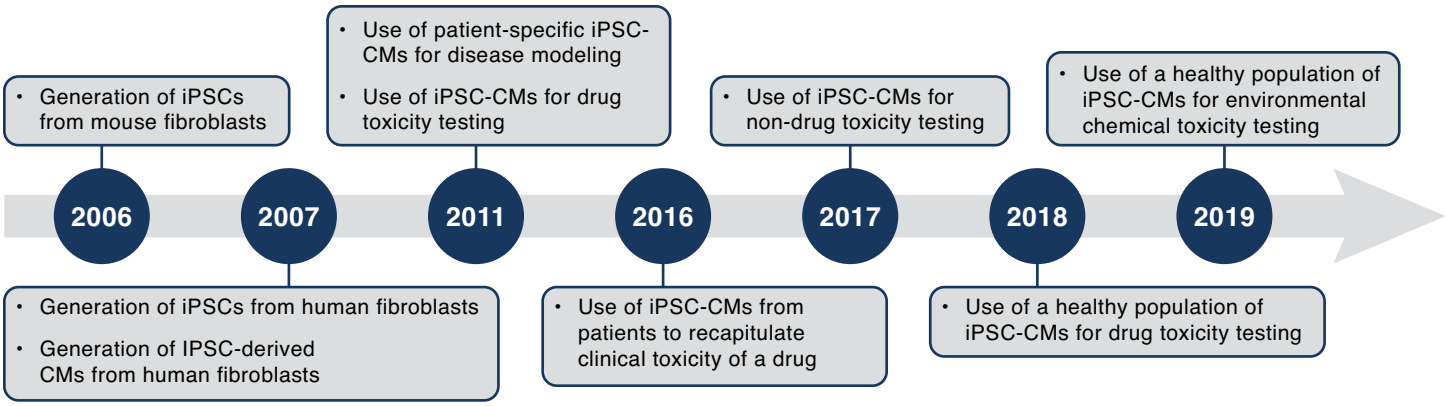

Figure 6 Developmental timeline of induced pluripotent stem cells derived cardiomyocytes (iPSC-CMs) for toxicity testing. [Source: Burnett, 2021]

Nonetheless, the potential for iPSCs to revolutionize biomedical science overall, and toxicology in particular, is well recognized, especially when coupled with the rapid development of advanced *in vitro* and microphysiological technology.

In vitro and Microphysiological Systems (MPS) Technologies

As discussed in "Toxicology Research Challenges", there is an increasing recognition that meeting the needs of toxicology will require expanding beyond the use of traditional preclinical *in vivo* rodent models. These technologies have been termed "New Approach Methods" (Environmental Protection Agency, European Chemicals Agency) or "Alternative Methods" (Food and Drug Administration), and all have the aim of increasing the rigor and predictivity of toxicity assessments while reducing the reliance on vertebrate models. Much of the progress in the last 15 years has been on high-throughput *in vitro* systems, exemplified by the Tox21 Consortium[4], which is a federal collaboration among U.S. Environmental Protection Agency, National Toxicology Program, National Center for Advancing Translational Sciences, and the Food and Drug Administration focusing on "driving the evolution of Toxicology in the 21st Century by developing methods to rapidly and efficiently evaluate the safety of commercial chemicals, pesticides, food additives/contaminants, and medical products." This effort made use of commercially available assay platforms across a wide range of targets, testing almost 10,000 compounds. The screening data generated across a wide diversity of chemicals and potential mechanisms of toxicity has resulted in hundreds of publications, with many lessons learned as to the opportunities and challenges in high-throughput screening data (Richard, 2021).

The workshop participants agreed that more advanced *in vitro* technologies, in particular microphysiological systems (Marx, 2016; Marx, 2020; Roth, 2022), represent the next great opportunity to advance toxicology (NASEM, 2021). An MPS model has been defined as one that "uses microscale cell culture platform for *in vitro* modeling of functional features of a specific tissue or organ of human or animal origin by exposing cells to a microenvironment that mimics the physiological aspects important for their function or pathophysiological condition."[5] These may include a wide variety of types of platforms, from mono-cultures to co-cultures and organoids, and also include so-called "organ-on-chip" models that include an engineered physiological micro-environment with functional tissue units aimed at modeling organ-level responses. These "chip" models consist of four key components:

- microfluidics to deliver target cells, culture fluid, waste discharge
- living cell tissues in either 2D or 3D, including scaffolding, physical, or chemical signals to simulate the microenvironment physiologically
- a system for delivering the drug or chemical, either through the same as the microfluidics delivering culture fluid, or via a separate channel (e.g., air-liquid interface)
- a sensing component that may be embedded (e.g., electrodes), visual (via transparent materials), or assayed from effluent

The mushrooming of MPS models has been fueled by stem cell technologies, 3D cultures (Alepee, 2014), microfluidics (Bhatia and Ingber, 2014), sensor technologies (Clarke, 2021), bioprinting (Fetah, 2019) and others. Figure 7 shows different ways of producing 3D cultures, which are key to creating organ architecture and functionality as key features of MPS. Notably, the MPS field has most recently started to organize itself by annual global meetings and an International MPS Society.[6]

3 https://cipaproject.org
4 https://tox21.gov
5 https://www.fda.gov/science-research/about-science-research-fda/advancing-alternative-methods-fda
6 https://mpsworldsummit.com

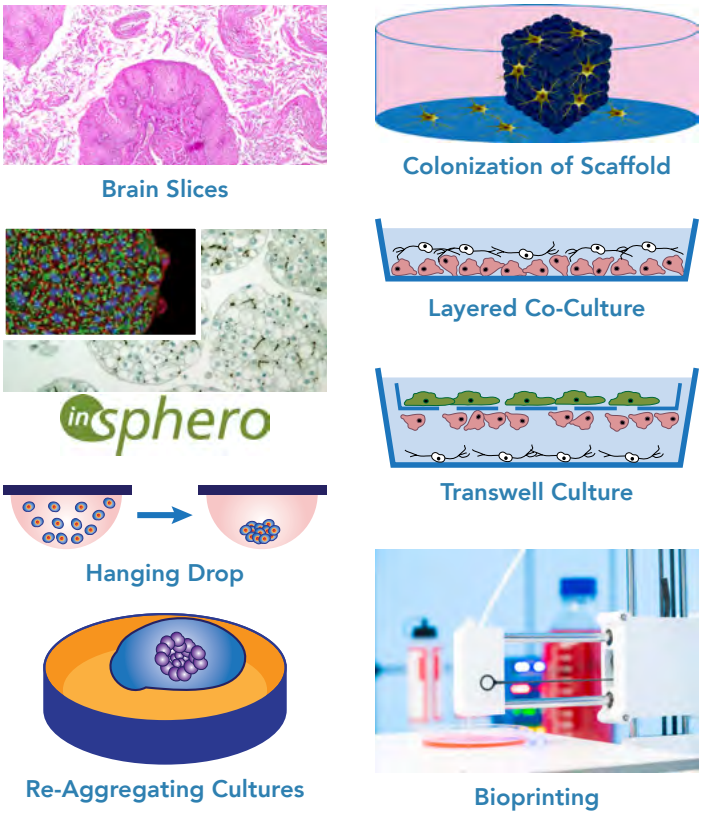

Figure 7 Ways to generate 3D cultures.

MPS models have been developed for nearly every human organ, and several have been linked together into multi-organ platforms (Hargrove-Grimes, 2021) (Figure 8).

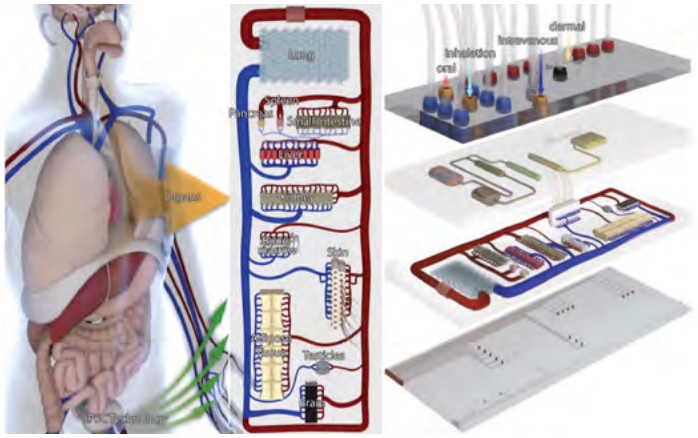

Figure 8 "Man-on-a-chip." [Source: Materne, et al., 2013]

Moreover, applications have been reported in drug development, disease modeling, personalized medicine, and assessment of environmental toxicants. However, many translational challenges remain that hinder the application of MPS in toxicology (Andersen, 2014; Watson, 2017; Nitsche, 2022). Efforts continue to improve external validation, reproducibility, and quality control and to enable technology transfer. Noteworthy, Good Cell and Tissue Culture Practice (GCCP 2.0, Pamies et al., 2022) has expanded these standards to MPS. Overall, the throughput remains low, and the cost remains high, hampering broader application of these technologies, particularly as benchmarking against simpler *in vitro* systems has not always revealed sufficient improvements to warrant the additional time, cost, and complexity. Nonetheless, emerging efforts to define appropriate "context of use" cases for MPS are promising through the continued interactions among researchers, regulators, and the private sector (Hargrove-Grimes, 2021; NAS, 2021).

Imaging and Other High-content Measurement Technologies

The high complexity of MPS and consequential lower throughput make them an ideal match to high-content measurement technologies, which provide through a comprehensive analysis of the biological system maximum insight into the Adverse Outcome Pathway (AOP) in play.

High-content imaging (HCI) combines automated microscopy with image analysis approaches to simultaneously quantify multiple phenotypic and/or functional parameters in biological systems. The technology has become an important tool in the fields of toxicological sciences and drug discovery because it can be used for mode-of-action identification, determination of hazard potency, and the discovery of toxicity targets and biomarkers (van Vliet, 2014). In contrast to conventional biochemical endpoints, HCI provides insight into the spatial distribution and dynamics of responses in biological systems. This allows the identification of signaling pathways underlying cell defense, adaptation, toxicity, and death. Therefore, high content imaging is considered a promising technology to address the challenges for the Tox-21c approach. Currently, HCI technologies are frequently applied in academia for mechanistic toxicity studies and in pharmaceutical industry for the ranking and selection of lead drug compounds or to identify/confirm mechanisms underlying effects observed *in vivo*.

Several ~omics technologies such as genomics, transcriptomics, proteomics, metabolomics, lipidomics etc. represent further high-content technologies allowing deep phenotypic characterization and mechanistic analysis. Hartung and McBride (2011) suggested earlier the use of combined orthogonal ~omics technologies to map pathways of toxicity (PoT) (Kleensang, 2014). Noteworthy, the PoT concept is reminiscent of the AOP approach, which were both proposed independently in 2011. However, there are some fundamental differences (Hartung, 2017c): AOP are designed by experts largely based on their understanding and review of the literature; they are for this reason very much biased by current knowledge/belief and typically not quantitative and difficult to validate experimentally. AOP are narrative, low level of detail, and largely a linear series of events. PoT, in contrast, are deduced from experimental data, especially pathway analysis from untargeted ~omics technologies. PoT are defined on molecular level with high level of detail, integrating emerging information, mainly describing network perturbation. They can be studied further by interventions in the experimental system and often allow quantitative description. This process is not free of biases either and the most promising combination of different omics technologies is still early in development.

Evidence-integrated Toxicology

Toxicology is at the intersection of application and basic science serving as an integrator of health sciences and public health. While this is a very powerful position, the dominance of the regulatory perspective constrains stakeholders. Traditionally, regulation needs predictions regarding single chemicals, but as a consequence, risk assessors are stuck in a system where they are tackling one chemical at a time. Because this is how toxicologists are trained and how regulatory requirements are formulated, toxicology has been shaped into a "one-chemical-at-a-time" science. The entire ecosystem of regulators, the public, and private industry have ended up focusing on understanding the impacts of each specific chemical on health or environmental outcomes, even leading to the creation of trade associations devoted solely to a single chemical. Breaking out of this paradigm, at minimum, requires that toxicologists share the data they collect so it can be assessed and integrated with other data to create a more holistic view of a chemical's risk profile. If a risk assessor choses a new tool, its integration requires broader discussion with regulators and often regulations must ultimately be updated. Ideally, this discussion and any information on the tool is public where others can comment on it. Since there is no centralized effort to do so currently, data sharing falls to the individual toxicologist and is not necessarily a common practice. True change has to come from moving public understanding and regulatory requirements with the field as one; which is very difficult to do at the same time.

Big Data

Eighty-four percent of all data in the world has been produced in the last six years. The scientific literature on the interaction of humans alone is enormous. For illustration: PubMed is estimated to cover 25% of biomedical literature. This database includes about one million new articles per year, of which ~100,000 describe exposures and ~800,000 include some effects of a substance on a biological system. Grey literature, such as the internet, databases of legacy data, -omics technologies, robotized testing, sensor technologies, image analysis etc. continuously add to this knowledge base. A critical challenge is in sharing of these data, which has been a notorious problem in toxicology. Often information is only in the possession of companies and shared with regulators in confidence, if at all. Not only do we have to overcome these hurdles, but we also need to establish data collection and/or meta data standards. This refers in essence to the FAIR principles, i.e., to make data available in a way that others can use them.

Toxicology is thus currently moving from a data-poor to a data-rich science, though too many things are still siloed. Raw data is often behind paywalls or regulatory walls, which can include being shielded from the public with claims that it contains confidential business information. Consequently, we only see the tip of the iceberg and data is often not accessible.

Adding to this, no ontologies or metadata allowing people to make use of each other's data are available. We generate a lot of it every day, and generally do not know how to integrate it unless it is highly curated. Data can be structured by chemical identity. With more and more data available, the field of toxicology becomes dynamic and needs consistent support, e.g., to host a central database online. Such a tool needs agreement regarding how to take data from across datasets. Data needs to be shareable and usable for machine learning. Ideally, a real-time assessment would be implemented based on monitoring (for example integrating data via application programming interfaces (API)), but such broader integration is hindered by various levels of technology used by and available in practice. A possible steward, semantics, standards, and definition of the level of information needed for human prediction are required. Initially, the focus might be on narrow chemical spaces with many studies/replicates.

A central problem of safety assessments is how to define something as safe. Typically, we have enough data to say something is toxic, but when do we know enough to say it is safe? The absence of evidence is no evidence of absence; i.e., a lack of evident toxicity does not mean that it could not manifest under different circumstances that are not adequately covered in the test systems. This calls, on the one hand, for post-marketing surveillance as done for drugs after market entry, or more generally for alertness towards consumer feedback and new scientific findings.

Systematic Review Methods

A central problem of toxicology is evidence integration (enabling integration of diverse, cross-disciplinary sources of information) as more and more methodologies and results, some conflicting and others difficult to compare, are accumulating. This is a challenge faced in more and more risk assessments, but also in many systematic review methods that need to combine different evidence streams (NASEM, 2011, 2017b, 2021; Woodruff and Sutton, 2014; Samet, 2020; EPA, 2020; EFSA and EBTC, 2018). Evidence integration is needed on very different levels of data, studies and to other stressors, as well as across evidence streams. The central opportunities are in quality assessment and AI, especially natural language processing (NLP). There needs to be a common platform, especially on the data side for dynamic modeling ("dynamic data requires dynamic models"), sharing, quality control, hardware and software, standards, metadata, automated annotation, continuous adaptation to AI progress (e.g. explainable AI), role model evidence-based medicine, composition of test strategies, validation, and probabilistic risk assessment. This collaborative open platform to transparently collect, process, share, and interpret data, information and knowledge on chemical and non-chemical stressors will enable real-time and rapid evidence integration, empowering all steps of protection of human health and the environment. The combination of tests and other assessment methods in integrated testing strategies (Hartung, 2013; Tollefsen, 2014; Rovida, 2015), a.k.a. IATA or DA by the Organisation for Economic Co-operation and Development, needed to integrate different types of evidence.

Role of Machine Learning and Artificial Intelligence

We need evidence integration on the levels of data, information, knowledge, and ultimately action. A system for integrating across different levels of information that are each integrated within

their own space, requires broad integration of quality information with the proper infrastructure to support this. The vision is to create an infrastructure with harmonized agreement on the levels of information and for what they may be best suited. For this and its broad use, more toxicologists with computational skills are needed. We also need common vocabularies across different levels of information, data architectural standards for release and utilization, real-time integration (e.g., through APIs), and annotation at different levels. Such annotation requires the connection of raw data to study metadata and the use of language that a computer can digest by NLP through ontologies, standardized "controlled" vocabularies, harmonized templates such as IUCLID (https://iuclid6.echa.europa.eu/), and a library of synonyms. A major question is what can be done to make sure data is encoded/tagged to make it useful? High-quality training sets for annotation to build knowledge graphs, causal networks, etc. need to be developed. However, the use of annotation/ structured databases is an old way of looking at things. It is almost impossible to get people to conform to data annotation guidelines, so instead the field will need to embrace methods to manage unstructured data.

We might rather spend energy on building better NLP and deep learning technologies to analyze unstructured data. The enormous progress on NLP in recent years means that we are moving very close to surpassing the Turing Test, if we have not already. The Turing Test is a deceptively simple method of determining whether a machine can demonstrate human intelligence. If a machine can engage in a conversation with a human without being detected as a machine, it has demonstrated human intelligence. Over the last two years, enormously large models have been trained (Hoffmann, 2022). They use 140 to 530 billion parameters and 170 billion to 1.4 trillion training tokens. Some of these models claim to have been trained on the entire Internet. They can respond in real-time to questions with high accuracy, write articles indistinguishable from those by human authors and even write code for computers. The first impact of the NLP breakthrough is that human knowledge becomes machine-readable. Our vision is that this enables the creation of similar models to virtually grasp the interaction of organisms with chemical substances.

Toxicologists can read and extract information better, but a computer can do this faster on many more sources. We now need to train computers to be as good as humans in interpreting data. AI is the best tool for evidence integration, and evidence integration must become the standard for risk and safety assessments. The big question is: how are people going to use this information generated by AI? Here we need to separate our vision from its implementation. Toxicological research areas and associated S&T advances can overcome hurdles to enable toxicology as a predictive science via evidence integration. From the explosion in the use of machine Learning and data science, the emerging use of NLP, knowledge graphs, and next-generation-omics analytics we need to move to explainable AI, embrace reinforcement learning and modern database management. The platform to be established will need an IT architecture, hardware and software, continuous deployment/support, decision support tools, expert systems etc. Evidence-based methodologies as furthered by evidence-based toxicology[7] (e.g., systematic review principles, risk of bias, meta-analysis, quality scoring, probabilistic approaches) can serve as role models for objective and transparent handling of evidence. Besides making sense of evidence pieces, such a platform can also guide the composition and validation of Testing Strategies (IATAs, DAs, AOP networks) and extraction of human relevant reference datasets.

Probabilistic Approaches

Recognizing that as science delivers only probability rather than absolutes, probabilistic tools lend themselves to all of these (Maertens, 2022; Chiu and Paoli, 2020), will enable us to move away from black/white, toxic/non-toxic dichotomies, as well as better support life cycle and socioeconomic analyses that require evaluation of incremental benefits or risks rather than "bright line" evaluations (NASEM, 2009; Chiu, 2017; Fantke, 2018, 2021). Substantial progress on developing and implementing probabilistic risk assessment approaches has been made in the last 10 years (Chiu and Slob, 2015; Chiu, 2018), with the publication of guidance from the WHO/IPCS (World Health Organization & International Programme on Chemical Safety, 2018). Conceptually, this involves replacing the fixed values currently used for both the initial 'point of departure" dose, as well as the "uncertainty factors" with distributions that reflect the state of the scientific understanding, incorporating and combining uncertainties quantitatively through statistical approaches (see Figure 9 for example applied to the Reference Dose). Several case studies illustrating the broad application of probabilistic approaches have been demonstrated (Blessinger, 2020; Chiu, 2018; Kvasnicka, 2019). Moreover, this conceptual approach to deriving toxicity values probabilistically can be extended to non-animal studies (Chiu and Paoli, 2020), as well as to incorporating population variability through genetically diverse models described above (Chiu and Rusyn, 2018; Rusyn, 2022). In this way, probabilistic approaches provide a framework that facilitates integration across different data types and sources.

7 https://www.ebtox.org

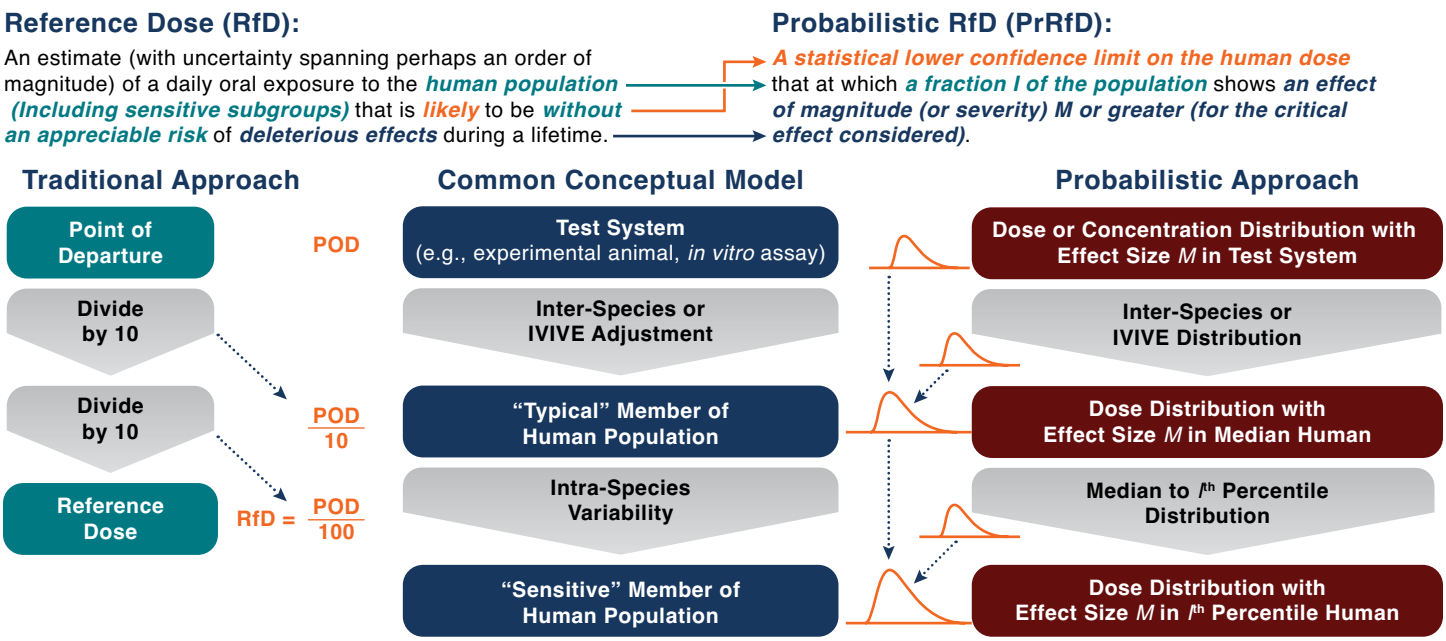

Figure 9 Illustration of the transition from deterministic to probabilistic approaches when deriving reference doses from toxicity data. The "Traditional Approach" refers to the practice attributed to Lehman and Fitzhugh (1954) to derive a "safe dose" by taking the dose level without significant effects in an animal study (a "point of departure") and dividing by a "safety factor" of 100. The "Common Conceptual Model" is an abstraction of this this procedure, whereby information from a test system (whether animal study or other type of data) is first adjusted to the "typical" in vivo human, and then adjusted to account for human variability in susceptibility, thereby deriving dose level that is protective of "sensitive" members of the human population. The "Probabilistic Approach" further incorporates quantitative uncertainty and variability into this conceptual model, using probability distributions at each step instead of single numbers, so that the result is a distribution (reflecting incomplete knowledge) for the dose that would cause on effect of magnitude "M" in the "I"th most sensitive percentile of the human population. [Adapted from World Health Organization & International Programme on Chemical Safety., 2018]

Toxicology Research Trajectory

Recognizing the broad research advances and opportunities that have arisen in the last 15 years since the 2007 Tox-21c report, the workshop participants outlined their vision for the future research trajectory needed to fulfill the promise of transforming toxicology into an exposure-driven, technology-enabled, evidence-integrated field that can better address population and precision health while ensuring safe pharmaceuticals and a safer environment. For each of the three research areas, participants delineated a 5-, 10-, and 20-year plan for building capabilities that would facilitate this transformation.

Exposure-driven Toxicology

Workshop participants identified several major areas of research focus to advance exposure driven-toxicology in the coming decades: 1) real-world-based exposure designs, 2) population-scale measurements, 3) strategies to ask the right questions, and 4) consideration of ethical and policy implications.

Real-world-based Exposure Designs

Developments in this area are needed to allow for better *in silico*, cellular, organoids, model organisms, as well as full populations-based longitudinal studies. These developments will allow studies to be conducted in a way that supports prediction of environmental transport and fate (including chemical transformations, inter-species comparisons, the application of the understanding of exposure levels and exposure mixtures) relevant to the population and its sub-groups. They will also allow us to apply that knowledge to the experimental setting. By using this real-world-based exposure design, the results of different approaches to answering similar questions will be easier to compare, and make it easier to utilize triangulation as a key strategy for assessing the health effect and relevant toxicity pathways of chemical and non-chemical exposures.

Population-scale Measurements

To understand the relevant exposures that lead to disease in general and specific populations, additional efforts are needed to develop biobanks (including biological specimens) and ecobanks (including environmental samples) that inform on the distribution of thousands of chemicals and non-chemical stressors in relevant populations. Factors of interest include relevant exposure scenarios, sociodemographic conditions, and relevant disease or health status. Beyond human populations, the inclusion of animals and the ecosystem for real-world exposure assessment is of relevance to human environmental health, as well as environmental health and toxicology, more broadly. Recent studies, for instance, have shown that exposure assessment efforts in companion animals, such as cats using non-invasive silicon tags, can contribute to the assessment of flame retardants in homes, and their potential role in feline hyperthyroidism (Poutasse, 2019).

Ask the Right Questions

One of the complexities in the current field of omics technology is how to prioritize the right questions in a way that leads to the correct computational approach. For instance, the question might be related to the total mixture, or to specific components of a mixture. Thinking strategically and with the right stakeholders (community, policymakers, interdisciplinary scientists), will contribute to developing those right questions in ways that are most useful for society and respecting privacy concerns that many have regarding the unintended consequences of data sharing.

Ethical and Policy Implications

An important amount of the workshop discussion focused on aspects related to the ethical and policy implications of toxicological research including the disproportionate burden of exposures affecting disadvantaged communities. Groups discussed the need for research to address those concerns by incorporating elements of community engagement, citizen support and environmental justice that must keep pace with the technology.

Anticipated Capabilities

Regarding the key anticipated capabilities for exposure-driven toxicology, the workshop participants anticipated the following achievements as shown in Table 1 and described here:

At 5 years:

- Scale-up technology for high quality inexpensive assessment of 1000-5000 chemicals that can be tagged to exogenous exposures including non-chemical stressors. Technology is currently slow and throughput is not high enough, which makes exposomic approaches expensive.
- Develop libraries that tag key information for those chemicals (meta data layering) to ensure their interpretation. There is currently a lack of validation for many chemical signatures that can be identified with untargeted technologies as to their prediction of health effects.
- These technology problems can be solved through effort and investment, similar to the genome project.

At 10 years:

- The availability of scalable technology for exposomics to achieve high throughput, that is also cheap, sensitive, and specific will allow us to apply this exposure-based approach to longitudinal studies and biobanks.
- Studies that can be both retrospective and prospective ensuring "FAIR" ness and linking exposome with health outcomes.
- Retrospective studies will allow us to go back decades and leverage biobanks. At the same time, we will be able to plan new prospective studies to evaluate the exposures of the future.

20 years:

- Exposome-disease prediction will integrate detailed exposure-based information with health outcome data in large scale and numerous populations. We will achieve a great level of precision in disease prediction that will be environment-based and can also leverage gene-environment interactions.
- This knowledge will provide us with new forms of exposome targeted prevention and treatment.

Table 1: Timeline for Key Exposure-driven Toxicology Developments

Key Capability	Near-term (5-yr) goal	Mid-term (10-yr) goal	Long-term (20-yr) goal
Analytical chemistry	Exposome assays (1000—5000k/person)	High throughput exposome assays (10,000/person)	Exposome disease prediction
Metabolomics, toxicology	Reference exposome library (meta-data layering)	Organ specific disease associated	Exposome targeted treatment and prevention
Epidemiology, clinical research	Disease associated metabolites	Retrospective and prospective studies ensuring "FAIR" and linking exposome with health outcomes	Exposome targeted treatment

Technology-enabled Toxicology

Workshop participants anticipate new technological capabilities in two key research areas to fulfill the promise of transforming toxicology (see Table 2). These include biological capabilities to provide a diversity of cells and tissues and bioengineering capabilities to develop relevant and reproducible assays. Moreover, in each, a set of supporting computational capabilities will need to be developed.

Biological Capabilities

The critical path for biological capabilities lies in the understanding of heterogeneity and susceptibility throughout the life course at multiple scales from cells to the whole organism. As genetics have turned out to be a much smaller factor in outcomes than originally anticipated, there is a need to better understand how non-genetic factors, such as epigenetic differences and social and environmental stressors, individually and collectively modulate development, pathology, and pathophysiology. Because of the diversity in the human population, an important resource for enabling this understanding will be reliable and reproducible sources of cells from multiple tissues representative of the population. Cell sourcing is particularly important because it is likely that *in vitro* microphysiological and other bioengineered systems (discussed below) will play an essential role in untangling these complex interactions. It is also recognized that in parallel, computational capabilities, including multi-omic databases and advances in interpretable AI, will need to be developed to move biological capabilities forward.

Bioengineering Capabilities

With respect to bioengineering capabilities, the main hurdles are: the lack of validated and standardized platforms with automated fabrication, and the lack of availability of individualized cell differentiation to enable personalized toxicological evaluation. It is recognized that a range of fit-for-purpose models, ranging from simple suspensions and monolayers to fully vascularized and innervated multi-organ microphysiological systems, will be developed over time. However, for any of them to be personalized, cell differentiation protocols are needed that can enable creation of multiple tissues from iPSCs from any individual. Coupled with the biological capabilities understanding heterogeneity and susceptibility, these bioengineering capabilities would enable modeling of the diversity of the human population through time. If the goals of automated fabrication and low cost are also achieved, then a "multiverse"-type model platform is envisioned, in which each person could have numerous "chip-based twins" that could predict a range of possible future states depending on different future exposures. As with biological capabilities, a parallel set of computational capabilities will be required, with the goal of creating "digital twins" to go alongside the suite of "chip-based twins."

Table 2: Timeline for Key Technology-enabled Toxicology Developments

Key Capability	Near-term (5-yr) goal	Mid-term (10-yr) goal	Long-term (20-yr) goal
		Biological	
Representative and reliable sources of human cells	National repository of human cells representing key tissues	International repository of human cells representing all major tissues	Repository of human cell types that is representative of population diversity
Determinants of heterogeneity and susceptibility to toxicants	Role of genetic, epigenetic, and social determinants	Understanding impact of timing of exposure and life stage	*In vitro*/*in silico* models for heterogeneity and susceptibility
Understanding of pathology, pathophysiology, and development at multiple scales	Faithful *in vitro* cell differentiation coupled with integrated multi-organ systems	Non-invasive, label-free biomarkers	Integrated biomarkers for modeling normal and diseased organismoids.
Computational support for biological capabilities	Multi-omic databases that are comparable and computable	Integrated multi-omic databases coupled with interpretable AI	

Table 2: Timeline for Key Technology-enabled Toxicology Developments

Key Capability	Near-term (5-yr) goal	Mid-term (10-yr) goal	Long-term (20-yr) goal
		Bioengineering	
Individual-specific cell differentiation protocols	Reproducible cell differentiation for all essential tissues for at least one donor	Multi-donor cell differentiation for all essential tissues	Individualized cell differentiation for all essential tissues
Menu of fit-for-purpose *in vitro* model platforms.	Models representing population diversity	Models representing both health and diseased populations	Models capable of modeling "multiverse" of potential future states
Validated, reproducible, and affordable multi-organ microphysiological "chips"	Platform standardization and automated fabrication for a dozen commercial platforms, validated for individual organs	Commercial/off-the-shelf single-organ chips; validated multi-organ chips; demonstrated personalize chips	Validated personalized "multiverse" chip
Computational support to bioengineering	Quantitative *in vitro* to *in vivo* extrapolation coupled with *in silico* modeling of model platforms	Digital/*in silico* organ modeling	Personalized digital twins

Evidence-integrated Toxicology

Evidence integration (animal studies, human studies, in vitro, and all other types of studies) is needed at different levels of integration, including: raw data, reports/scientific papers (meta-analysis), and data on exposure and hazard. We integrate raw data by interpreting it, and transforming it into information. We integrate different pieces of information in our studies and reports and create knowledge from the body of available studies and papers. Ultimately, we act based on this knowledge, but where do we integrate? Integration across evidence levels requires a systematic review, based on structured data submissions. Curated data sources and models fall between raw data and study reports. The hope is that twenty years from now we will have a system for integrating all these data and factors. This would enable better risk management for public health with enormous societal consequences. But we will also need to address the challenges of communicating the results. Any evidence integration that does not lead to simple classification is a potential communication problem. However, chemicals cannot be simply put into black and white bins (toxic vs. non-toxic) because they exist on a spectrum best characterized by the probability of hazard.

To make Big Data common and encourage its use broadly in toxicology in 10-20 years, we must incentivize people to share information that today is still considered proprietary to businesses. A lot of companies are already required to submit structured information as regulatory data, but there needs to be regulatory change to make the information accessible (possibly with acceptable "blinding" of certain data). A key role of public-private partnerships for data sharing, accessibility, modeling, and cross-sector harmonization was identified. The application of blockchain technology to encrypt pharma/health data and federated model building should be explored. For the animal part of toxicology, we have a moral obligation to make results public. Experimenters received an exemption from society to do something they normally would not (harm an animal), so they owe society the data/outcome in return. The OECD QSAR Toolbox is a pioneering tool that could serve as a model since industry and regulators are less often using tools offered by vendors, especially those tools which use commercial data that's not publicly available. In addition, there are differing levels of transparency requirements (open access vs. open data vs. open source). Cost could also be an issue, and promoting data collection, processes, and trusted data brokers will be needed. Training models with synthetic data sets as done more often with patient/clinical data might be a promising avenue to explore. The FAIR principles (Wilkinson et al., 2016) give important guidance for the accessibility of structured and annotated data to make them useful. Efficient utilization relies on real-time data integration and updates as well as structured, annotated input data to unlock the information contained.

The opportunities come from Big Data (characterized by the 3V's, or volume, variety, and velocity). It is expected that NLP for data extraction will play a big role. Currently, most AI is a black box. To make progress, we will need to move from this paradigm to an explainable AI. Increasingly there is a need to combine prospective and retrospective testing strategies. Policy does not keep pace with the speed of technology. Strategies for enabling evidence integration, such as better integration of various ~omics data from mechanistic toxicology, ~omics data layering at the individual level is needed. We need to understand how to incorporate less understood but equally important variables into the equation, such as microbiome, circadian rhythm, and age. A goal is to develop mechanistic models by changing from data-driven to mechanism-driven. Challenges include:

- Finding incentives and reward structures in institutions to encourage integration of big data approaches
- Defining relevant toxicants information to move through three areas of measurement: time, space, and population. This includes expansion to global populations.
- Small portable technologies to capture genetic and environmental heterogeneity

- Designing a resource-efficient tiered strategy composed of various methods for gathering human data for supervised learning
- Identification of enabler technologies that make analyses affordable and reliable
- Integration of human, animal, *in vitro*, etc. studies in cohesive projects, allowing crosstalk and feedback
- Testing, data analysis, treatment and prevention informed by predictive modeling (recursively applied to testing)
- Cloud-based computing platforms for continuous data and model integration
- Common language so that information can move between the three areas of exposure, technology, and evidence-integration
- Robust informatic infrastructures: graph databases and novel ML over structured and non- structured
- Need for Quality Assurance/Quality Control and validation
- Quality control of MPS reporting input into AI

Table 3 Timeline for Key Evidence-integrated Toxicology Developments

Key Capability	Near-term (5-yr) goal	Mid-term (10-yr) goal	Long-term (20-yr) goal
CompTox literacy	Trainers and trainees with CompTox skills	Establish curricula broadly	Highly skilled CompTox workforce
NLP for decision making	Annotation enabling resources (e.g., synonyms, ontologies, standardized vocabularies); NLP to automatically retrieve/parse study methods [unstructured data]; NLP to learn about relationships in currently structured data	NLP to automatically extract/parse study results; accessible networks of NLP-defined causal networks	NLP to automatically interpret/ combine study results; NLP associations create datasets that inform risk assessments
Data sharing	Global use of IUCLID and other structured repositories and APIs	Platform of networked annotated databases; community engagement in exposure/health data sharing and tracking	Real-time update and analysis via networked platform
Explainable AI for evidence integration	Explainable AI algorithms and modeling pipelines	Interactive decision-support tool that integrate evidence streams	Widespread implementation of AI/ML in decision making; probabilistic risk assessment

Accelerating Progress

Realizing the Tox-21c 2.0 vision needs the Entrepreneurial State[8] (i.e., massive government investment) to create dedicated grants for research, training, and implementation, in addition to efforts to overcome institutional barriers. The evident role model is the Human Genome Project, which largely transformed biomedical science (Hood, 2013). Its costs have been estimated at up to $3 billion.[9] A Battelle report from 2011 appraised the large and widespread economic and functional impacts[10]: "Between 1988 and 2010 the human genome sequencing projects, associated research and industry activity—directly and indirectly—generated an economic (output) impact of $796 billion, personal income exceeding $244 billion, and 3.8 million job-years of employment. In the 2013 update, these numbers increased to economic (output) impact of $965 billion, personal income exceeding $293 billion, and 4.3 million job-years of employment." The transformation of toxicology envisioned here as a Human Exposome Project (HEP) represents a similar opportunity, promising to identify an even larger fraction of causes of disease and opening up new opportunities for prevention and cure.

Governance: The necessarily multi-disciplinary and international character of toxicology requires strategic steering. A cross-agency alliance in the US could play a central role. New metrics for success that measure impact/implementation must be developed. Public-private partnerships are the most promising avenue given their economic prospects. In its build-up, dedicated preparatory programs, infrastructure, partnerships, and engagement with the community have to be developed. To start, a centralized effort is needed to facilitate data sharing and to create the analysis platforms discussed in the evidence integration section.

Education and Training: The challenge in educating and training skill set development is a cross-cutting issue. The lack of multidisciplinary skills was mentioned across all workshop groups, as was the need to strike a balance between what you can and cannot share (privacy, IP, crowdsourcing). A continued dialogue through workshops, papers, strategic plans, policy advice, and implementation is needed. The implementation especially requires training in computational toxicology skills and user comfort with computational tools. Most university curricula are not up-to-date in this respect. The younger generation is very receptive. A portfolio of CompTox training materials would be helpful. It is also a communication challenge to both the workforce and through outreach/engagement (two-way to understand needs; multi-directional communication and training on all levels is key) to the public and decision-makers.

Communication: The assembly of the components for transforming toxicology and revamping risk sciences is a premier communication challenge. Already, personal risk and public health are difficult to communicate to the general public and policymakers. Identifying drivers of disease must present itself not as an anti-industry stance, but a societal need with business opportunities. Communication with end-users to understand and overcome institutional barriers is required. We need to articulate in terms of the problems we are trying to solve.

8 https://marianamazzucato.com/books/the-entrepreneurial-state
9 https://web.ornl.gov/sci/techresources/Human_Genome/project/budget.shtml
10 https://web.ornl.gov/sci/techresources/Human_Genome/project/economics.shtml

Conclusion

This workshop is visionary, looking 10-20 years into the future. Incredibly powerful novel methodologies exist to revamp toxicology; the challenge is their implementation. We have to enable toxicology to keep pace and benefit from cross-sector advances. There is a need for proof-of-concept examples around information retrieval, evidence integration, quality assessment, and decision support. A special opportunity lies in crowdsourcing. Investment in technical infrastructure will facilitate decision support tools (interpretable, actionable, probabilistic, flexible) that integrate multiple evidence streams.

The implementation of this vision is based on several key expectations:

1. *Biomonitoring* and *exposomics* can evolve and be scaled to make toxicology and environmental health more exposure-driven
2. Relevant human model systems can be bioengineered to study disease etiologies and interventions, especially of exposure, as microphysiological systems
3. Computational approaches allow us to scale assessments of chemicals and drugs
4. Evidence integration from these disruptive technologies can guide risk assessment and management

Long-term Impacts

Ultimately advances in these areas would enable transformation in toxicology with lasting impacts in three major ways:

- **Safer Chemicals and Drugs.** Much of toxicology today is focused on ensuring the safety of xenobiotic exposures, whether they be pharmaceuticals intentionally administered or chemicals to which one is incidentally exposed through the environment, consumer products, or occupation. Thus, the most direct impacts of predictive toxicology would be improvements in safer chemicals and drugs through higher throughput and/or higher relevance *in vitro* or *in silico* assays, particularly ones that are better at identifying intrinsic and extrinsic susceptibilities.
- **Precision Health.** Similarly, there is substantial investment already in precision medicine in the form of personalized drug treatment. However, the research described here could broaden this to the concept of precision health, which would not only include personalized pharmacological treatment, but also personalized preventive interventions and non-pharmaceutical therapies. Moreover, while current efforts in precision medicine focus on pharmacogenomics or poly-pharmacy, advances in the frontiers of toxicology discussed above could enable that individualization to extend to the epigenome and exposome, as well as to interventions connected with community health and well-being including access to green-spaces, clean air and water.
- **Targeted Public Health Interventions and Environmental Regulations.** Finally, the concepts of precision health could be expanded to support public health with better targeted public health interventions and environmental regulations. This would better elucidate the toxicological impacts of the genome, epigenome, and exposome, all in combination. Thus, not only could there be better assurance that interventions and regulations are protective of the most vulnerable, but novel approaches may also be revealed that enable better targeting of such measures so that scarce resources can be allocated to achieve the greatest overall benefit.

Ultimately, the workshop participants envision a future for toxicology as a Human Exposome Project in which collaborative, technology-enabled open platforms transparently generate, collect, process, share, and interpret data, information, and knowledge of real-world chemical and non-chemical stressors to enable real-time and rapid evidence integration, empowering all steps of protection of human health and the environment.

Glossary

This is the section provides the definitions for terms used in the body of the report (see also Ferrario et al.,2014; Sille et al., 2020).

Adverse Outcome Pathway (AOP): An AOP is a sequence of events from the exposure of an individual or population to a chemical substance through a final adverse (toxic) effect at the individual level (for human health) or population level (for ecotoxicological endpoints). The key events in an AOP should be definable and make sense from a physiological and biochemical perspective. AOPs incorporate the toxicity pathway and mode of action for an adverse effect. AOPs may be related to other mechanisms and pathways as well as to detoxification routes.

Biomarker: Indicator signaling an event or condition in a biological system or sample and giving a measure of exposure, effect, or susceptibility.

Biomonitoring: The measurement of the body burden of toxic chemical compounds, elements, or their metabolites, in biological substances

Evidence-based toxicology (EBT): EBT is a process for transparently, consistently, and objectively assessing available scientific evidence in order to answer questions in toxicology. Particularly EBT: a) promotes the consistent use of transparent and systematic processes to reach robust conclusions and sound judgments; b) displays a willingness to check the assumptions upon which current toxicological practice is based to facilitate continuous improvement; c) recognizes the need to provide for the effective training and development of professional toxicologists; d) acknowledges a requirement for new and improved tools for critical evaluation and quantitative integration of scientific evidence; e) embraces all aspects of toxicological practice, and all types of evidence of which use is made in hazard identification, risk assessment, and retrospective analyses of causation; f) ensures the generation and use of best scientific evidence; g) includes all branches of toxicological science: human health assessment, environmental and ecotoxicology, and clinical toxicology; h) has the potential to address concerns in the toxicological community about the limitations of current approaches to assessing the state of the science; i) acknowledges and builds upon the achievements and contributions of Evidence Based Medicine/Evidence Based Health Care.

Exposome: Concept describing the totality of exposure experienced by an individual during their life and the health impact of those exposures (Wild, 2005), redefined (Miller and Jones, 2014): The cumulative measure of environmental influences and associated biological responses throughout the lifespan, including exposures from the environment, diet, behavior, and endogenous processes.

Hazard: 1) A biological, chemical, or physical agent with the potential to cause an adverse health effect (European Commission, 2002). **2)** The inherent characteristic of a material, condition, or activity that has the potential to cause adverse effects to people, property, or the environment.

Metabolomics/Metabonomics: Evaluation of cells, tissues, or biological fluids for changes in metabolite levels that follow exposure to a given substance in order to determine the metabolic processes involved, to evaluate the disruption in intermediary metabolic processes that results from exposure to that substance, or to determine the part of the genome that is responsible for the changes. Note: Although "metabolomics" and "metabonomics" are frequently used as synonyms, there is a growing consensus that there is a difference in that "metabolomics" places a greater emphasis on comprehensive metabolic profiling, while "metabonomics" is used to describe multiple (but not necessarily comprehensive) metabolic changes caused by a biological perturbation.

Risk assessment: A scientifically based process consisting of four steps: hazard identification, hazard characterization, exposure assessment, and risk characterization.

Threshold of toxicological concern (TTC): Human exposure threshold value for a group of chemicals below which there should be no appreciable risk to human health.

Toxicokinetics: Generally, the overall process of the absorption (uptake) of potentially toxic substances by the body, the distribution of the substances and their metabolites in tissues and organs, their metabolism (biotransformation), and the elimination of the substances and their metabolites from the body. In validating a toxicological study, the collection of toxicokinetic data, either as an integral component in the conduct of non-clinical toxicity studies or in specially designed supportive studies, in order to assess systemic exposure.

Validation: The process by which the reliability and relevance of a particular approach, method, process, or assessment is established for a defined purpose.

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Appendix I—Workshop Attendees

Workshop Co-chairs

Ana Navas-Acien	Columbia University
Weihsueh Chiu	Texas A&M University
Thomas Hartung	Johns Hopkins University

Workshop Participants

Tony Atala	WakeForest University
Dana Dolinoy	University of Michigan
Lauren Heine	ChemForward
Salman Khetani	University of Illinois at Chicago
Marianthi Kioumortzoglu	Columbia University
Nicole Kleinstreuer	NIEHS NTP
Koren Mann	McGill University
Uwe Marx	TissUse
Patrick McMullen	Scitovation
Gary Miller	Columbia University
Katie Paul- Friedman	US Environmental Protection Agency
Jennifer Sass	NRDC
Kris Thayer	US Environmental Protection Agency
Cavin Ward-Caviness	US Environmental Protection Agency
Cheryl Walker	Baylor University
Katrina Waters	Pacific Northwest National Laboratory
Hao Zhu	Rutgers University

Government Observers

Bindu Nair	OUSD(R&E), Basic Research Office
Jean Luc Cambrier	OUSD(R&E), Basic Research Office
Shanni Silberberg	OUSD(R&E), Basic Research Office
Daniel Osburn	OUSD(R&E), Basic Research Office
Betsy Melebrink	OUSD(R&E), Basic Research Office
Anna Lowit	Environmental Protection Agency
Mark Johnson	US Army Public Health Center
Rabih Jabbour	US Army Edgewood Chemical Biological Center
Natalie Vinas	US Army Engineer Research and Development Center
Louis Scarano	Environmental Protection Agency
Rachel Gooding	Department of Homeland Security, Chemical Security Analysis Center

VT-ARC Team

Matthew Bigman	Virginia Tech Applied Research Corporation
Jordan Brown	Virginia Tech Applied Research Corporation
Christina Houfek	Virginia Tech Applied Research Corporation
Kate Klemic	Virginia Tech Applied Research Corporation
Lynne Ostrer	Virginia Tech Applied Research Corporation

Workshop Participant Short Biography

Anthony Atala, Director, Wake Forest Institute for Regenerative Medicine
Wake Forest School of Medicine
aatala@wakehealth.edu
Anthony Atala, MD, is the G. Link Professor and Director of the Wake Forest Institute for Regenerative Medicine, and the W. Boyce Professor and Chair of the Department of Urology at Wake Forest University. His work focuses on growing human cells, tissues and organs. Fifteen applications of technologies developed in Dr. Atala's laboratory have been used clinically. Dr. Atala was named by Scientific American as one of the world's most influential people in biotechnology, by U.S. News and World Report as one of 14 Pioneers of Medical Progress in the 21st Century, by Life Sciences Intellectual Property Review as one of 50 key influencers in the life sciences intellectual property arena, and by the journal Nature Biotechnology as one of the top 10 translational researchers in the world.

Weihsueh A. Chiu, Professor
Texas A&M University
wchiu@cvm.tamu.edu
Weihsueh A. Chiu, Ph.D. is a professor in the Department of Veterinary Physiology and Pharmacology at Texas A&M University. Before joining the university in 2015, he worked at the U.S. Environmental Protection Agency (EPA) for more than 14 years, most recently as branch chief in the Office or Research and Development. His research in human health risk assessment includes toxicokinetics, physiologically-based pharmacokinetic modeling, dose-response assessment, characterizing uncertainty and variability, systematic review, and meta-analysis, with particular interest in Bayesian and probabilistic methods. Dr. Chiu has participated or chaired expert review panels for multiple government agencies, including NTP, CalEPA, the FDA, and ATSDR. He has also served on numerous national and international committees and workgroups for Health Canada, the World Health Organization, the Organisation for Economic Cooperation and Development, and the U.S. National Academies of Sciences, Engineering and Medicine.

Dana Dolinoy, Chair and Professor
University of Michigan
ddolinoy@umich.edu
Dana C. Dolinoy is Professor of Environmental Health Sciences and Nutritional Sciences and NSF International Chair of Environmental Health Sciences at the University of Michigan School of Public Health as well as Faculty Director of the Epigenomics Core at Michigan Medicine. Her research focuses on how nutritional and environmental factors interact with epigenetic gene regulation to shape health and disease. In 2015, she received the 2015 NIH Director's Transformative Research Award to develop piRNA epigenetic editing technologies and in 2018 received the Society of Toxicology Achievement Award and has recently co-edited the book ToxicoEpigenetics: Core Principles and Applications. She has authored >130 manuscripts and 10 book chapters, and served as Chair of the Gordon Conference in Cellular and Molecular Mechanisms of Toxicity. She has mentored 14 doctoral students, one of whom recently received a F31 award, and 6 post-doctoral fellows, one of whom recently received a NIEHS K99/R00 award, as well as several masters and undergraduate students.

Thomas Hartung, Professor and Chair
Johns Hopkins University
https://www.jhsph.edu/faculty/directory/profile/2308/thomas-hartung
thartun1@jhu.edu
Thomas Hartung is the Doerenkamp-Zbinden Chair for Evidence-based Toxicology in the Department of Environmental Health and Engineering at Johns Hopkins Bloomberg School of Public Health, Baltimore, with a joint appointment at the Whiting School of Engineering. He also holds a joint appointment for Molecular Microbiology and Immunology at the Bloomberg School. He is adjunct affiliate professor at Georgetown University, Washington D.C.. In addition, he holds a joint appointment as Professor for Pharmacology and Toxicology at the University of Konstanz, Germany; he also is Director of Centers for Alternatives to Animal Testing (CAAT, http://caat.jhsph.edu) of both universities.

CAAT hosts the secretariat of the Evidence-based Toxicology Collaboration (http://www.ebtox.org), the Good Read-Across Practice Collaboration, the Good Cell Culture Practice Collaboration, the Green Toxicology Collaboration and the Industry Refinement Working Group. As PI, Dr. Hartung headed the Human Toxome project funded as an NIH Transformative Research Grant and the series of World Summits for Microphysiological Systems started in 2022. He is Field Chief Editor of Frontiers in Artificial Intelligence. He is the former Head of the European Commission's Center for the Validation of Alternative Methods (ECVAM), Ispra, Italy, and has authored more than 625 scientific publications (h-index 105).

Lauren Heine, Director of Science and Data Integrity
ChemFORWARD
www.chemforward.org
lauren@chemforward.org

Lauren Heine applies green chemistry, green engineering, alternatives assessment and multi-stakeholder collaboration to develop tools that result in safer and more sustainable chemical products and processes. Her work with ChemFORWARD builds on prior experience developing GreenScreen's for Safer Chemicals, a pioneering method for chemical hazard assessment to enable informed substitution; and CleanGredients, a web-based information platform for identifying greener chemicals for use in cleaning products; both tools were designed to scale access to information needed to develop materials and products that are safe and circular. Lauren worked closely with the US EPA Safer Choice Program to facilitate development of ingredient and hazard criteria for the Safer Choice Program.

Salman Khetani, Associate Professor and Director of Graduate Studies
University of Illinois at Chicago
mtm.uic.edu
sketani@uic.edu

Salman Khetani is an associate professor of Biomedical Engineering at the University of Illinois at Chicago where he directs the Microfabricated Tissue Models (MTM) laboratory that is engaged in developing *in vitro* models of various tissues (liver, cardiac, intestine, brain, and placenta) for drug screening, disease modeling, and regenerative medicine. Prior to academia, Dr. Khetani co-founded and directed research at Hepregen Corporation, which launched engineered models of the human liver that continue to serve the pharmaceutical industry for elucidating drug metabolism, toxicity, and efficacy for liver diseases. Dr. Khetani's research is currently funded by the US National Science Foundation and National Institutes of Health. His laboratory focuses on engineering the microenvironmental cues around mammalian cells towards stabilizing their long-term phenotype *in vitro* for applications in drug development and regenerative medicine. He has developed model systems to mimic key aspects of diseases *in vitro* and elucidate underlying molecular mechanisms of disease progression as a function of cell-cell, cell-ECM, and cell-soluble factor interactions. His liver models have been translated to the commercial realm through licensing of issued patents and patent applications to companies. Recent work on iPSC-derived atrial cardiomyocytes are used to study the underlying genetic determinants of atrial fibrillation in close collaboration with leading cardiologists.

Marianthi-Anna Kioumourtzoglou, Assistant Professor
Columbia University
https://www.publichealth.columbia.edu/people/our-faculty/mk3961
mk3961@cumc.columbia.edu

Marianthi-Anna Kioumourtzoglou is an environmental engineer and epidemiologist. She holds a Master of Science in Public Health (MSPH) from the Environmental Sciences and Engineering Department at the University of North Carolina at Chapel Hill and a Doctor of Science (ScD) in Environmental Health from the Harvard TH Chan School of Public Health, where she also conducted her post-doctoral fellowship. She is currently an Assistant Professor at the Department of Environmental Health Sciences at Columbia University's Mailman School of Public Health. Her research focuses on applied statistical issues related to environmental epidemiology, including quantifying and correcting for exposure measurement error, exposure prediction uncertainty propagation, and assessment of high-dimensional and complex exposures in health analyses. Her studies mainly (albeit not exclusively) focus on air pollution exposures and, additionally, on identifying vulnerable sub-populations and characterizing how risks may vary across neighborhood-level and other urban characteristics, as well as in a changing climate.

Nicole Kleinstreuer, Director
NICEATM
https://www.publichealth.columbia.edu/people/our-faculty/mk3961
nicole.kleinstreuer@nih.gov
Nicole Kleinstreuer is the acting director of the NTP Interagency Center for the Evaluation of Alternative Toxicological Methods (NICEATM), the US federal resource for alternatives to animal testing. At NICEATM, she leads domestic and international efforts to develop novel testing and analysis strategies that provide more rapid, mechanistic, and human-relevant predictions of potential environmental chemical hazards. Kleinstreuer's research focuses on mathematical and computational modeling of biological systems and their susceptibility to perturbations that result in adverse health outcomes. She has a secondary appointment in the NIEHS Division of Intramural Research Biostatistics and Computational Biology Branch, and adjunct faculty positions in the Yale University School of Public Health and the Eshelman School of Pharmacy at UNC Chapel Hill.

Koren Mann, Professor and Chair
McGill University, Department of Pharmacology and Therapeutics
koren.mann@mcgill.ca
Koren Mann is a Professor and Chair of the Department of Pharmacology and Therapeutics at McGill University, and a Senior Investigator at the Lady Davis Institute for Medical Research in Montreal, Quebec, Canada. She received her doctorate in Pathology and Immunology from Boston University in 1999 and was a postdoctoral fellow at McGill University from 1999-2004. Her laboratory focuses on studying the health effects of metal exposure, although recent studies include other environmental pollutants. She focuses on how modulation of the immune system results in pathology, especially cardiovascular toxicity. The overarching theme of her lab is to integrate toxicology questions within the framework of the epidemiology, providing relevance and feed-forward questions to further interrogate in human cohorts.

Uwe Marx, MD
TissUse GmbH
www.tissuse.com
uwe.marx@tissuse.com
Uwe Marx is the founder and CSO of TissUse, a 2010 spin-out from the Technische Universitat Berlin dedicated to the development of human organ and body-on-a-chip systems for drug testing and precision medicine approaches. The solutions aim to shorten the drug development process and to reduce animal experiments. With more than 30 years of experience in protein drug development and tissue engineering Dr. Marx has published about 150 scientific papers and numerous reviews and book chapters. He is an inventor in more than 30 patent families. Uwe Marx received his doctorate degree in immunology from the Charite of the Humboldt-University in Berlin in 1991 after finishing his medical and biochemistry training. His academic research at the Charite Berlin, the University of Leipzig and the Technische Universitat Berlin focused on human monoclonal antibodies, tissue engineering and human multi-organ chip solutions respectively. Between 2000 and 2010, Uwe Marx joined ProBioGen, a biotech Company he founded in 1994 - as CSO. He served as a reviewer for various German governmental biotech programmes and received several awards for the development of animal-free technologies. Dr. Marx is a serial entrepreneur and co-founder of numerous German biotech companies.

Patrick McMullen, Director of Computational Toxicology
ScitoVation
www.scitovation.com
patrick.d.mcmullen@gmail.com
Patrick McMullen is the Director of Computational Toxicology at ScitoVation. Dr. McMullen works with diverse stakeholders spanning government, non-profit, and industry groups to bring new approaches to toxicology, with the goal of improving chemical safety decision-making processes. His research and consulting work combine high-content biological experiments with statistical and computational approaches to advance the understanding of biological fundamentals that underlie chemical safety challenges. Dr. McMullen's background in molecular biology, engineering, and computational science has been instrumental in interpreting and communicating complex data problems in diverse applications. Dr. McMullen manages a diverse computational biology team that uses modeling and cell-based experiments to deepen our understanding of how chemicals interact with biological systems. Dr. McMullen earned his Ph.D. in Chemical and Biological Engineering from Northwestern.

Gary Miller, Vice Dean for Research Strategy and Innovation
Columbia University
https://www.publichealth.columbia.edu/people/our-faculty/gm2815
gm2815@cumc.columbia.edu
Gary Miller serves as Vice Dean for Research Strategy and Innovation and Professor of Environmental Health Sciences at the Columbia University Mailman School of Public Health. He also has appointments in the Department of Molecular Pharmacology and Therapeutics and the Department of Neurology in the Vagelos College of Physicians and Surgeons. He is an international leader on the exposome, the environmental analogue to the genome. Dr. Miller founded the first exposome center in the U.S. and wrote the first book on the topic. He has helped develop high-resolution mass spectrometry methods to provide an omic-scale analysis of the human exposome. He served as Editor-in-Chief of Toxicological Sciences, the official journal of the Society of Toxicology from 2013-2019 and is now Editor-in-Chief of Exposome, the first journal in the field. He is on the Scientific Advisory Board of NIHs All of Us Research Program, the NIH Human Health Exposure Analysis Resource (HHEAR), and the Human Biomonitoring for the European Union (HBM4EU) project.

Ana Navas-Acien, Professor
Columbia University
https://www.publichealth.columbia.edu/people/our-faculty/an2737
an2737@cumc.columbia.edu
Ana Navas-Acien is a physician-epidemiologist (MD, University of Granada, Spain '96) with a specialty in Preventive Medicine and Public Health (Hospital La Paz, Madrid '01) and a PhD in Epidemiology (Johns Hopkins University '05). Her research investigates the long-term health effects of environmental exposures (arsenic and other metals, tobacco smoke, e-cigarettes, air pollution), relevant molecular pathways, and effective interventions for reducing involuntary exposures. She collaborates with major cohort studies such as the Strong Heart Study, a study of cardiovascular disease and its risk factors in American Indian communities, and the Multi-Ethnic Study of Atherosclerosis (MESA), a study of cardiovascular, metabolic and lung disease in urban settings across the US, and with the TACT2 study (a clinical trial assessing the cardiovascular benefits of metal chelation). Both in the US and internationally, she evaluates exposure to tobacco smoke including e-cigarettes through toxicological and epidemiological research strategies. Her goals are to contribute to the reduction of environmental health disparities in underserved and disproportionately exposed populations.

Katie Paul-Friedman, Toxicologist
US EPA
Paul-friedman.katie@epa.gov
Dr. Katie Paul Friedman joined the Center for Computational Toxicology and Exposure in the Office of Research and Development at the US EPA in August 2016, where she is currently focused on application of new approach methodologies to chemical safety assessment, with additional interests in uncertainty in alternative and traditional toxicity information, endocrine bioactivity and developmental neurotoxicity prediction, and *in vitro* kinetics. One of her roles in the Center is to run the ToxCast program. Previously, Dr. Paul Friedman worked as a regulatory toxicologist at Bayer CropScience with specialties in neuro-, developmental and endocrine toxicity, and predictive toxicology. She has been actively involved in multi-stakeholder projects to develop adverse outcome pathways, alternative testing approaches, and the regulatory acceptance of new approach methodologies. Her laboratory background includes development of high-throughput screening assays, the combined use of myriad *in vitro* and *in vivo* approaches, including receptor-reporter and biochemical assays, primary hepatocyte cultures, and targeted animal testing paradigms, to investigate the human relevance of thyroid and metabolic adverse outcome pathways using probe chemicals. Dr. Paul Friedman received a Ph.D. in Toxicology from the University of North Carolina at Chapel Hill.

Jennifer Sass, Senior Scientist
Natural Resources Defense Council
jsass@nrdc.org
Jennifer Sass is a Senior Scientist at the Natural Resources Defense Council (2001-2021) and part-time faculty at George Washington University Milken School of Public Health (2008-2021). She has published over 50 articles. She holds BSc, MSc, and PhD (1998) degrees from the University of Saskatchewan, College of Medicine, Department of Anatomy and Cell Biology, and a Post-Doctoral Certificate (2000) from the University of Maryland, College of Medicine, Program in Human Health and the Environment.

Kristina Thayer, Director

US EPA, Chemical and Pollutant Assessment Division (CPAD)
thayer.kris@epa.gov

Kristina Thayer is Director of the Chemical and Pollutant Assessment Division (CPAD) at the U.S. Environmental Protection Agency (https://www.epa.gov/aboutepa/about-chemical-and-pollutant-assessment-division-cpad). CPAD occupies an essential position in EPA's Office of Research and Development between researchers generating scientific data and EPA's program and regional offices that make decisions regarding the protection of public health and the environment. CPAD scientists develop a range of fit-for-purpose human health risk assessment products based on the evaluation, synthesis, and analysis of the most up-to-date scientific information. Products include the Integrated Risk Information System (IRIS) and Provisionally Peer Reviewed Toxicity Values (PPRTV) assessments. These products are developed through interactions with EPA's program and regional offices, other agencies, the scientific community, industry, policymakers, and the public. Once finalized, they serve as a major scientific component supporting EPA's regulations, advisories, policies, enforcement, and remedial action decisions. CPAD also conducts cutting-edge research to develop innovative human health risk assessment methods (e.g., systematic review) that facilitate careful evaluation of scientific evidence, as well as tools and models (e.g., benchmark dose modeling software).

Cheryl Walker, Director

Baylor College of Medicine, Center for Precision Environmental Health
cheryl.walker@bcm.edu

Cheryl Walker holds the Alkek Presidential Chair in Environmental Health and is the founder and Director of the Center for Precision Environmental Health at Baylor College of Medicine. She also directs the NIEHS P30 Gulf Coast Center for Precision Environmental Health. Dr. Walker has over 200 publications in the scientific literature and is an elected member of the National Academy of Medicine. Her research on gene:environment interactions and environmental epigenomics has been continuously funded by the NIH, DOD, and Foundations and advocacy groups for over 25 years Her laboratory actively investigates gene x environment interactions and their role in diseases such as cancer, fibroids and NAFLD. The TSC2 tumor suppressor, and its role in cell signaling, has been one of the areas of interest for her lab, in addition to the study of TSC2-linked pathways that regulate key cellular functions.

Cavin Ward-Caviness, Computational Biologist

US EPA
ward-caviness.cavin@epa.gov

Cavin Ward-Caviness is a Principal Investigator in the Public Health and Integrated Toxicology Division of the US Environmental Protection Agency. With a background in computational biology and environmental epidemiology, Dr. Ward-Caviness seeks to understand the environmental factors which influence health in vulnerable populations and the molecular mechanisms that influence environmental health risks. He is the PI of the EPA CARES research resource, which allows researchers to study environmental health effects in vulnerable patient populations, using large electronic health record databases. Ward-Caviness also leads the Environmental Health Domain Team for the National Covid Cohort Consortium. He is also interested in how epigenetics and metabolomics can serve as an early indicator of adverse health effects from chemical and social environmental exposures and in particular how molecular biomarkers can give us insight into how the environment may accelerate the aging process and thus contribute to chronic disease.

Katrina Waters, Director

Pacific Northwest National Laboratory
https://www.pnnl.gov/people/katrina-m-waters
katrina.waters@pnnl.gov

Katrina Waters is a Laboratory Fellow and Director for Biological Sciences Research at the Pacific Northwest National Laboratory (PNNL). Her research interests are focused at the intersection of environmental exposures and infectious disease on human health. Her current programs include the study of health effects of chemicals at Superfund sites and personal environmental exposure assessment for epidemiological studies in disadvantaged communities. She recently completed a Department of Energy research program focused on airborne and environmental transmission of COVID-19. She has also led numerous research efforts in Computational Modeling, Bioinformatics, and Data Management for a NIAID Center for Predictive Modeling of Infectious Diseases and a Department of Homeland Security program for Predictive Modeling of Viral Infections. Dr. Waters holds joint faculty appointments with OSU and the University of Washington.

Hao Zhu, Professor
Rutgers University-Camden
hao.zhu99@rutgers.edu

Hao Zhu is a Professor of Chemistry at the Rutgers University-Camden. His major research interest is to use cheminformatics tools to develop predictive models. All resulted models can be used to directly predict the chemical toxicity based on the public big data and molecular structure information. His current research interests also include data-driven modeling, artificial intelligence algorithm development and computer-aided nanomedicine design. He is the Principal Investigator of several prestigious research grants (NIH R01, R15 and etc). Dr. Zhu is author/co-author of 81 peer-reviewed journal articles and 7 book chapters with over 5,600 citations. His research was recognized with different awards, such as Rutgers Chancellor's Award for Outstanding Research and Creative Activity, National Institute of Environmental Health Sciences (NIEHS) Extramural Paper of the Month (two times, 2019 and 2020) and Drug Discovery Today top citation paper of the year.

Appendix II—Workshop Agenda and Prospectus

Basic Research Innovation Collaboration Center
4100 N. Fairfax Rd. | Fourth Floor| Suite 450
Arlington, VA 22203

DAY 1—THURSDAY, APRIL 28, 2022

Time	Title	Speaker
8:00—8:15	Check-in and Continental Breakfast	
8:15 - 8:20	Welcome and Introductions and Expectations	Thomas Hartung, JHU
8:20 -8:45	Workshop Framing Talk	Co-chairs
8:45—9:00	Breakout Instructions and Morning Break	
9:00—10:45	Working Group I: Define the Problem *Small group discussions to frame a vision for toxicology as a predictive science and identify the greatest hurdles to achieving it.* Group A—Exposure-driven Toxicology Group B—Technical Advances Group C—Evidence Integration	
10:45—11:00	BREAK - *Transition to main conference room and leads prepare outbriefing*	
11:00 –12:00	Working Group 1: Outbriefing	
12:00—1:00	LUNCH *(provided for participants)*	
1:00—3:45	Working Group II: Technical Capabilities and Opportunities *What are the promising research directions for moving to a more predictive toxicology? What are the potential capabilities in the 10- to 20-year horizon?* Group A—Exposure-driven Toxicology Group B—Technical Advances Group C—Evidence Integration	
3:45—4:00	BREAK - *Transition to main room and leads prepare outbriefing*	
4:00—4:45	Working Group II: Outbriefing	
4:45—5:00	Summary of Day	Co-chairs
5:00	MEETING ADJOURNED FOR THE DAY	

DAY 2—FRIDAY, APRIL 29TH, 2022

Time	Title	Speaker
8:00—8:15	Check-in and Continental Breakfast	
8:15—8:30	Welcome and Day 1 Recap	Co-chairs
8:30 -9:30	**'White Space' Discussion I** Discussion of topics which did not fit into the framework of day 1 but need to be discussed.	
9:30—10:30	**'White Space' Discussion II** Discussion of particularly far-out (or long-term), high-risk, high-impact ideas.	
10:30—10:45	BREAK	
10:45—11:45	Discussion of Key Ideas/Components for Report	
11:45—12:00	Closing Remarks	Co-chairs
12:00	DEPARTURE	

Future Directions Workshop: Advancing the Next Scientific Revolution in Toxicology

Basic Research Office, Office of the Under Secretary of Defense (RandE)

28-29 April 2022
Basic Research Innovative Collaboration Center
4100 N. Fairfax Road, Suite 450 Arlington, VA 22203

Co-Chairs: Thomas Hartung (Johns Hopkins), Ana Navas-Acien (Columbia), Weihsueh Chiu (Texas A&M)

In the nearly two decades since the human genome was sequenced, the field of toxicology has undergone a transformation, taking advantage of the explosion in biomedical knowledge and technologies to move from a largely empirical science aimed at ensuring the absence of harmful effects to a mechanistic endeavor aimed at elucidating disease etiology. However, a substantial gap remains between the promise of mechanistic toxicology and the actualization of the field as a predictive science. For instance, high-throughput *in vitro* and *in silico* toxicity testing remains largely focused on prioritization of individual chemicals for future investigation. Moreover, efforts to translate such data into hazard or risk have been hampered by inadequate coverage of important biological targets, inadequate consideration of population heterogeneity, and aiming still to provide assurances of safety rather than quantification of effects across the population. Furthermore, there has been little progress on understanding the complex interactions among chemicals and between chemicals and other intrinsic and extrinsic factors that affect population health, such as genetics and non-chemical stressors, including marginalization and other social determinants of health.

This Future Directions Workshop on Advancing the Next Scientific Revolution in Toxicology aims to establish a new overarching vision for toxicology as a predictive science. This vision entails a major paradigm shift in how toxicology is both conceived and practiced, recognizing the multi-factorial, multi-causal nature of toxicity. Specifically, this vision involves two critical steps:

- Moving away from reductionist interrogation of single chemicals, individual model systems, and discrete biological targets, which ultimately cover only a minute sliver of relevant human experiences.
- Striving for a holistic understanding of the interactions among chemicals, non-chemical stressors, heterogeneous populations, and life-stages, in order to prospectively identify and quantify their impacts on the incidence and severity of human disease.

A key outcome of this Workshop will be a roadmap of key basic science research needs that, if addressed in the next 10-20 years, can substantially advance this transformational vision. The discussions and ensuing distributed report will provide valuable long-term guidance to the DoD community, as well as the broader federal funding community, federal labs, and other stakeholders. Workshop attendees will emerge with a better ability to identify and seize potential opportunities in the different fields addressed. This workshop is sponsored by the Basic Research Office within the Office of Secretary of Defense, along with input and interest from the Services and other DoD components.

Agenda

Rather than a standard conference format, the workshop design emphasizes interactive dialogue with primarily small-group breakout sessions followed by whole-group synthesis of ideas.

Day One: The majority of the first day will be spent in small-group breakout sessions on fundamental challenges to progress and technical capabilities. The three breakout themes include:

1. **Exposure-driven Toxicology**
   Populations are exposed to multiple environmental agents, including chemical agents through air, water, food and soil, and non-chemical agents such as noise, light, and social stressors (e.g., racism, socioeconomic deprivation, climate). Toxicological research that embraces an exposure-driven approach, characterizing real-life exposure scenarios including exposure mixtures and how these agents work together affecting multiple mechanistic pathways and health outcomes is needed. A key opportunity is the expansion of exposomic approaches to include this broader landscape of exposures. The interplay of environmental and social stressors with genetic and molecular variants, and the contributions of this research towards the identification and evaluation of effective interventions will be critical elements for discussion.

2. **Technical Advances and Challenges**
   Predictive toxicology requires expanding the "toolbox" in several directions. First, because adverse outcomes involve interactions of environment (see above), genes, and lifestage, we need our "model systems" to cover "gene" and "lifestage" more broadly.

Example technologies include genetically diverse population-based *in vitro* and *in vivo* resources, and expansion of experimental designs to cover different stages of development, as well as developmental origins of health and disease. Additionally, our approaches currently cluster at the beginning (e.g., high throughput assays) and the end (e.g., *in vivo* apical endpoints) of the pathophysiological process, neglecting the modulating and stochastic factors that influence outcomes that lie between. Thus, approaches that provide access to intermediate states, perturbations, and outcomes are needed to better understand the progression to disease. Example technologies include novel biomarkers, microphysiological systems (e.g., organ on a chip), and *in silico* models (e.g., systems toxicology/virtual experiments, AI/Machine Learning). Finally, a key challenge is characterizing the predictive accuracy, precision, and relevance of new approaches, as well as understanding their domains of applicability.

3. **Evidence Integration**

   Toxicology is currently transitioning from a data-poor to a data-rich science with the curation of legacy databases, "grey" information in the internet, mining of scientific literature, sensor technologies, ~omics, robotized testing, high-content imaging and others. Key questions include how to handle these new types of information sources, which may be incomplete, how to weigh (evidence strength, risk of bias, quality scoring etc.), and how to integrate this evidence. For instance, probabilistic risk assessment integrates across sources of evidence resulting in a more holistic probability of risk/hazard, though challenges include how to validate (real-life, fit for purpose, ground truthing, qualification, triangulation) and communicate these probabilities. Additional challenges, such as data curation and storage, mining, analysis and visualization will be discussed.

Day Two: The second day of the workshop is a half-day consisting of white-space, whole group discussions on topics that did not fall into the Day 1 framework or were especially ambitious and/or high-risk. Participants will also discuss cross cutting themes and the trajectory of the field over the next 10-20 years. At the end of the day, the whole group will discuss the overarching themes of the workshop that should be included in the final workshop report.

Cross Cutting themes to discuss

- What disease endpoints are the most promising in terms of developing the knowledge (e.g., availability of human biomarkers, understanding of genetic/non-genetic risk factors) and technologies (e.g., microphysiological systems, computational models) needed to enable predictive toxicology?
- Where are the greatest opportunities for synergies between toxicology and other disciplines including the social sciences?
- How to ensure standards (e.g., reporting standards, systematic review, meta-analysis, risk of bias analysis, etc.) that retain quality assurance (best practices, validation and other aspects of QA and QC) and public health protection in a mechanistic toxicology?