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Perspective

Making the Case for Quantum Mechanics in Predictive Toxicology—Nearly 100 Years Too Late?

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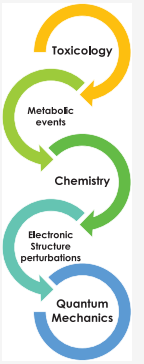
ABSTRACT: The use of quantum mechanics (QM) has long been the norm to study covalent-binding phenomena in chemistry and biochemistry. The pharmaceutical industry leverages QM models explicitly in covalent drug discovery and implicitly to characterize short-range interactions in noncovalent binding. Predictive toxicology has resisted widespread adoption of QM, including in the pharmaceutical industry, despite its obvious relevance to the metabolic processes in the upstream of adverse outcome pathways and advances in both QM methods and computational resources, which support fit-for-purpose applications in reasonable timeframes. Here, we make the case for embracing QM as an indispensable part of a toxicologist's toolkit. We argue that QM provides the necessary orthogonality to alert-based expert systems and traditional QSARs, consistent with calls for animal-free integrated testing strategies for safety assessments of commercial chemicals. We outline existing roadblocks to this transition, including the need to train model developers in QM and the shift toward service-based toxicity models that utilize high-performance computing clusters. Lastly, we describe recent examples of successful implementations of QM in hazard assessments and propose how *in silico* toxicology can be further advanced by integrating QM with artificial intelligence.



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INTRODUCTION

In three-year's time (the year 2026), we will mark the 100th anniversary of the Schrödinger equation, a formalism that allows us to derive the energy of a chemical system from its electronic structure (i.e., from the subatomic motion of electrons). This is significant because electronic energy, corrected for thermal effects (to capture molecular motions in the real world), and entropy (to account for the many states molecules can occupy) can be used to estimate how likely and how fast any chemical process is going to be. The totality of the natural world around us, its evolution, and its instantaneous representation at any given moment are a direct reflection of these two guiding principles. This should feel familiar and likely revive old fears in any former chemistry student who had to make the leap from the visually friendly building blocks of organic chemistry to the calculus-heavy quantum mechanics in their junior year in college.

In recent years, toxicology has initiated the sensible pivot from whole organism testing to mechanistic studies of toxicity to reduce the ethical and economic burden of animal models.[1−4] Case in point, as of late 2022, new pharmaceuticals do not need to be tested in animals to receive U.S. Food and Drug Administration (FDA) approval.[5] This transformation is both knowledge-driven (we have developed sound methods that enable a closer look at the underlying biochemistry in toxicity pathways) and knowledge-inducing (we can better understand organism-level effects by quantifying the key events triggering animal or human response). In that light, it is fair and true to state that all modern toxicology is fundamentally chemistry (of these key events), and because all chemistry is guided by the subatomic motion of electrons, we require quantum mechanics (QM) to capture toxicological phenomena (Figure 1). From Figure 1, what we will demonstrate below is that this conceptual

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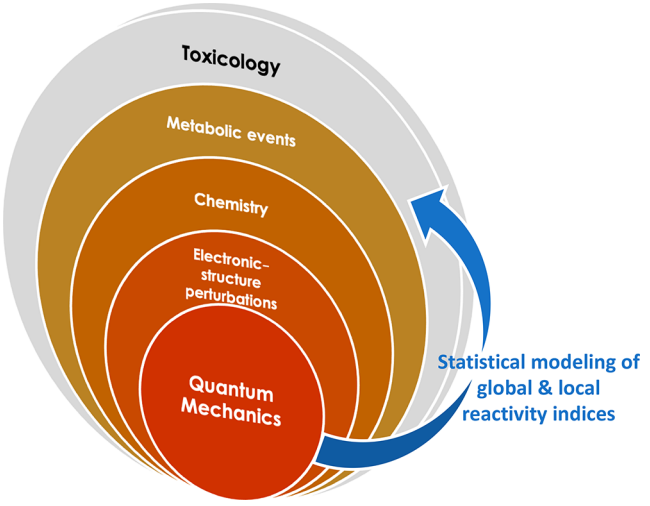

Figure 1. Links between toxicological phenomena and quantum mechanics (QM) based on fundamentals of underlying processes (nested circles) and practical implementations in predictive-toxicology solutions (blue arrow).

link between QM and toxicity can be exploited in practice by computing reactivity indices, derived from electronic structure, in support of risk and hazard assessment.

In computational modeling, the relationship between QM theory and solutions to chemistry problems in the real world has been explored for some time and successfully leveraged across many different fields, from calculating energetics of organic and enzymatic reactions to computer-aided drug discovery (CADD).[6,7] The progression of CADD is particularly interesting, as the field started with relatively simple structure−activity relationships (SARs) over 50 years ago,[8] eventually evolving into a complex modern toolbox based on explicit models of host−guest interactions (featuring techniques such as Monte Carlo or molecular dynamics simulations) and statistics-driven approaches (e.g., quantitative SARs (QSARs), and artificial intelligence (AI) in the more recent years), employed at different stages of the drug discovery process.[9−11] QM is projected to play an increasingly important role in CADD as the efficiency/accuracy of real-world implementations has improved considerably (see, for example, mixed quantum and classical, QM/MM, methods used to determine free-energy landscapes of enzymatic reactions or applied in virtual screening for covalent drug discovery).[12] Furthermore, available computational power continues to rise exponentially at diminishing costs (viz. Moore's law about transistor density on a microchip), supporting the use of more expensive (i.e., accurate) QM methods.[12,13]

Surprisingly, this transformation of *in silico* modeling in CADD was not mirrored in predictive toxicology, which has largely relied on (Q)SARs based on structural features and/or physicochemical properties (not unlike CADD in the 1960s) for much of its existence.[14] This is true even for end points characterized by covalent reactivity in key initiating events.[15−18] The lack of technology transfer in practical applications compelled us to outline the self-evident benefits of QM and the modeling of molecular interactions for the purposes of predictive toxicology in 2018.[19] We were not the first to point at this unrealized opportunity,[20,21] but a quick survey of the topic and citations of the few key publications to date suggest QM has never truly arrived in toxicology.

SELF-IMPOSED LIMITATIONS OF "QM BY PROXY" IN TRADITIONAL PREDICTIVE TOOLS

Let me preface this section by stating that existing approaches used in predictive toxicology (i.e., atomistic QSARs, expert systems, or read-across) incorporate some QM implicitly (and perhaps mindlessly). For example, many physicochemical properties used in QSARs (such as polarizability, molecular volume, or the various partition coefficients like log $K_{o/w}$) are a manifestation of the electronic structure and its interactions with the environment. Similarly, expert rules for chemical interactions rest on observations that fundamentally stem from (or align with) QM theory.[22] These approximate methods are valuable in virtual screening of large data sets, when seeking to prioritize a subset of promising compounds for further experimental follow-up. However, in deterministic tools that need to provide absolute metrics, one must be concerned about the relevance of these "proxy variables" to the specific transformations at hand (i.e., the key events in the adverse outcome pathway) and about the quality of their estimation. For example, polarizability, i.e., the ability to distort a molecule's electron cloud, can reflect permeation through lipid membranes or binding of biological targets driven by induced-dipole interactions.[23] It is commonly predicted from the number of valence electrons rather than computed directly from electron density.[23] The former is instantaneous, but stacking a model on top of another model propagates error; compounds uncertainty; and obscures applicability.[23] The latter is much more involved and may require advanced, computationally costly methods (though faster solutions, such as quantum theory of atoms in molecules, QTAIM, have now been developed to deliver robust and physically insightful QSAR models).[24] Critically, a principle-based approach is not limited by the chemicals in a training set of a statistical model. The same can be said about other properties frequently invoked in toxicokinetics and dynamics (e.g., dipole moment, surface area, volume, electrostatic potential, electron affinity. etc.).[24] Derivation of these properties from electron density, such as outlined by Matta,[24] is the better approach if there is limited data to train a model and/or the chemicals used in training are different from those the model will be eventually applied to. Here we arrive at the crux of the matter—in practical terms, it is difficult to externally evaluate these two conditions (training-set limitations and chemical-space applicability), as the makeup of predictive models may be proprietary (or inscrutable for the nonexpert user) and because molecular behavior varies dramatically across the chemical landscape (e.g., in comparing commodity chemicals vs pharmaceuticals).[25] As these concerns are challenging to fully alleviate with applicability domain definitions, direct application of QM is nearly always the more reliable option.[19,25]

Aside from how the calculation is carried out, which impacts both computational cost and accuracy, the relevance of the computed properties matters too. Physicochemical properties used in QSARs are usually poor proxies for the biochemical phenomena and systems they try to approximate (e.g., ligand polarizability used to capture protein binding affinity[26] or the octanol−water partition coefficient used to gauge skin permeability).[27] This is because molecular processes in real systems are different from and far more complex than the standard "roster" of physicochemical properties available to model developers, inevitably leading to the same outcomes as noted in the previous paragraph—a dubious (and likely severely

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limited) applicability domain.[25] For example, log $K_{o/w}$ is a popular descriptor of passive diffusion across a lipid matrix used in many existing tools (e.g., OECD QSAR Toolbox). However, the properties of octanol are not identical with the components of lipid membranes, such as free fatty acids, ceramides, or cholesterol. Thus, a more accurate approach is to replace log $K_{o/w}$ with interaction energies between the xenobiotic and these media, which can be obtained from molecular simulations used in conjunction with mixed quantum and classical calculations.[28]

"QM by proxy" in (the most competent) expert systems and read-across can best be understood through the lens of organic chemistry coursework. Even before being introduced to the postulates of QM in physical chemistry, students learn to recognize molecular patterns, i.e., functional groups, each linked to a specific chemical behavior and reactivity. While they might not yet understand QM, they nonetheless, albeit unwittingly and in a superficial manner, adhere to the principles of QM in drawing reaction mechanisms and moving electrons across Lewis structures. This "pattern recognition" propagates into expert systems and read-across and can be encoded in an autonomous computer program to enable predictions. Unfortunately, the limitations outlined for physicochemical proxies above apply to (2D) structure-based expert systems and read-across as well, in that neither can robustly propagate beyond existing knowledge into a new chemical space (without prior understanding of the outcomes that define that space) or quantitatively distinguish nuanced effects of molecular structure on biological activity (e.g., the subtle and often confounding effects of molecular substitution around a functional group).[25] The latter explains why these approaches cannot reliably predict potency of xenobiotics,[29] regardless of the toxic end point, a feature that is key to hazard and risk assessment.[30] Recent studies on the skin sensitization of peptide couplers[31] and carcinogenicity of *N*-nitrosamines[32] exemplify these limitations.

TOWARD EXPLICIT QM IN PREDICTIVE TOOLS

Nowadays, there is no reason to be overly reductionist in our model development, representing complex biochemical processes with simple rules or generic properties. Instead, we can focus on modeling molecular interactions, which, provided that all metabolic processes involve covalent interactions (i.e., the breaking and making of bonds) and even noncovalent interactions with biological targets rely on the polarization of electronic density, calls for a QM approach of some kind. We can subsequently use expert knowledge to perform a "sanity check" on the predictions of our models in simple systems. Historically, we shied away from such techniques due to limited compute, but that is no longer the case; we are now able to parallelize complex tasks across multiple CPUs and take advantage of the immense computational resources of high-performance computing clusters (HPCC). In doing so, we can better extrapolate beyond current knowledge; capture nuanced effects of chemical microenvironments; expand our understanding of biochemical phenomena; and alleviate the Achilles heel of (Q)SAR predictive tools, the oft ill-defined and limited applicability domain.[33−36] We have shown in our recent reports that judicious use of QM can effectively "soften" applicability-domain constraints.[31,37]

What is then the hesitancy behind incorporating explicit QM modeling into predictive toxicology? Scientific communities are humanlike in that they resist change, which is both a feature and a "bug" of our collective research enterprise.[38] Aptly, Herman von Helmholtz, who is known for the Helmoltz equation of basic wave propagation (that gave rise to the Schrödinger equation and QM), wrote that "new ideas need more time for gaining general assent the more original they are".[39] Ironically, Helmoltz subsequently rejected ideas proposed by Max Planck, who is recognized as the originator of the quantum theory, because he did not understand them.[40] While these historical digressions may appear superfluous here, they indicate that our reluctance to accept new paradigms has often little to do with the science itself and more to do with our scientific training.[38] Scientific training is worth highlighting here, as our reductionist educational model, which segregates knowledge into disciplines, creates divides we must actively engage to bridge to generate understanding across different fields.[41] This is critical if we want, for example, toxicologists to understand the value of quantum chemistry as a solution to toxicological problems.

In practice, outside of science education and communication, we recognize two main barriers to the progress of QM-focused tools in predictive toxicology. One, extensive use of QM in model development necessitates investment in both knowledge (i.e., hiring modelers with expertise in QM) and resources (i.e., developing infrastructure for the execution of QM calculations at scale). Two, adoption of QM-based models requires changes to the current paradigm of using predictive tools. In that regard, we need to move away from self-contained programs that offer instantaneous predictions on personal computers to an infrastructure that facilitates data transfer and remote execution on HPCC. In client engagement, we have observed that '"not being able to run your own calculations" is often viewed as a deficit of emerging toxicology tools that require greater processing power. Our rebuttal is that these approaches are now common in computational chemistry and biology and work perfectly well.[42−46] Furthermore, remotely executed tools offer distinct advantages over standalone counterparts in terms of software updates, data curation, and outcome interpretation. We have previously written about data-sharing as the major hurdle in the progress of *in silico* model development, noting this is a largely irrational obstacle (as are negative sentiments about not running the software yourself!), given that highly secure protocols exist to transfer data in a way that protects the intellectual property of all parties involved.[47] Last but not least, we should abolish the notion that computational tools are cheap; while even the most complex QM-based models are more affordable than *in vivo* or *in vitro* tests, they have greater operational costs than traditional (Q)SARs.

PRACTICAL CONSIDERATIONS FOR SUCCESSFUL IMPLEMENTATIONS

Although we made the claim that our computational resources and capabilities are vast, they remain finite with respect to cost and time available for any hazard or risk assessment. To that end, a prudent choice of a QM method and a modeling approach is necessary. For example, it is useful to characterize key toxicity-initiating events using reaction-pathway modeling at a high level of QM theory to gain better understanding of the process[48,49] or to expand the training set of chemicals.[50] However, this approach does not scale well in hazard assessments, where a "deconstruction" into QM-based reactivity indices, which can be obtained at a fraction of the computational cost, is likely the better option.[28,37,51,52] The studies cited above illustrate this QM adaptation for modeling of *N*-nitrosamines' metabolic activation into DNA-alkylating (i.e., mutagenic/carcinogenic) diazonium byproducts and for the reactivity of Michael

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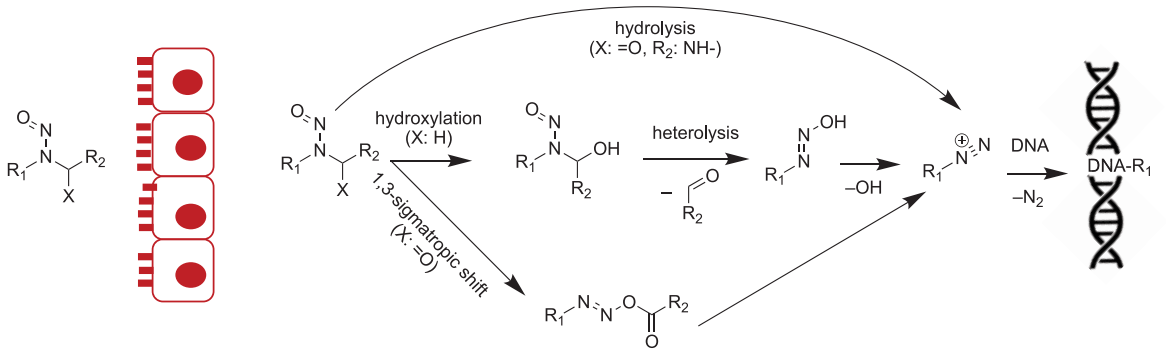

Figure 2. Proposed metabolic pathways for *N*-nitroso compounds leading to mutagenesis that necessitate trade-offs between model predictivity and mechanistic applicability in view of limited training-set data.

acceptors with skin proteins (i.e., the initiating event in dermal sensitization).

QM models in predictive toxicology must also grapple with uncertainty and variability of biological data.[53] While these tools may be very accurate in capturing well-defined and well-understood biochemical events,[54] many adverse outcome pathways (AOPs) are incomplete (e.g., respiratory sensitization),[55,56] and toxic end points used to fit models can be dominated by unreliable experimental data.[57,58] In the *N*-nitrosamine example above, rodent cancer bioassays used to determine carcinogenic potency (i.e., tumor dose—TD_{50} values) have a mere 57% reproducibility.[59] While some issues can be alleviated with curation,[35,60] and QM is particularly amendable to this as a "first-principle" approach that does not need ample data to be robust,[37] no predictive tool should be held to a higher standard (of predictivity) than the underlying data upon which it was built. In dealing with uncertainty (vs data quality or variability), one can tune model specificity to balance resolution of predictivity (i.e., what metrics can be reliably predicted) with breadth of mechanistic applicability (i.e., what processes can be captured by the model). This is useful when we do not understand all relevant mechanisms of key events but also when there are too many well-characterized mechanisms for the size of available data, and so model overfitting would be an issue if we attempted to describe all transformations explicitly. For example, in the case of *N*-nitroso metabolism, one might calculate atom-based QM indices to capture reactivity at specific molecular sites in α- or β-hydroxylation, heterolysis, or DNA-binding events (Figure 2).[37] However, one might also want to incorporate global reactivity metrics, such as those derived from frontier molecular orbital theory (FMOT),[61] to account for uncertainty in the mechanistic pathway and to capture the known competing processes, such as 1,3-sigmatropic shift or hydrolysis available for some *N*-nitroso compounds,[62] albeit with a lower resolution. Lastly, one needs to account for bioavailability (e.g., GI tract absorption). The resultant trade-off is decreased resolution of predictivity to a few potency categories (vs specific tumor dose values), in return for greater applicability across the diverse *N*-nitroso chemical class.[37] While this trade-off is necessary to develop a robust model, it is also perfectly sensible given the variability and uncertainty of the underlying experimental data.

For the practitioner, the value proposition of QM does not rest on a single exemplar of the *N*-nitroso model above. QM-based reactivity indices developed either as global (i.e., whole-molecule) descriptors derived from FMOT or local (i.e., atom-based) metrics leveraging the Fukui function[63,64] have resulted in robust predictive models across toxic endpoints (e.g., for skin[28] and respiratory sensitization,[65] acute and chronic aquatic toxicity,[66,67] and mutagenicity and carcinogenicity[37]). Ecotoxicity is of particular relevance here due to the many underlying modes of action (MOA), the high number of tested chemical classes (49), and the end point's modern role as an alternative to vertebrate animals for hazard assessment.[1] In comparing ecotoxicity models, Melnikov et al. showed that a QM-based approach outperforms other tools by a considerable margin in external testing,[67] underscoring QM applicability to both specific transformations in toxicity pathways (e.g., inhibition of acetylcholinesterase as an ecotoxicity MOA) as well as processes with poorer mechanistic resolution (e.g., narcosis or general electrophilicity MOAs).[66]

The reader could raise several objections here. First, QM-based reactivity indices do not explicitly consider sterics, and indeed all models above integrate steric factors (e.g., atom-based solvent-accessible surface area or volume) along with electronic parameters. Second, many key events for the aforementioned toxic end points are Lewis acid−base reactions, with toxicants acting as electrophiles. These are the proverbial "low-hanging fruits", readily supported by the theory of Hard and Soft Acids and Bases (HSAB) in QM calculations of electrophilicity indices.[68] In contrast, nucleophilicity and radical-chemistry indices are less explored in the literature, though recent studies have demonstrated their respective utility to describe organic reactivity[69] and metabolic activation and depletion mechanisms.[37,50,65] Lastly, there will be cases where insufficient data and knowledge limits training of QM-based predictive models. To that end, QM can still deliver supporting evidence in hazard assessments (e.g., in electronic structure read-across),[37] as was shown for carcinogenicity, neurotoxicity, oxidative stress, or general cytotoxicity.[37,68,70]

THE ROAD AHEAD

One could define the dilemma of future model development by how we decide to allocate our large (but finite) resources. We can invest into physics-driven (QM) models or data-driven approaches based on AI (artificial intelligence), which has been reshaping our scientific enterprise in recent years, from predicting outcomes of chemical reactions[71] to supporting vaccine research and clinical trials.[72] In principle, QM and AI can coexist in predictive toxicology,[73] whereby QM is used to calculate descriptors and AI constructs relationships between descriptors and toxicity outcomes. However, both approaches are quite power-hungry, so, in practice, the need for big data by AI limits generation of computationally demanding QM

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descriptors. Concurrently, AI in predictive toxicology suffers from the limited amount of high-quality (whole-animal) data that is available now and for the foreseeable future if we eliminate animal testing. Case in point, there are fewer than 100 *N*-nitrosamines with reliable carcinogenicity studies to support model development.[32,37] This is a constraint that may never be fully alleviated by high-throughput screening (HTS) of *in vitro* and *in chemico* assays, as these tools offer a partial glimpse into organism-level effects[74] and might be better suited either to validate specific events in the training of *in silico* models or to contribute to a weight of evidence approach.[25] Conceptually, our concern with AI as a tool to predict toxicity outcomes is that while the optimization algorithms are comprehensible, the relationships constructed between model inputs and outputs are inscrutable, undermining confidence in external predictivity. Crucially, these models do not further our toxicological knowledge, i.e., our understanding of molecular mechanisms that lead to adverse outcomes, as they are the proverbial "black boxes".

There exists, however, an alternate paradigm, where AI is not used to predict toxicity but is applied instead to improve the accuracy of QM methods at lower computational cost. In this case, we have ample data to train AI models (and we can readily generate more data as models are trained on computational vs experimental outcomes). To this end, Bogojeski et al. leveraged machine learning (ML) to calculate highly accurate coupled-cluster (CC) energies from density functional theory (DFT), reducing computational errors from 2−3 kcal/mol to under 1 kcal/mol.[75] They showed that the use of point groups reduced the amount of training data required (Achilles heel of ML), and the lower computational cost allowed for integration of these methods into molecular simulations to capture system dynamics, which we showed is key to modeling key events in predictive toxicology.[28,37,65] In a different example, aiming to advance drug and materials design, Smith and co-workers approached CC accuracy for a broad range of organic chemistry by training a neural network on DFT data and then using transfer learning techniques to retrain the model on a higher level of theory.[76] In the same vein as the two studies above, AI can be used to target physicochemical properties derived from molecular energy. For example, Isert et al. reported that ML can be learnt on calculated log $K_{o/w}$ values to obtain a computationally affordable, QM-based estimation of lipophilicity.[77] Thus, AI and QM have a shared future that can benefit *in silico* toxicology, so long as we keep in mind their fundamental differences and limitations. In this regard, while QM has evolved in the Darwinian sense from human knowledge and understanding of the natural world and has been extensively tested over the past nearly 100 years, AI is a difficult-to-verify construct of the machine world that requires large data to ensure robustness. Marie Curie, the first woman to win the Nobel Prize, famously said that "nothing in life is to be feared, it is only to be understood. Now is the time to understand more, so that we may fear less." For many practitioners in toxicology, this statement applies to both QM and AI if we want to move beyond current structure−activity relationships and develop *in silico* frameworks robust enough to replace animal tests.

A WIN−WIN SCENARIO

In conclusion, let me address the risk and hazard assessors, who are the end-users and immediate (though not the ultimate) beneficiaries of *in silico* tools in predictive toxicology. Whatever your hesitancy might be, the adage that "the proof is in the pudding'" holds true, and so I encourage you to give QM a try, whatever the model, to judge for yourself the validity of our claims based on real-world results. While QM may not be the "magic bullet" for your purposes (though it might very well be!), the strategy of incorporating QM into your workflow is consistent with the widely accepted framework of integrated testing strategies (ITS) in support of safety assessments.[78,79] There is a clear benefit to combining *orthogonal* (i.e., differentially derived) modeling approaches to gauge potential risk from untested chemicals, as was recently showcased by a study on the skin sensitization potency of peptide coupling agents, which won the Occupational and Public Health Specialty Section of Society of Toxicology Paper of the Year Award for 2022.[31] By incorporating QM into hazard assessment, these models can help elucidate structure−activity relationships and pinpoint dubious experimental outcomes that may warrant retesting.[31] In the end, bringing QM fully into the fold of predictive toxicology is a "win−win" scenario for both fields.

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