Chemical Research in Toxicology

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Perspective

Going All In: A Strategic Investment in *In Silico* Toxicology

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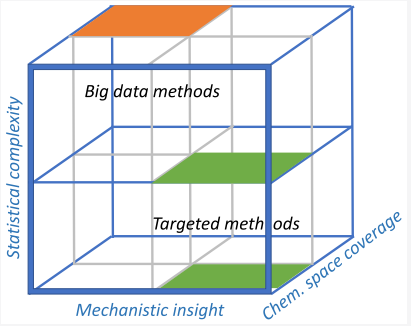
ABSTRACT: As vast numbers of new chemicals are introduced to market annually, we are faced with the grand challenge of protecting humans and the environment while minimizing economically and ethically costly animal testing. *In silico* models promise to be the solution we seek, but we find ourselves at crossroads of future development efforts that would ensure standalone applicability and reliability of these tools. A conscientious effort that prioritizes experimental testing to support the needs of *in silico* models (versus regulatory needs) is called for to achieve this goal. Using economic analogy in the title of this work, we argue that a prudent investment is to go all-in to support *in silico* model development, rather than gamble our future by keeping the *status quo* of a "balanced portfolio" of testing approaches. We discuss two paths to future *in silico* toxicology—one based on big-data statistics ("broadsword"), and the other based on direct modeling of molecular interactions ("scalpel")—and offer rationale that the latter approach is more transparent, is better aligned with our quest for fundamental knowledge, and has a greater potential to succeed if we are willing to transform our toxicity-testing paradigm.



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INTRODUCTION

Our current toxicity-testing paradigm struggles to cope with the need to protect public health from unintended adverse effects of industrial chemicals at acceptable socioeconomic cost. It is estimated that upward of 700 chemicals are registered for commerce annually in the United States alone, which makes animal testing, our traditional means of assessing chemical hazard, an impractical and unsustainable solution that leaves over 85% of chemicals without any health or safety data.[1] Similar trends are seen in Europe, where the REACH regulations have failed to generate the expected quantities of new animal data on chemical hazards due to high cost of testing.[2,3] Recognizing the fallacy of the *status quo*, the U.S. EPA's Administrator recently signed a memo to significantly reduce mammalian testing with the goal of completely eliminating it by 2035.[4] *In vitro* assays and *in silico* models, recently defined collectively as New Approach Methodologies (NAMs),[5] are alternatives that we will rely on to deliver on this public charge and to resolve our economically and ethically costly predicament. Albeit faster, less expensive, and mechanistically more relevant than whole-animal tests, we must ask ourselves whether these methods are sufficiently developed to take up the proverbial baton in safeguarding public health, and do so without the support of animal models. Many have argued that they are not: molecular-level interpretation of *in vitro* assay outcomes and their *in vivo* extrapolations is still insufficient,[6] and *in silico* models are plagued with training-set biases and thus questionable predictivity beyond current knowledge.[7,8] In essence, the much-needed thread connecting animal tests, *in vitro* assays, and *in silico* models is currently broken in many places. This thread is critical in relating whole-

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animal effects to biochemical events responsible for toxicity and enabling standalone applications of *in vitro*, and especially *in silico* models. To that end, despite formidable progress in molecular toxicology over the past two decades, we do not have a clear, detailed picture of what actually happens in most toxicity pathways on the molecular level. We propose this is a problem we are capable of addressing if we rethink our current paradigm of testing and model development. Here, we outline the future directions of *in silico* model development in the context of *in vivo* and *in vitro* models, and provide analysis supporting advancement of direct modeling efforts that rely on detailed description of molecular mechanisms underlining key events in toxicity pathways.

DISCUSSION

Promise and Challenges of *In Vitro* Methods. A landmark report by the National Academies in 2007 envisioned high throughput *in vitro* assays on human cells or cell lines to increase efficiency and decrease animal usage using robotics.[9] The U.S. EPA subsequently funded an impressive project, "Toxicity Testing in the 21st Century", to develop new high throughput technologies based on *in vitro* methods. As a result, over the course of the past decade the U.S. EPA has generated a substantial quantity of *in vitro* data with the goal to drive (i) preliminary hazard identification, (ii) adverse outcome pathway elucidation, linking molecular initiating events to toxicological outcomes, and (iii) relative priority and safety assessments from risk-based ratios of bioactivity and exposure.[10] Consequently, significant advances have been made toward using *in vitro* tests to replace, reduce, and refine (3R) *in vivo* tests; prioritize chemical hazards; categorize chemicals according to their modes of action; and provide mechanistic information.[11,12] However, *in vitro* tests are yet to fully attain regulatory acceptance and implementation, as they lack convincing results from validation studies on *in vivo* data.[5,13] There are a number of examples in the literature that outline the challenges in clearly linking *in vivo* and *in vitro* toxicity outcomes.[6,14,15] Despite the fact that *in vitro* assay data interpretation is still not sufficiently developed to replace *in vivo* models, *in vitro* assays have played, and will continue to play an increasingly important role in chemical safety risk assessment. In a complementary role, *in vitro* data has been successfully used to either corroborate other pieces of evidence or to support the development of *in silico* models.

Promise and Challenges of *In Silico* Methods. Considered broadly, *in silico* models are invaluable because they can, in theory (pun intended), be used to assess thousands of chemicals for multiple hazard endpoints quickly and at relatively low cost. However, in practice, the use of *in silico* models by the U.S. EPA and within EU's REACH is still largely limited to supporting experimental outcomes,[16] much like *in vitro* models. While regulatory agencies provide guidelines for the rigor, reliability, and transparency of *in silico* models, wider adoption is stifled by negative perceptions and experiences practitioners have using existing models. This distrust is fueled by difficulty interpreting model rigor and applicability by the (non-expert) user. Although most *in silico* models report metrics of accuracy and predictivity (the former being based on the fit to the training set, while the latter reflecting validation based on data external to the training set), the interpretation of these metrics is not always straightforward in the literature. This is particularly true of metrics reflecting predictive power, as modelers report various types of validations, and some are much less relevant to predictive power than others.[17] In addition, end-users have to consider that the predictive power will also be affected by how the model was developed: whether experimental data used was sufficient in quality and quantity; how it was compiled and curated; how many compounds were used in validation and how structurally diverse they were; whether the model was "over-fitted"; whether the descriptors used are linked to the molecular mechanism of action of the endpoint being modeled; and whether the compounds of interest fall inside the "applicability domain" of the model.[18,19] Such analysis can be very difficult to conduct provided all model-development data is disclosed, and it is impossible to carry out for the many proprietary commercial models, which are effectively "black boxes" to the end-users. Furthermore, as will be discussed later, the use of complex statistical methods to build models on big datasets, such as neural networks and machine learning, make it even more challenging to interpret predictivity metrics and judge model development[20] because developers posit that these models should not be evaluated by the criteria used to assess more traditional statistical models.[21] All considered, the only way a (nonexpert) user can accurately judge the capabilities of a predictive model is after extensive testing in his or her chemical space of interest.

When lack of expertise and (experiential) distrust combine with historically normalized reliance on animal models,[22] the impression created is that of a large confidence gap between *in vivo* and *in silico* approaches. We would argue this distinction—many toxicologists' mantra—is far from obvious, as animal tests are often not rigorously validated against human data, and generally do not indicate great predictivity for complex human endpoints, ca. 60−70% according to a recent report.[22] As we will outline later, overreliance on animal testing and the distrust associated with computational models, while in many cases experientially justified, is unfortunate because the future of *in silico* modeling is directly tied to cooperative engagement across all available methods and our willingness to strategically support *in silico* model development with limited economic resources. Despite the skepticism many of us have about the use of *in silico* models in toxicology, our state-of-the-art indicates that computational modeling can be highly accurate in describing discrete biochemical phenomena, and the incorporation of *in silico* models in the drug-discovery process by nearly every pharmaceutical company should provide sufficient proof.[23] The issue with the specific use of computational models in toxicology is the increased complexity of the problem being addressed: whereas a target-specific activity of a single compound is desired and optimized in drug discovery, in toxicology we seek to predict outcomes related to complex toxic endpoints for a wide range of chemicals and chemical classes. Furthermore, toxic endpoints for many industrial chemicals are not pathway-specific but rather have nonspecific modes of action, which is to say that the specific targets responsible for observed change in biological activity can be many and are not (yet) well-understood. While one could argue that precisely the same biochemistry applies in drug development as it does in hazard assessments, toxicity concerns are treated differently for drugs versus industrial chemicals. Since we have grown to accept high costs of developing new drugs, their unintended biological effects can be effectively probed using a battery of (expensive!) tests in the development process, and for some life-saving drugs these effects can be tolerated considering their therapeutic benefits.[24] None of this is true for industrial chemicals, which have a far-less favorable cost-benefit ratio, and must remain relatively inexpensive to develop

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and produce, thus severely limiting the budget for toxicity testing.

Current and Future Directions of *In Silico* Modeling in Toxicology. From an economic standpoint, *in silico* methods are the least-expensive solution to testing industrial chemicals, assuming these models can cope with biochemical complexity and mechanistic uncertainty. In the face of such challenges, *in silico* models have largely relied on statistics. These (Q)SARs, or (quantitative) structure−activity relationships, and their many modern variants, were pioneered in the early 1960s,[25] and represent the incumbent workhorse of computational toxicology. In their core principle, all (Q)SARs link structure-based descriptors (be it structural fragments or various physicochemical and electronic properties) with measured biological activity; however, the quality and mechanistic relevance of computed descriptors can vary greatly.[7] While an apt discussion of the evolution of (Q)SARs can be found elsewhere,[7] it is important to note that the future direction of *in silico* modeling proposed in this work rests on the foundation established by mechanistic (Q)SAR methods. These methods have pioneered the concept of molecular initiating events in predictive toxicology (long before it was formalized by Ankley et al. in 2010)[26] as means of extracting useful information about the initial interaction between a xenobiotic and its biological target(s) that can be causally linked to a toxicological outcome via a pathway.[27−31]

While transformative at the time, the reliance of traditional QSAR methods on structural features and/or physicochemical properties of the xenobiotic to describe complex biochemistries has been a double-edged sword: it has allowed for crude screenings of large datasets across complex toxic endpoints in order to "weed out" bad actors,[32] but it has also neglected the specificity and complexity of molecular interactions that may be important in new-chemical design.[33] While it is true that the difference in activity between toxicants originates in their different structure (regardless of the complexity of the biological interactions responsible for the toxic response),[7] any correlation of descriptors derived solely from the xenobiotic will carry a magnitude of uncertainty and training-set bias, which erode confidence in the model's true predictive power.

Focusing primarily on structural features of the xenobiotic in model building and ignoring the biological "dancing partner" is a missed opportunity given the boom of computing resources and methodological advancements in computational biochemistry. In our view, there is much to be learned from the trajectory of the pharmaceutical industry, which has shown that structure-based design, which considers the biological target(s) explicitly, often outperforms ligand-based approaches,[34] with some models capable of accurately capturing the effects of minute structural changes on biological activity.[35] At present, the application of similar approaches in toxicology is still limited to a handful of isolated case studies,[36−39] which represent but a small fraction of applicable, pathway-specific modes of action for known toxicant classes and toxic endpoints.

A Broadsword vs a Scalpel. Looking onward, we can outline two conceptually distinct directions being pursued simultaneously in the quest for the holy grail of reliable *in silico* methods in toxicology, as contrasted broadly in Figure 1 and detailed further in Figure 2. The first one, i.e., the broadsword, rests on the premise that we can collect enough experimental data to blanket a "sufficient" portion of the chemical space of interest. When the toxicity outcome of a compound is predicted, it is then based on close structural proximity to one or several tested analogs. In the ideal case of a complete or near-complete

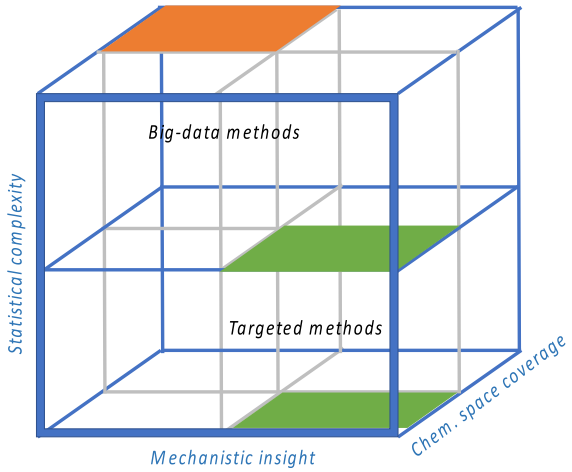

Figure 1. Big-data methods, which rely extensively on statistics, versus targeted methods, which rely on explicit modeling of molecular interactions, categorized in terms of mechanistic relevance and requirements for training-dataset size.

chemical-space coverage by measured outcomes, we thus effectively circumvent the need for mechanistic understanding of the xenobiotic's role in the living organism. In such a case, the accuracy of predictions, as determined from the model's (statistical) training approach, becomes the only relevant metric of success. On the other hand, if we possess perfect knowledge of toxicity mechanisms and how these biochemical processes translate into cellular, organ or organismal changes, then we do not need to rely heavily on experimental testing and complex statistics. We can simply utilize the best-available computational chemistry techniques, i.e., the scalpel, to describe the relevant individual biochemical processes, limited only by the accuracy of the applied theory. Since neither ideal has been attained, current approaches seek a compromise from either end of the spectrum. The following discussion aims to compare these two directions, commenting on their relative viability in terms of obtaining sufficiently large and quality datasets versus sufficient mechanistic detail to support reliable model development.

The Broadsword. The strive toward the first ideal is perhaps best represented by the boom of state-of-the-art machine learning (and other related pattern-recognition strategies such as deep learning, neurocomputing, artificial intelligence, etc.), which focus on utilizing available "big data".[40] These methods are revolutionizing industries and our daily lives, and are inevitably part of our future; however, despite their promise and potential power, these methods' reliance on extensive coverage of the chemical space of interest is problematic in toxicology for several reasons. The first concern is related to variable data quality, which arises from the necessity to combine different data sources and test types, and which cannot be analyzed effectively for such large datasets.[19] Big-data models are inherently plagued by the challenge of securing minimal threshold of data quality.[41] This problem has not gone unnoticed in toxicology, as a number of efforts have been put forth to categorize and quantitate data reliability and relevance, e.g., the ToxRTool, TRAM (Toxicological data Reliability Assessment Method) or fuzzy expert systems based on the Klimisch scoring approach.[42−44] However, these assessments are time-consuming to effectively and systematically apply to large datasets, and input data required by these models is often difficult to acquire or is lacking entirely.[45] The data quality challenge is perhaps best illustrated

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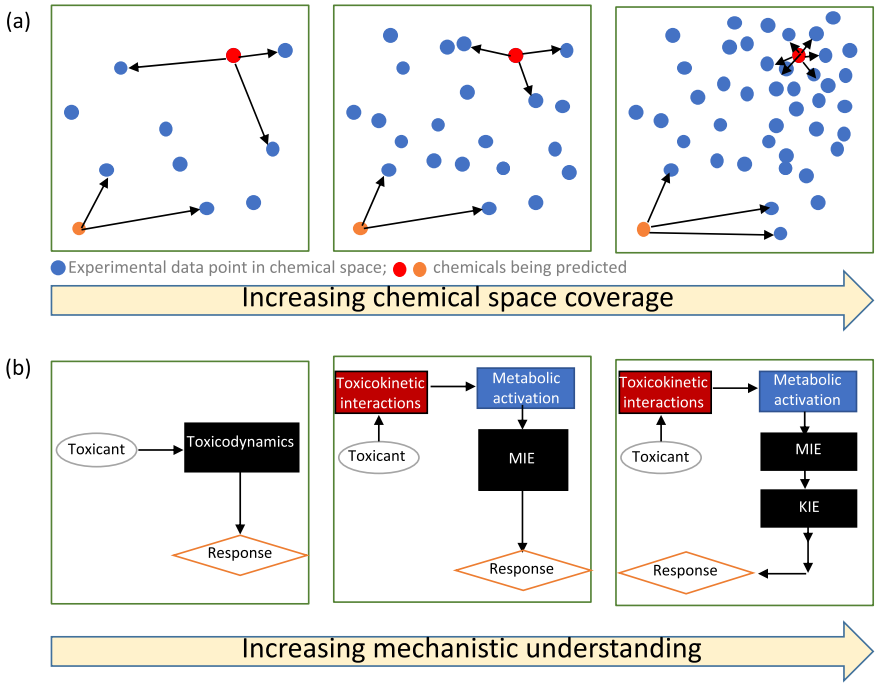

Figure 2. Strategies for increasing predictive power of the two types of predictive approaches (a) In the big-data approach, increasing chemical space coverage of experimental data can increase predictive power as long as chemical of interest falls in a densely populated area of chemical space (true for red, not true for orange compound). (b) In the mechanistic approach, increasing mechanistic understanding in AOP (adverse outcome pathway) results in models with increased predictive power, limited only by the accuracy of the applied theory. MIE = molecular initiating event; KIE = key intermediate event.

by the effort put forth by the European Union to collect significant volumes of new animal test data required through the REACH legislation. A recent study found that of the chemical dossiers submitted, nearly two-thirds lack any experimental data on reproductive and developmental toxicity, instead relying on read-across predictions, and the majority of those that report data source it from studies up to 20 years old, which may not meet current testing standards.[2]

Data quality is not just an issue for toxicological thresholds: inaccuracies in the reporting of chemical structures, ionization state, purity or solvent used can be just as detrimental to the predictive power of big-data models. While some errors can be identified though labor-intensive efforts, many cannot; yet, studies on this topic indicate that error rates in reporting are too large to ignore. For example, for data published in the WOMBAT medicinal chemistry database, Olah et al. found two structural errors per publication average (error rate as high as 8%),[46] while in another analysis of public and commercial databases error rates ranged from 0.1 to 3.4%.[47] Careful curation of data from non-peer-reviewed sources, like those that are needed to feed big-data models, is going to be a challenge that is difficult to address with sophisticated statistics and labor-intensive curation. There may be a more realistic opportunity to improving the reliability of peer-reviewed studies, but this will require strengthening the dialogue between regulators, toxicologists, and modelers, as well as standardizing reported protocols in the literature.

Aside of data quality, relying on big data in model development poses a challenge of how much is in fact "enough" to avoid training-set bias in models that have dubious mechanistic underpinning. As chemical space constantly evolves, questions must be raised about the applicability domain of any tool that is defined by its training-set versus one that is mechanism-driven and the resulting bias, which is at best minimized but never eliminated by training on large datasets. Outlined in Figure 2a, even big-data models struggle with asymmetrical data distributions; there will always be outliers with large distances to nearest structural neighbors, for which predictions are dubious. Furthermore, close structural similarities between chemicals do not imply correspondingly similar activities. Thus, machine learning and statistical approaches struggle with "activity cliffs", i.e., steep changes in the structure–activity landscape that are difficult to anticipate and negatively impact model development.[48,49] Most importantly, being overly focused on optimizing accuracy of predictions within the training set limits, these methods may offer limited insight into the fundamentals of natural processes.

In contrast to statistical tools, computational models in (bio)chemistry were never very good at telling us something new; they can, however, excel at telling us why something happens. In this regard, and somewhat paradoxically, the Achilles heel of machine-learning and related approaches coincides with what is viewed as their biggest advantage—efficient big-data processing. When considering big data, one cannot apply sophisticated, highly mechanistic descriptors because these may be too expensive (both in terms of human and computer resources) on that scale.[50] However, without mechanistic and highly specific descriptors, most current statistical and machine learning approaches in toxicology may suffer from inscrutable (and thus questionable) relationships with the biochemical processes they aim to describe, which limits their potential to help us advance the fundamental science that underlines these phenomena.

The Scalpel. The alternative path to extensive data coverage and the use of big-data approaches is that of perfect mechanistic knowledge, which would support *explicit* modeling of key

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biochemical events in toxicity pathways. This ideal may seem equally naïve: after all, aren't we just trading one unfeasibility (impossibly large training sets and data quality issues) for another (impossible mechanistic complexity)? In our view, perhaps not entirely: modern computational chemistry has built a vast toolkit over the past several decades, which can be used to accurately describe complex biochemical processes. Furthermore, computing resources have expanded considerably, allowing application of these models in reasonable time frames. Crucially, this direction of explicit description of molecular initiating and other key events in toxicity pathways is in line with our strive for fundamental understanding of natural phenomena. It could also be argued that mechanistic insights provided by *in vitro* and *in chemico* methods better align with the needs of explicit mechanistic models than highly sophisticated statistical approaches, by providing vital clues for the key toxicokinetic and toxicodynamic events implicated in the toxic response (Figure 2b).[6,51]

Lastly, a strong case can be made that such highly mechanistic models do not require extensive training sets, alleviating issues associated with data quality and time-consuming curation efforts. Since one of the key attributes of any model's predictive power is its applicability domain, which sets the boundaries of predictivity based on training-set data, one might argue that relying on smaller training sets will lead to narrow applicability domains. However, applicability-domain constraints are far less relevant to highly mechanistic models, which describe molecular interactions explicitly, than to statistical models; rather than being based on training set compounds, their applicability domain is defined by the limitations of the underlying theoretical principles. Consequently, explicit-modeling efforts are far less likely to suffer from activity cliffs, leading to greater stability and wider applicability across chemical space. The latter is owed to the vast collection of benchmarking studies published by computational chemists, which support selection of the appropriate theoretical method for the type of system being described.

While arguments for changes to chemical testing to better serve the needs of future mechanistic models will be made in the next section, assessments of the large number of models developed for the skin sensitization endpoint can already demonstrate the viability of this direction. For this endpoint, highly mechanistic approaches, which consider biological-target chemistry yet are based on relatively small but well-curated training sets, tend to outperform models that are less mechanistically relevant even when those models are based on large training sets.[52−54] The difference, ca. 30% improvement in performance metrics, is considerable, and can readily be interpreted as the difference between reliable and unreliable predictive tools. Same outcome of similar magnitude was noted when comparing prominent models for acute aquatic toxicity.[55] This is particularly true when evaluating these models within chemical space that is very different from the models' training sets. Active pharmaceutical ingredients and their synthetic intermediates are a great example to illustrate this point because of their complex functionalization, conformational variability, and multiple protonation states. The biochemistry of these chemicals is "unpredictable" from the perspective of models that rely on structural descriptors and/or physicochemical properties due to the intricate intra- and intermolecular interplay of many steric and electronic factors. Regrettably, publication record cannot support our claim due to the proprietary nature of these chemicals, which keeps testing outcomes confidential. However, having assessed thousands of drug-like compounds using *in silico* models over many years, we can assure the reader that models that ignore biological target(s) chemistry consistently fail to accurately capture adverse outcomes of pharmaceuticals regardless of the size of the training set.

While existing models that strive for better mechanistic concordance with molecular initiating events and try to model these events explicitly show compelling promise, their full potential is far from realized. The main hindrance is not the quantity but the type of available experimental data and the type of chemicals being tested. To this end, for modelers to succeed in developing the next generation of mechanistic models, we need to ensure better connectivity of knowledge across whole-animal, *in vitro*, *in chemico*, and *in silico* methods, which implies closer bridging of different scientific communities. The brief discussion that follows is a proposal for changing the way we test chemicals in support of *in silico* model development and the financial investment in education, academic research, and interdisciplinary collaborations that must inevitably come along with it.

Changing the Paradigm of Model Development and Testing. If the premise of advancing explicit-modeling efforts that use no or minimal statistics is indeed the most promising approach to reduce reliance on *in vivo* testing, then what we require to build reliable tools is a transformation of thinking and process. Focused on computational methods that can describe complex biochemistry in toxicity pathways, we should recalibrate our skepticism about *in silico* methods, and invest into what these methods can become when properly supported by experimental studies. It must be stressed that explicit-modeling methods (versus largely statistical or machine-learning approaches) are fully transparent, which is both their strength and weakness, as their limitations are in plain view, devoid of any mathematical obfuscations. For these methods to become reliable mainstays in predictive toxicology, we need to invest in both education and research.[56,57] Making computational chemistry a standard part of the undergraduate curriculum and including toxicology concepts in undergraduate chemistry curricula are the key two steps we can take to boost our confidence in theoretical models; adequately train future innovators, who can bridge the chemistry and toxicology fields, and, consequently, pave the way to a more sustainable toxicity-testing future.[58,59] Only with the right mental-paradigm shift will we gain the necessary courage to subsequently transform the process, i.e., to rewrite our approach to experimental testing.

In research efforts, the mental preparedness to change the *status quo* is perhaps best illustrated by the following statement: in moving toward minimizing animal testing while protecting environmental and public health from adverse effects of chemicals, it is imperative that future experimental testing is aligned with the needs of *in silico* model development. Currently, chemicals and corresponding toxicity thresholds within most models' training sets originate from data that was selected and tested for regulatory purposes. In most instances, such data represents a chemical space that is far from ideal of what a computational model requires: a rationally selected series of structural and functional analogs, informed by modeling exercises based on current knowledge, to assess relative trends in the magnitude of toxic response. This endeavor is not necessarily overly costly; as stated previously, a highly mechanistic model does not need an extensive training set. It does, however, need a carefully selected and curated experimental data, which probes the relative trends within and

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across chemical classes, in turn allowing the *in silico* model to extrapolate safely and reliably based on underlying theory. Thus, investment must be made into testing compounds we may "feel" we do not need to test to immediately protect human or environmental health but are critical for robust model development. To this end, modelers need to work far more closely with experimentalists during chemical selection and experimental design than is the case now, mirroring successful collaborative strategies in drug discovery. The eventual payoff of interconnecting highly sophisticated computer models with experimental design is a harmonized iterative loop that provides a two-way check on the outcomes of each individual approach and, in the end, a reliable predictive model. While presenting some logistical challenges, this strategy is perfectly in line with the call for more integrated testing approaches in toxicology[22,60] and our most basic thirst for greater mechanistic insight into biological phenomena. It must be reiterated at this point that for such approach to succeed, transformation of thinking must come first. To that end, we cannot leave the development of *in silico* models unattended; realizing this approach is the most cost-effective option to effectively ensure human and environmental health safety, we ought to optimize the conditions that maximize chances of its success.

To offer a metaphor in concluding our narrative, existing *in silico* models are the equivalent of a teenage athlete: this individual represents the next generation that we must inevitably rely on; however, for her to grow into a competitive adult, the right nutrition (rather than copious amounts of junk food) and proper training ought to be provided. Furthermore, one may argue it is the responsibility of her predecessors to ensure attention is paid to her development and to ensure successful transition when the torch is passed on. Only such cooperative engagement by all stakeholders in the scientific community will eventually allow *in silico* models to take up the mantle of protecting human and environmental health from adverse effects of chemicals.

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Notes

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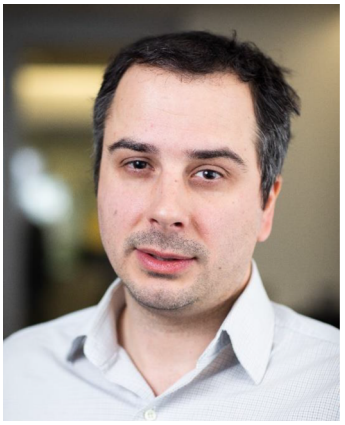
Biographies



Jakub Kostal is an Assistant Professor of Chemistry at The George Washington University, with a research focus on the rational design of functional and benign commercial chemicals. To that end, his group develops *in silico* predictive models that take advantage of advanced computational techniques to model discrete steps in the toxicokinetic and toxicodynamic interactions that lead to adverse outcomes. He is an NSF CAREER awardee for an effort to design biodegradable pesticides, as well as PI of a current NSF award on upgrading biomass using *in silico* designed ionic liquids. Dr. Kostal is Founder and Principal of Designing Out Toxicity (DOT) LLC, which provides consulting services for filling toxicological data gaps using *in silico* predictive tools. He holds a Ph.D. from Yale University (2012) under the supervision of William Jorgensen in Theoretical and Biophysical Chemistry and a B.S. in Chemistry and Biochemistry from Middlebury College (2006). Under the supervision of Paul Anastas and Julie Zimmerman, he completed postgraduate work at Sustainability A to Z and the Yale Center for Green Chemistry and Green Engineering, focusing on the development of mechanistic predictive models for toxicological endpoints.

Adelina Voutchkova-Kostal is an Associate Professor of Chemistry at The George Washington University. Her research group aims to develop catalytic processes that contribute to a circular economy, which necessitates concerted efforts in both development of efficient catalytic processes, and design of functional chemicals that are inherently benign and degradable. Her research efforts in the area of design of chemicals for safety have focused on developing predictive tools for toxicologically relevant endpoints using NMR data. In the area of *in silico* predictive toxicology, Dr. Voutchkova-Kostal has helped develop commercial mechanistic models for a number of human and ecotox endpoints. She completed her graduate work at Yale University (PhD, 2008) under the supervision of Robert Crabtree in Organometallic Chemistry, prior to which she attended Middlebury College, where she obtained her degree in Chemistry and Biochemistry (B.A. 2004). Her postgraduate work at

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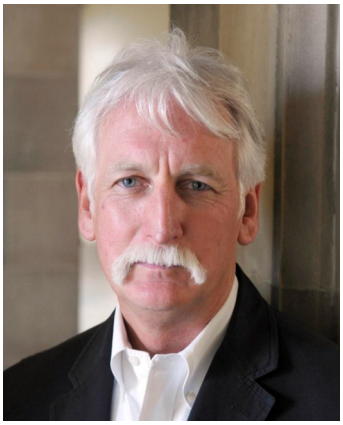
the Yale Center for Green Chemistry and Green Engineering with Paul Anastas and Julie Zimmerman (2009−2011) focused on the rational design of commercial chemicals that fulfill the desired function but are minimally hazardous to humans and the environment.



***Mentor Biography*: William L. Jorgensen** is the Sterling Professor of Chemistry at Yale University. His extensive research career has defined the frontiers of organic, medicinal, and computational chemistry, including simulations of organic and enzymatic reactions, computer-aided drug design, and synthesis and development of therapeutic agents targeting infectious, inflammatory, and hyperproliferative diseases. His work was astutely characterized as providing "piercing insights, often accompanied by characteristic simplicity". His dedication to advancing the field of physical organic chemistry dates back to his graduate training under the tutelage of E. J. Corey at Harvard University (1990 Nobel Prize in Chemistry). Dr. Kostal completed his graduate work under Professor Jorgensen, which focused on computational method development and applications in the areas of organic and enzymatic reactions to provide mechanistic insight into fundamental chemical and biochemical processes. In Dr. Kostal's recollections, one of Professor Jorgensen's statements stands out, paraphrasing: "Make sure your academic work does not turn into an academic exercise." This necessity to ensure one's work has real-world relevance has greatly impacted Dr. Kostal's career trajectory.

***Mentor Biography*: Paul T. Anastas** is the Teresa and H. John Heinz III Professor in the Practice of Chemistry for the Environment at Yale University, with appointments in the School of Forestry and Environmental Studies, Department of Chemistry, and Department of Chemical Engineering. He is also the Director of the Center for Green Chemistry and Green Engineering at Yale. An organic chemist with a Ph.D. from Brandeis University, he had a momentous career in public service, during which he and John Warner coined the term "Green Chemistry" and proposed the fundamental principles that define it, including the rational design of safer commercial chemicals. He has since been a thought leader in cutting-edge frontiers of sustainability, with emphasis on the design of safer commercial chemicals. Dr. Voutchkova-Kostal served as postdoctoral and research associate under his guidance at the Center for Green Chemistry and Green Engineering at Yale, where she gained experience in toxicology and the development of predictive models. Dr. Kostal also completed postdoctoral training with Professor Anastas, working on the development of predictive *in silico* models for mutagenicity, carcinogenicity, and skin sensitization.

***Mentor Biography*: Julie B. Zimmerman** is a professor jointly appointed to the Chemical & Environmental Engineering Department of the School of Engineering and Applied Sciences and the School of Forestry and Environmental Studies. She is also the Senior Associate Dean for Academic Affairs and Deputy Director at the Yale Center for Green Chemistry & Green Engineering. Professor Zimmerman's research has holistically addressed numerous challenges of sustainability, including processing and lifecycle assessment of renewable sources of fuels and chemicals, as well as water purification technologies that enhance resource quality and quantity. She has also led efforts in the design of safer chemicals and nanomaterials. As a co-mentor to both Drs. Voutchkova-Kostal and Kostal during their postdoctoral research appointments, she significantly impacted their perspectives on how to evaluate effectiveness and impediments of new methods to advance sustainability. Professor Zimmerman joined Yale after years of public service at the U.S. EPA, and holds a joint Ph.D. from the University of Michigan in Environmental Engineering and Natural Resource Policy.

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■ ABBREVIATIONS

AOP, adverse outcome pathway; MIE, molecular initiating event; KIE, key intermediate event; NAM, new approach methodology

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