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Transforming regulatory safety evaluations using New Approach Methodologies: A perspective of an industrial toxicologist

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Abstract

Spurred by the vision articulated in the National Academy of Sciences report Toxicity Testing in the 21st Century (2007) and the availability of advanced methods for biological profiling of 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 National Academy of Sciences 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 to require development and implementation of tiered approaches for risk-based safety evaluations and for the Environmental Protection Agency to develop, evaluate, and use New Approach Methodologies. Considerable efforts are now being devoted to interpreting results, determining how these relate to potential health effects, and integrating bioactivity with predicted or measured human exposures for product safety evaluations. This article presents a number of my perspectives on the transformation that New Approach Methodologies are catalyzing in regulatory safety evaluations and product stewardship of commodity and consumer product chemicals.

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Developing scientific confidence in New Approach Methodologies

New Approach Methodologies (NAMs) are defined as any non-animal technology, methodology, approach, or a 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 (HTS), fit-for-purpose cell-based assays, genomics, proteomics, metabolomics, advanced toxicokinetic methods, and exposure prediction modeling (ECHA, 2016 [1]; 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, this has been derived from the standardization and validation of assays and specific decision frameworks. However, because 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 used?

The need to establish confidence in scientific methods is not new, and numerous guidance documents have been developed to validate and demonstrate confidence in methods. These include, for example, the Organization for Economic Cooperation and Development (OECD) Guidance Document 34 Validation and International Acceptance of New or Updated Test Methods for Hazard Assessment [2]; OECD Principles for the Validation, For Regulatory Purposes, of (Quantitative) Structure-Activity Relationship Models [3]; ECHA's Read-Across Assessment Framework [4]; OECD Guidance Document on the Reporting of Defined Approaches To Be Used Within Integrated Approaches to Testing and Assessment [5]; OECD Guidance Document for Describing Non-Guideline In Vitro Test Methods [6]; USEPA's Strategic Plan to Promote the Development and Implementation of Alternative Test Methods Within

the Toxic Substances Control Act (TSCA) Program [7], and so on. Given this apparent abundance of guidance, which is understandable as these various approaches were developed with different assays and specific aims in mind, there is 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 [8]. The stepwise components of this framework include the following: (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 end point(s) of interest from NAMs; (4) transparency: dissemination of the data, inference models, and so on 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 (SCF) has been cited and elements have been adapted in the USEPA's Strategic Plan to Promote the Development and Implementation of Alternative Test Methods within the TSCA Program [7]. Such an 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 with 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. If test method developers do not work closely with NAM users, such as regulatory agencies and private-sector product stewardship professionals, new methods may fall short in providing sufficient scientific certainty needed for product safety decision-making. Therefore, it would be beneficial to the regulatory science community and the NAM development community if consensus on implementation of such a universal SCF for NAMs could be reached.

Challenges and opportunities for using NAMs

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 analog and category approaches for read-across to be used to make inferences about toxicity end points (*e.g.*, Low *et al.*, 2013, Shah *et al.*, 2016). Read-across inference models can then be developed using data sets comprising substances that have both biological profiling results from NAMs and existing data for the toxicity end point(s). Modeling of properties or end points 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 [9,10]. Using chemical structure analyses and almost 1,000,000 chemical property hazard data points, Luechtefeld and colleagues [11] developed read-across models that achieved 70–95% balanced accuracies for classification of nine hazard end points.

Transcriptomics as a measure of bioactivity

In vitro transcriptomics produces complex data sets 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 articles 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 [12–14]. Discussions in these series of articles deal with the need to document test conditions, doses/concentrations, and numerous aspects of quality assurance and quality control. The research by USEPA'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 [15] as will the Tox21 High-Throughput Transcriptomics project (https://tox21.gov/projects/; [16]).

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. Some commodity chemicals may have biological effects, and these typically manifest at much lower potencies and much higher concentrations than pharmaceuticals, both *in vivo* and *in vitro*. Thus, as Thomas *et al.* [17] point out, for commodity chemicals, it will be critically important to use 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 nonselective 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 less than *in vivo* traditional point of departures (PODs) (on a mg/kg/day basis), indicating transcriptomics-derived PODs seem to be adequately health protective to support risk-based prioritization and screening [18].

Is it necessary to ground truth *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 is a 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 is not always necessary, nor would it be scientifically justified, to ground truth NAMs by comparing with existing animal toxicity data sets. As the scientific community's knowledge of biological pathways improves, ground truthing 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 [19] and how many cell and tissue types would be optimal to provide confidence comparable with those of screening toxicity using an *in vivo* laboratory 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 coculture or organ-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.

Complex substances and unknown or variable composition, complex reaction products, or biological substances

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 composition, complex reaction products, or biological substances, and there are significant challenges in studying such complex substances. Unknown or variable composition, complex reaction products, or biological substances and multi-constituent materials cannot be represented by unique structures or defined molecular formulas and typically comprise many different individual constituents, each of which may possess different physicochemical, 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.* [20] 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 with the findings from agrochemicals tested in ToxCast, enabling a comparison of bioactivities of ToxCast chemicals with 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 were supported in part by the Long-range Research Initiative (LRI) of the American Chemistry Council (ACC), show that (1) compounds in fruits and vegetables affected multiple *in vitro* end points, (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 because health impacts (be they positive, in the context of a healthful diet, or negative) are influenced by both.

Integrated approaches to testing and assessment

Another promising area for the use of NAMs is within integrated approaches to testing and assessment (IATAs). In the recent (2016) amendments to the US 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 HTS results to predict adverse effects for use in risk assessment is 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 for 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 with the actual or modeled human exposure to calculate a margin of exposure. In the study by Becker *et al.* [21], 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 less 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 *et al.*, again as part of the ACC LRI. In addition, to better recapitulate the complexity of estrogen signaling compared with ToxCast assays, this assay can be used to generate concentration response data for calculating points of departure that can then be subjected to *in vitro* to *in vivo* extrapolation (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 with 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 that *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 with human exposures, and can be used for risk-based decision-making to inform the need, or lack thereof, for further testing [22].

Along similar lines, Andersen *et al.* [23] 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; and 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 multidimensional or multicellular assays. Exposure information is integrated with bioactivity data and each level to enable risk-based decision-making. Consistent with the 2007 National Academy of Sciences report [29], level 4 consists of tailored, traditional *in vivo* animal toxicity testing.

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 tens of thousands of substances [30], 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 the TSCA, where it can be considered for use in priority setting or for filling data needs. For priority setting, Patlewicz *et al.* [24] processed more than 7000 substances through the TTC workflow (which accounts for known exclusions to the TTC and structural alerts) and then compared the high-throughput modeled exposures of these compounds (exposure estimates from the study by Wambaugh *et al.* [25]) with 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 used 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.

Hazard prediction modeling

The replacement of traditional toxicity testing with NAMs for determining hazards and risks is rapidly

advancing. However, as noted previously, to establish scientific confidence in these methods, it 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 programing skills, such as structured query language (SQL), R, python, and so on. 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 quantitative structure activity relationship (QSAR) Toolbox (https://www.oecd.org/chemicalsafety/risk-assessment/oecd-qsar-toolbox.htm), and so on.

The PATwas used to analyze ToxCast/Tox21 data for the IARC Key Characteristics of Carcinogens in the study by Becker *et al.* [26]. The International Agency for Reserach on Cancer's (IARC) approach for using 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 noncarcinogens. Hence, it was 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 noncarcinogen. 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. The IARC's failure to cite or address these scientifically established shortcomings, documented in the article by Becker *et al.* [26] and in their subsequent article [27], 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 World Health Organization's International Programme on Chemical Safety MOA framework [28] in conjunction with the unified theory of carcinogenicity (Wolf *et al.*, 2019). Next, the assays, including NAMs, associated with the key characteristics of carcinogens are mapped to appropriate key events. Then, the data are analyzed using evolved Bradford Hill causal considerations. The MOA confidence scoring method [26] is an example of using objective and transparent approaches for integrating mechanistic information into a chemical cancer hazard evaluation.

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 nongovernmental organizations — are needed to harness the power of NAMs to improve regulatory and product stewardship decision-making. Resources also need to be allocated to education and outreach efforts. These encompass traditional approaches, such as publishing articles in scientific journals and presentations at meetings, and nontraditional approaches, such as open community collaborations and real-time peer review.

Conflict of interest statement

The author had complete control over development of this manuscript. The contents of this manuscript are solely the responsibility of the author and do 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.

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