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Spurred by the vision articulated in the National Academy of Science's (NAS) report Toxicity Testing in the 21st Century (2007) and the availability of advanced methods for biological profiling chemicals, large volumes of data describing in vitro effects have been generated over the last 10 years for thousands of chemicals. Additional important drivers in transforming toxicity testing and safety evaluation include the NAS report Using 21st Century Science to Improve Risk-Related Evaluations (2017) and the passage of the Frank R. Lautenberg Chemical Safety for the 21st Century Act, which amended the Toxic Substances Control Act (TSCA) to require development and implementation of tiered approaches for risk-based safety evaluations and for EPA to develop, evaluate and use of New Approach Methodologies (NAMs). Considerable efforts are now being devoted to interpreting results, determining how these relate to potential health effects, and integrated bioactivity with predicted or measured human exposures, for product safety evaluations (Thomas et al., 2019). This article presents a number of my perspectives on the transformation that NAMs are catalyzing in regulatory safety evaluations and product stewardship of commodity and consumer product chemicals.

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Transforming Regulatory Safety Evaluations Using New Approach Methodologies: A Perspective of an Industrial Toxicologist

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Conflict of Interest

The author had complete control over development of this manuscript. The contents of this manuscript are solely the responsibility of the author and does not necessarily reflect the views or policies of his employer. The author is employed by the American Chemistry Council, an industry trade association that represents a diverse set of companies engaged in the business of chemistry.

Abstract

Spurred by the vision articulated in the National Academy of Science's (NAS) report Toxicity Testing in the 21st Century (2007) and the availability of advanced methods for biological profiling chemicals, large volumes of data describing *in vitro* effects have been generated over the last 10 years for thousands of chemicals. Additional important drivers in transforming toxicity testing and safety evaluation include the NAS report Using 21st Century Science to Improve Risk-Related Evaluations (2017) and the passage of the Frank R. Lautenberg Chemical Safety for the 21st Century Act, which amended the Toxic Substances Control Act (TSCA) to require development and implementation of tiered approaches for risk-based safety evaluations and for EPA to develop, evaluate and use of New Approach Methodologies (NAMs). Considerable efforts are now being devoted to interpreting results, determining how these relate to potential health effects, and integrated bioactivity with predicted or measured human exposures, for product safety evaluations (Thomas et al., 2019). This article presents a number of my perspectives on the transformation that NAMs are catalyzing in regulatory safety evaluations and product stewardship of commodity and consumer product chemicals.

1. Developing Scientific Confidence in New Approach Methodologies

NAMs are defined as any non-animal technology, methodology, approach, or combination of these that can provide information on chemical hazard and risk assessment. NAMs include in silico approaches, in chemico and in vitro assays, high-throughput screening, fit for purpose cell based assays, genomics, proteomics, metabolomics, advanced toxicokenitic methods, and exposure prediction modeling (ECHA, 2016; EPA 2018). Having scientific confidence in the methods and results of an assay or an integrated evaluation is *sine qua non* for regulatory and product stewardship decision making. Traditionally, such this has derived from the standardization and validation of assays and specific decision frameworks. However, because New Approach Methodologies (NAMs) are rapidly evolving, may be proprietary, or involve unique procedures and instrumentation, traditional time-consuming round-robin validation is not optimal for harnessing the power of NAMs to support cutting-edge, best available science product safety evaluations. In addition, the rapid parallel advancement of our knowledge of biological pathways

involved in toxicity and 21st century *in silico* and *in vitro* methods has contributed to new thinking in the chemical safety assessment community concerning approaches applicable to establishing scientific confidence in NAMs to support specific decisions. How do we go about objectively evaluating, transparently documenting, and communicating scientific certainty for NAMs if traditional validation is not employed?

The need to establish confidence in scientific methods is not new, and a numerous guidance documents have been developed to validate and demonstrate confidence in methods; these include, for example, OECD Guidance Document 34 Validation and International Acceptance of New or Updated Test Methods for Hazard Assessment (OECD, 2005); OECD Principles for the Validation, For Regulatory Purposes, of (Quantitative) Structure-Activity Relationship Models (OECD 2004); ECHA's Read-Across Assessment Framework (RAAF) (ECHA, 2017), OECD Guidance Document on the Reporting of Defined Approaches To Be Used Within Integrated Approaches to Testing and Assessment (OECD 2017); OECD's Guidance Document for Describing Non-Guideline In Vitro Test Methods (OECD, 2014), USEPA's Strategic Plan to Promote the Development and Implementation of Alternative Test Methods Within the TSCA Program (USEPA, 2018), etc. Given this apparent abundance of guidance, which is understandable as these various approaches were developed with different assays and specific aims in mind, there's an opportunity to consider the overarching principles involved, and integrate these in a manner that would support more universal application.

With such a goal in mind, we have suggested using an explicit Scientific Confidence Framework (Patlewicz et al, 2015). The step-wise components of this framework include: 1) problem formulation – description of the purpose of the evaluation and the specific decision the results will be used for; 2) analytical validation – documentation of assay sensitivity, specificity, reliability, and domain of applicability; 3) documentation of prediction models –communication of performance characteristics of qualitative and/or quantitative models for predicting/inferring the endpoint(s) of interest from NAMs; 4) transparency – dissemination of the data, inference models, *etc.*, in such a manner that an expert could independently replicate the analyses and documentation of independent scientific peer review(s); and 5) a written narrative explaining why there is sufficient scientific confidence in the NAM to support specific decisions. This Scientific Confidence Framework has been cited, and elements adapted, in the US EPA's Strategic Plan to Promote the Development and Implementation of Alternative Test Methods within the TSCA Program (US EPA, 2018). Such a SCF provides the rigor needed for transparency and the flexibility needed for application in different decision contexts. For example, a higher level of confidence could be required for a regulatory hazard assessment decision compared to a priority setting decision. Moreover, these five components of the SCF are sufficiently broad to be applied widely, but at the same time, are specific enough to ensure the scientific rigor and transparency to underpin safety evaluation decision making. Often, test method developers labor too far removed from NAM users, such as regulatory agencies and private sector product stewardship professionals, which can lead to development of methods that miss the mark in being able to provide sufficient scientific certainty for product safety decision making. Therefore, it would be beneficial to the regulatory science community and the NAM development community if consensus on implementation such a universal SCF for NAMs could be reached.

2. Challenges and Opportunities for Using NAMs

A. Read-Across

How and where will NAMs be used? One of the most likely uses will be to fill data needs for hazard characterization or risk assessment using read across approaches. NAMs could be used for rapid and cost effective *in silico* or *in vitro* biological activity profiling to support the analogue and category approaches for read across to be used to make inferences about toxicity endpoints (e.g. Low et al 2013, Shah et al, 2016).. Read-across inference models can then be developed using datasets comprised of substances that have both biological profiling results from NAMs and existing data for the toxicity endpoint(s). Modeling of properties or endpoints may be qualitative (or quantitative. In the simplest of cases, establishing sufficient confidence in a NAM-based approach for read across could entail results from one specific type of biological profiling data stream (e.g., a receptor binding assay). However, the more likely case will involve evaluating evidence from NAM results from multiple assays, such that a more complicated prediction model or weight of evidence assessment procedure is required to integrate the many different pieces of relevant information. The utility of integrating complementary biological data streams in a read-across has been shown (Ball et al, 2016; Zhu et al., 2016). Using chemical structure analyses and almost 1,000,000 chemical property hazard data points, Luechtefeld et al., 2018 developed read-across models that achieved 70%–95% balanced accuracies for classification of nine hazard endpoints.

B. Transcriptomics as a Measure of Bioactivity

In vitro transcriptomics produces complex datasets that have received considerable attention for use in biological profiling of chemicals. Gathering, evaluating, and interpreting transcriptomics data can be very challenging; a series of papers stemming from a 2016 ECETOC workshop cover many of the key areas required toward achieving scientific confidence in such data to support chemical risk assessments (Kauffmann et al. 2017; Gant et al. 2017; Bridges et al. 2017). Discussions in these series of papers deal with the need to document test conditions, doses/concentrations, and numerous aspects of quality assurance and quality control. The research by US EPA's National Center for Computational Toxicology in conducting concentration response studies using whole cell transcriptomics in MCF-7 cells for more than 1000 compounds will provide valuable insights (Shah, 2018) as will the Tox21 High Throughput Transcriptomics project (https://tox21.gov/projects/; Thomas et al., 2019).

Unlike pharmaceuticals, which are designed to have high biological potency to interact with target sites in cells, commodity chemicals are developed and used to impart functionality to commercial and consumer products. While some commodity chemicals may have biological effects, these typically manifest at much lower potencies and much higher concentrations than pharmaceuticals, both *in vivo* and *in vitro*. Thus, as Thomas *et al*. (2013) point out, for commodity chemicals it will be critically important to utilize NAMs with data evaluation and interpretation methods that can distinguish between selective interaction with a biological pathway (*e.g.*, determination of a putative mode of action (MOA) and non-selective responses at relatively high concentrations (*e.g.*, interactions/perturbations of multiple cellular processes). With these challenges in mind, the case is being made that the points of departure derived from in vitro transcriptomics studies are about 100-fold lower than in vivo traditional PODs (on a mg/kg-day basis), indicating transcriptomics-derived PODs seem to be adequately health protective to support risk-based prioritization and screening (Paul-Friedman, 2018).

Is it necessary to groundtruth in vitro transcriptomics (or other high content data derived from in vitro cell-based NAMs) to in vivo toxicity results? Not necessarily. In fact, in some cases, such an effort would be fatally flawed. For example, there's negative value in trying to establish confidence by comparing in vitro omics data to toxic effects in animal models and/or at high dose levels of little to no relevance to humans. Animal toxicity studies can be subject to considerable variability and inconsistencies, especially if they are not conducted using established test guidelines and good laboratory practices. Therefore, it's not always necessary, nor would it be scientifically justified, to groundtruth NAMs by comparing to existing animal toxicity datasets. As the scientific community's knowledge of biological pathways improve, groundtruthing can be more relevantly established by linking responses of specific substances to knowledge of the dose dependent perturbations in biological pathways and systems that have been established with archetypical substances. As such, the gold standard of toxicity will need to evolve from histopathological diagnoses of adverse effects to knowledge-based interpretations of human relevant biological pathways, taking into consideration both toxicokinetics and toxicodymanics at the pathway and systems levels.

In using transcriptomics for broad biological profiling, there remains the question of how to visualize and interpret the data (McMullen et al., 2019), and how many cell and tissue types would be optimal to provide confidence comparable to that of screening toxicity using an *in vivo* lab animal model? In the human body, there are ~200 different cell types. Would one need to test each of these cell types to cover the relevant biological space? Or would a subset of cell types suffice, and if so which types? Are NAM approaches using isolated cell types sufficient to recapitulate an *in vivo* environment, or does one need to use co-culture or organs-on-a-chip methods to obtain more biologically relevant representative data for profiling the activity of commodity chemicals? These are questions that are ripe for further research as the development of 'omics-based NAMs advance.

C. Complex Substances and UVCBs

Approximately 20% of chemicals on the recently updated EPA active TSCA inventory (https://www.epa.gov/tsca-inventory/how-access-tsca-inventory#download) are substances of unknown or variable compositions, complex reaction products, or biological substances (UVCBs), and there are significant challenges in studying such complex substances. UVCBs and multi-constituent materials cannot be represented by unique structures or defined molecular formulas and are typically comprised of many different individual constituents, each of which may possess different physico-chemical, fate, and toxicological properties. In what may prove to be a particularly enlightening evaluation of complex substances, particularly from the standpoint of contextualizing responses by specific chemicals in such assays, Wetmore *et al*. (2019) evaluated the biological activity of extracts from 30 organically grown fruits and vegetables in terms of their concentration-response using BioMAP assays. These results were then compared to the findings from agrochemicals tested in ToxCast enabling a comparison of bioactivities of ToxCast chemicals to substances (extracts from fruits and vegetables) that are essential components of a healthy diet and are considered to be safe. The results of this research, which was supported in part by the ACC LRI, shows that 1) compounds in fruits and vegetables affected multiple *in vitro* endpoints, 2) these extracts exhibited qualitatively and quantitatively different bioactivity profiles than ToxCast single chemicals (possibly because these extracts are complex mixtures of bioactive phytochemicals), 3) dose-responses arise from the superposition of responses to different components in the complex substance, and 4) bioactivity does not necessarily equate with an adverse response, reinforcing the findings that bioactivity *in vitro* does not equate on a one-to-one basis with

adverse health effects. Given that such healthful substances produce a wide array of bioactivities *in vitro* provides a note of caution when using high-throughput toxicity testing approaches as part of hazard characterization. An additional lesson learned is the value of considering bioactivity in the context of known or expected levels of exposure, since health impacts (be they positive, in the context of a healthful diet) or negative are influenced by both.

D. Integrated Approaches to Testing and Assessment

Another promising area for the use of NAMs is within integrated approaches to testing and assessment (IATA). In the recent (2016) amendments to the US Toxic Substances Control Act (TSCA), the law requires encouraging and facilitating the "use of scientifically valid test methods and strategies that reduce or replace the use of vertebrate animals while providing information of equivalent or better scientific quality and relevance…" (15 USC Ch. 53 §2603(h).) The new TSCA also requires use of "best available science," "weight of the scientific evidence," and "tiered screening and testing process, under which the results of screening-level tests or assessments of available information inform the decision as to whether or more additional tests are necessary…." While the application of high throughput screening (HTS) results to predict adverse effects for use in risk assessment are still aspirational, scientific support is growing for more immediate applications in priority settings to differentiate those substances that may need more testing or evaluation from those that do not. While some IATAs may be strictly geared to evaluating biological activity (as a surrogate for *in vivo* toxicity), it is preferable to explicitly include and integrate exposure at each decision node within an IATA.

For example, as an initial step in such an IATA, ToxCast results for estrogen activity of a chemical can be converted to an oral equivalent dose and compared to the actual or modeled human exposure to calculate a margin of exposure. In Becker *et al.* (2015), we provided a proof-of-concept of this approach, in which we included a comparison of the exposure:activity ratio of a specific compound to the exposure activity ratio of genistein. This enables risk-based and context-based prioritization for further screening. Substances with a wide margin of exposure or substances with an exposure:activity ratio much lower than that of genistein would be deprioritized. For prioritized substances, the next step in such an IATA would be evaluation using an *in vitro*, fit-for-purpose cell-based assay for estrogenic responses. Such a system has been developed by Clewell and colleagues, again as part of the ACC LRI. In addition, to better recapitulate the complexity of estrogen signaling compared to ToxCast assays, this assay can be used to generate concentration response data for calculating points of departure that can then be subjected to IVIVE for derivation of human equivalent exposures. And again, as part of the decision node in this second tier of testing, this oral equivalent dose can be compared to the actual or modeled human exposure to calculate a margin of exposure. If there is an adequate margin of exposure, no additional testing would be envisioned. In this manner, IATAs for evaluating endocrine activity can efficiently and cost-effectively take into account bioactivity, potency, and exposure to support regulatory and product stewardship decision making. A recent IATA case example published by the OECD combined read across with HTS and exposure:activity profiling has also demonstrated *in silico* and *in vitro* data can be used to screen for estrogenic potential, can be used for estimating *in vivo* points of departure, compared to human exposures and be used for risk-based decision making to inform the need, or lack thereof, for further testing (OECD, 2018).

Along similar lines, Andersen et al., 2019 present a functional roadmap that identifies where NAMs are expected to be adequate for chemical safety decision-making. This roadmap describes levels and

scenarios for applying NAMs in different risk contexts. Level 1 focuses solely on computational methods; Level 2 contains in vitro assays that can provide broad coverage of responses; Level 3 provides more quantitative estimates of dose responses using in vitro-fit-for-purpose cellular assays selected based on presumptive modes of action. Level 3 is expected to evolve and is likely to consist of in vitro NAMs using more complex multi-dimensional or multi-cellular assays. Exposure information is integrated with bioactivity data and each level to enable risk-based decision making. Consistent with the 2007 NASA report, Level 4 consists of tailored, traditional in vivo animal toxicity testing.

E. Integrating Exposure with NAMs to Support Risk-Based Safety Evaluations

The ability to use tools such as IVIVE to convert *in vitro* effect concentrations to human equivalent doses, coupled with advances made in predicting exposure for 10,000s of substances are incredibly enabling technologies for risk-based decision making. One of the most significant challenges in safety evaluation of commodity chemicals has been quantifying exposures, and without information on exposure, risk-based decision making is impeded. These prediction exposure models have helped to bridge that divide. One NAM that may not be all that new, the threshold of toxicological concern (TTC), has now emerged as an important tool for potential application in TSCA, where it can be considered for use in priority setting or for filling data needs. For priority setting, Patlewicz *et al*. (2018) processed more than 7000 substances through the TTC workflow (which accounts for known exclusions to the TTC as well as structural alerts) and then compared the high throughput modeled exposures of these compounds (exposure estimates from Wambaugh et al. 2014) to the appropriate class-specific TTC. None of the substances categorized as Cramer Class I or Cramer Class II exceeded their respective TTC values, and no more than 2% of substances categorized as Cramer Class III or acetylcholinesterase inhibitors exceeded their respective TTC values. This investigation showed that coupling the TTC with high-throughput exposure modeling can be employed as a pragmatic first step in ranking substances as part of a risk-based prioritization approach. In general, an IATA or similar assessment using basic exposure tools (*e.g.*, simple exposure calculations, default values, conservative assumptions) can be conducted as the first tier of a safety evaluation assessment (*i.e.*, a screening-level assessment). The assessor can then determine whether the results of the screening-level assessment warrant further evaluation through refinements, such as replacing exposure assumptions, by using more advanced models or by conducting additional bioactivity or toxicity testing on flagged substances.

F. Hazard Prediction Modeling

The replacement of traditional toxicity testing with NAMS for determining hazards and risks is rapidly advancing. However, as noted above, to establish scientific confidence in these methods often requires robust statistical and analytical tools to evaluate and document prediction model performance. For those with prediction analytic expertise, a vast array of computational tools, such as R packages, are available for detecting, analyzing, quantifying, and visualizing associations and other relations. To make these powerful tools more accessible to the toxicological sciences community, Cox Associates developed the Prediction Analytics Toolkit (PAT), an Excel add-in developed with partial support from the American Chemistry Council. The PAT provides a point-and-click interface for doing advanced prediction analysis from Excel using R packages; knowledge of R is not required. PAT, along with web-based tutorials, is now available at no charge on a cloud-based web platform; no software or add-ins are necessary, and there are no downloads (http://cox-associates.com/patkit/). Others too have taken steps to make tools available that facilitate the use and interpretation of such NAM data in the absence of data scientist

skills such as SQL, R, python, etc. Examples of these efforts include the EPA CompTox Chemicals Dashboard (https://www.epa.gov/chemical-research/comptox-chemicals-dashboard), the NICEATM Integrated Chemical Environment tool (https://ntp.niehs.nih.gov/pubhealth/evalatm/test-method-evaluations/comptox/ct-ice/ice.html), the OECD QSAR Toolbox (https://www.oecd.org/chemicalsafety/risk-assessment/oecd-qsar-toolbox.htm), etc.

PAT was used to analyze ToxCast/Tox21 data for the IARC Key Characteristics of Carcinogens in Becker *et al*. (2017). IARC's approach for employing the Key Characteristics of Carcinogens is essentially a judgment-based grouping of mechanistic evidence for use in implicit causal inference. But this IARC inference model was implemented without first determining whether the potential to elicit these characteristics could distinguish chemical carcinogens from non-carcinogens. Hence, e explicitly tested how well mechanistic data from ToxCast/Tox21 could predict cancer classifications. Using the IARC approach for mapping ToxCast/Tox21 assays to Key Characteristics of Carcinogens, we analyzed ToxCast data using PAT, and the results showed the key characteristics of carcinogens were no better than chance alone in predicting cancer; the key characteristics of carcinogens provided no evidentiary value for distinguishing a carcinogen from a non-carcinogen. This does not mean that mechanistic data have no role to play in informing determinations of potential carcinogenic risks of chemicals. Quite the contrary. The problem is that the IARC approach falls short because it does not explicitly incorporate understanding of the causal linkages of the sequence of key events and biological responses (including dose-response and temporal relationships) involved in carcinogenesis. IARC's failure to cite or address these scientifically established short comings documented in Becker et al., 2017 in their subsequent paper (Guyton et al., 2018) could lead some to raise serious concerns about the scientific integrity and objectivity of the IARC processes. A more rigorous, hypothesized mode-of-action approach is needed. The first step requires identification of biologically plausible MOAs and key events using the WHO/IPCS MOA framework (Meek et al., 2014) in conjunction with and the unified theory of carcinogenicity (Wolf et al., 2019). Next, the assays, including NAMs, associated with the key characteristics of carcinogens would be mapped to appropriate key events. Then the data is analyzed using evolved Bradford Hill causal considerations. The MOA confidence scoring method (Becker *et al*., 2017) is an example of using objective, and transparent approaches for integrating mechanistic information into a chemical cancer hazard evaluation.

3. Need for Additional Collaborations to Further Advance the Transformation of Chemical Safety Sciences

Many NAMs are now poised to be applied to meet the increased global demand for developing safety evaluations of chemicals. NAMs, including IATAs, transcriptomics and rapid exposure prediction modeling, are already transforming tiered, risk-based safety evaluations. As this scientific journey continues, there will be a greater need to integrate mechanistic data into biological pathway inference models and this will spur more reliance on data from NAMs and less reliance on traditional data approaches. There is a shared responsibility to develop and communicate the scientific strengths and limitations of NAMs for each specific type of decision these methods will support. More collaborations across all sectors — academia, industry, government, and non-governmental organizations — to harness the power of NAMs to improve regulatory and product stewardship decision making — are needed. Resources also need to be allocated to education and outreach efforts. That encompass traditional approaches, such as publishing articles in scientific journals and presentations at meetings, and non-traditional approaches, such as open community collaborations and real-time peer review.

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CURRENT OPINION IN TOXICOLOGY

Author declaration

[Instructions: Please check all applicable boxes and provide additional information as requested.]

1. Conflict of Interest

☒ Potential conflict of interest exists:

We wish to draw the attention of the Editor to the following facts, which may be considered as potential conflicts of interest, and to significant financial contributions to this work:
The nature of potential conflict of interest is described below:

The author is employed by the American Chemistry Council, an industry trade association that represents a diverse set of companies engaged in the business of chemistry. The author had complete control over development of this manuscript. The contents of this manuscript are solely the responsibility of the author and does not necessarily reflect the views or policies of his employer.

☐ No conflict of interest exists.

We wish to confirm that there are no known conflicts of interest associated with this publication and there has been no significant financial support for this work that could have influenced its outcome.

2. Funding

☐ Funding was received for this work.

All of the sources of funding for the work described in this publication are acknowledged below:
List funding sources and their role in study design, data analysis, and result interpretation]

☒ No funding was received for this work.

3. Intellectual Property

☒ We confirm that we have given due consideration to the protection of intellectual property associated with this work and that there are no impediments to publication, including the timing of publication, with respect to intellectual property. In so doing we confirm that we have followed the regulations of our institutions concerning intellectual property.

4. Research Ethics

☒ We further confirm that any aspect of the work covered in this manuscript that has involved human patients has been conducted with the ethical approval of all relevant bodies and that such approvals are acknowledged within the manuscript.

Not applicable

☐ IRB approval was obtained (required for studies and series of 3 or more cases)

☐ Written consent to publish potentially identifying information, such as details or the case and photographs, was obtained from the patient(s) or their legal guardian(s).

5. Authorship

The International Committee of Medical Journal Editors (ICMJE) recommends that authorship be based on the following four criteria:

1. Substantial contributions to the conception or design of the work; or the acquisition, analysis, or interpretation of data for the work; AND
2. Drafting the work or revising it critically for important intellectual content; AND
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