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REVIEW

The evolving role of *in silico* toxicology in science and industry: A narrative review

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ABSTRACT. Modern toxicology is experiencing a major paradigm shift driven by the demand for efficient chemical safety assessment and the ethical and economic limits of animal testing. *In silico* toxicology, understood as the use of computational tools and computer-based simulations to predict and analyze toxic effects, has become a core element of this transformation. By enabling rapid, cost-effective, and mechanistically based hazard assessment, computational approaches bridge science, industry, and regulation. This development echoes the idea of "heartbroken toxicology", where the scientific, industrial, and regulatory domains have grown apart. *In silico* methods offer a way to reconnect these dimensions and foster a shared framework for decision-making. This narrative review summarizes current advances, methodological foundations, and key challenges of *in silico* toxicology, emphasizing its expanding role in regulatory and industrial contexts and the need for harmonized validation, standardization, and education to secure its place as a cornerstone of 21st-century toxicology.

Keywords: *In silico models; Computational toxicology; Computer simulation; Risk assessment; Toxicity tests.*

Modern toxicology faces enormous challenges: we live in a world exposed to thousands of chemical substances and countless mixtures.[1,2] Ensuring chemical safety requires innovative solutions, particularly because traditional *in vivo* toxicity testing (animal studies) is time-consuming, costly, and constrained by ethical considerations.[3,4] The use of experimental animals has become increasingly difficult to justify, both socially and scientifically, especially given the limited predictive performance of classical animal models.[5] At the same time, industry and regulatory agencies must evaluate the safety of thousands of substances (e.g., under the EU REACH program),[6] which, within a traditional testing paradigm, would require the use of tens of millions of animals. Such an approach is not only unethical but also impractical.

In the face of these challenges, a shift in the toxicological paradigm becomes essential, moving away from a model that relies primarily on *in vivo* experiments toward strategies based on *in silico* methods, *in vitro* tools, and a mechanistic understanding of toxic action.[7] In recent years, the urgent need for such a shift has been widely recognized. This vision requires a radical rethinking of toxicology, effectively turning traditional procedures upside down.

Historically, toxicity assessment began with *in vivo* studies (often treated as a "black box") and only later proceeded to mechanistic analyses.[8] The new approach proposes the opposite strategy: to begin with *in vitro* and *in silico* studies that identify toxicity pathways, and only then (if necessary) advance to narrowly targeted animal tests. This reversed "toxicological funnel"[1] represents a fundamental transformation of toxicology from a largely descriptive discipline (supporting regulatory decision-making) into a full-fledged biological science grounded in the understanding of underlying mechanisms.

The need for a new approach arises not only from ethical and regulatory pressures but also from emerging scientific challenges. Modern chemistry and the pharmaceutical industry continuously generate new compounds. For example, in the field of new psychoactive substances (NPS), dozens of previously unknown chemicals appear each year. Traditional methods cannot keep pace with the assessment of their risks, creating gaps in public, clinical, and forensic safety. Another category involves the chemistry of chemical warfare agents, which relies almost exclusively on archival data from the 1940s, even though these compounds are widely used as "natural" components.

Given these circumstances, both science and industry recognize that the time has come for a genuine paradigm shift in toxicology. In line with the concept of "heartbroken toxicology" described in 2008,[1] modern toxicology can be seen as a discipline whose scientific, applied, and regulatory "souls" have become disconnected. This separation has led to stagnation, limited innovation, and communication barriers between academic science, industrial needs, and regulatory expectations. In this context, *in silico* methods emerge as one of the few tools capable of restoring this dialogue. They enable rapid, cost-effective, and mechanistically grounded assessment of chemical safety at early stages of development, providing a shared platform for scientific discovery, industrial application, and regulatory evaluation. Thus, computational toxicology may serve as a bridge, capable of reuniting the "broken heart" of the field and advancing a more integrated and forward-looking paradigm for 21st-century toxicology.

This article provides a narrative overview of this transition—from traditional methods to modern *in silico* and related strategies. The subsequent sections summarize the current state of knowledge in this field, outline the challenges and limitations of *in silico* approaches, present examples of their applications (in regulation, industry, and risk assessment), and conclude with recommendations for fully leveraging the potential of i*n silico* toxicology.

METHODS

This article adopts a narrative review approach aimed at synthesizing current knowledge on the development and application of *in silico* toxicology. Relevant literature was identified through a structured search of major scientific databases, including PubMed, Scopus, and Web of Science, using combinations of keywords such as "in silico toxicology" and "computational toxicology". Only peer-reviewed publications written in English were considered. Studies were included if they addressed methodological aspects, applications, or regulatory perspectives related to *in silico* approaches in toxicology.

Initial search results were screened based on titles and abstracts, followed by full-text review to confirm eligibility. Additional references were identified through manual searches of cited literature in key review articles. The gathered evidence was narratively synthesized, emphasizing thematic organization rather than quantitative analysis. The discussion highlights major trends, challenges, and perspectives emerging from the reviewed literature.

DISCUSSION

Current state of knowledge: from in vivo testing to in silico approaches

Traditionally, toxicology has relied on animal experimentation. For decades, it was assumed that a comprehensive battery of *in viv*o tests (often described as the "gold standard") was essential for assessing chemical hazards in humans.[9] This model can be illustrated by the metaphor of a funnel: at the top lies a broad collection of untested substances subjected to standard animal studies, and only a narrow stream of results ultimately yields a limited number of well-characterized chemicals and mechanisms.[1] The remaining substances are classified as less hazardous or remain poorly defined. This classical toxicological funnel reflects an approach in which findings from animal studies trigger subsequent, often mechanistic, investigations of selected compounds.

Today, however, new concepts and technologies are reshaping this paradigm. *In vitro* methods—including assays using cell lines, organoids, and organ-on-a-chip systems—and *in silico* approaches, which incorporate computational simulations, mathematical models, and artificial intelligence (AI) to predict toxic effects, now play a central role. Within the modern toxicological framework, these methods should represent the first line of toxicity assessment. They provide information about potential mechanisms of action before any animal testing is considered. This model assumes that animal studies should be used only to fill remaining data gaps after advanced *in vitro* testing and *in silico* analyses have been completed, essentially the reverse of the traditional approach. This shift is often described as a breakthrough that marks the entry of toxicology into the twenty-first century. The term "Toxicology for the 21st Century"[1,2] refers to a set of new methods and concepts designed to make toxicological evaluation more efficient, data-driven, and human-relevant. A central element of this transformation is the transition from treating toxicity testing as a "black box" to adopting a mechanistic framework. In practice, this means focusing on toxicity pathways and adverse outcome pathways (AOPs),[10] which outline sequences of biological events beginning with an initial molecular interaction and culminating in an adverse effect. Initiatives such as the Human Toxome Project (HTP)[11] and the AOP program of the Organisation for Economic Co-operation and Development (OECD) aim to catalogue these pathways and their associated biomarkers. With a well-defined map of key biological events, it becomes easier to connect *in vitro* and *in silico* data with potential *in vivo* outcomes.

Predictive in silico methods in modern toxicology

In silico methods encompass a broad range of approaches. The term extends far beyond traditional QSAR models (quantitative structure–activity relationship models), which predict a chemical's biological activity based on its molecular structure. *In silico* toxicology includes any activity performed using a computer—from experimental planning and statistical analysis to sophisticated computational modeling.[8] Simple tasks such as determining the number of replicates needed to achieve adequate statistical power or applying algorithms for data processing are everyday *in silico* activities in a toxicology laboratory. However, the greatest interest focuses on predictive methods that can replace or reduce the need for animal studies.

Predictive *in silico* tools include a variety of methods that enable the estimation of toxicological properties without direct animal testing. These approaches include:

1. **QSAR models**. Predict a chemical's biological or toxicological activity based on its molecular structure and can estimate outcomes such as acute or chronic toxicity.[8]
2. **Read-across**. Infers the properties of an untested chemical based on data from structurally or functionally similar substances and is widely used in regulatory frameworks such as REACH.[8]
3. **IVIVE (*in vitro–in vivo* extrapolation)**. Translates concentrations causing effects *in vitro* to equivalent *in vivo* doses using toxicokinetic information, such as physiologically based pharmacokinetic (PBPK) models, to simulate absorption, distribution, metabolism, and excretion. The success of this method depends on the relevance of the *in vitro* systems and the availability of tools for reverse toxicokinetics, which allow calculation of *in vivo* doses corresponding to active *in vitro* concentrations.[12]

In parallel, the volume of experimental data has expanded dramatically. Modern computational techniques make it possible to manage and analyze very large datasets, opening new opportunities for discovering SAR but also creating a challenge: how to extract meaningful conclusions from such extensive data. The goal is not only to generate big data but also to generate "big sense", meaning biologically and regulatorily useful insight.

In silico methods: challenges and limitations

Despite substantial progress, *in silico* toxicology still faces several challenges and limitations that must be critically considered. First, the performance of any *in silico* model depends directly on the quality of its input data.[8] A computational model can never exceed the quality of the data on which it is built, a concept captured by the well-known principle "garbage in, garbage out". *In silico* methods inevitably inherit the weaknesses of their underlying datasets, which are often derived from *in vitro* or *in vivo* studies. For example, a QSAR model constructed from a small or chemically homogeneous dataset may lack predictive power outside that chemical space, illustrating the problem of a restricted applicability domain. Similarly, if an *in vitro* system fails to reflect essential physiological characteristics (such as a cell line with limited metabolic capacity) then even the most sophisticated IVIVE approach cannot convert poor-quality experimental data into a reliable *in vivo* prediction. In short, poor-quality data combined with advanced methodology still yields poor-quality predictions.

Another major challenge is the validation and acceptance of *in silico* methods.[8] The scientific community and regulatory agencies often adopt a cautious stance toward new technologies. Before a QSAR prediction can be considered equivalent to an animal study result, the method must undergo rigorous evaluation for reproducibility, reliability, and fitness for purpose. The traditional validation paradigm, historically applied by organizations such as the European Centre for the Validation of Alternative Methods (ECVAM) for alternative methods, relied on comparing the results of a new method against the "truth" defined by *in vivo* tests.[13] For *in silico* approaches, however, this type of comparison may be insufficient or even inappropriate, as the goals and assumptions of these methods can differ fundamentally from those of classical animal tests. This is why an evidence-based validation framework is increasingly advocated as a continuous, systematic evaluation process that integrates all available data from multiple sources. In practice, this includes systematic reviews, assessment of data quality, meta-analyses, and expert consensus to determine whether a method is suitable for decision-making. The ideal is to base decisions not on the historical authority of animal models but on the best possible combination of modern methods that together provide the most reliable prediction of hazard. An additional challenge is persuading stakeholders, including industry and regulators, that these new approaches can ensure at least the same level of protection as traditional methods. This is a nontrivial task given decades of reliance on animal tests as the mentioned earlier "gold standard".[9]

A significant limitation also arises from the inherent complexity of biological systems and the fact that our understanding remains incomplete. The human body is an intricate network of organs, pathways, feedback systems, and

interactions. Many of these elements, such as homeostatic compensatory mechanisms, immune responses, and microbiome-driven effects, are extremely difficult to model *in silic*o.[8] Although advanced technologies, including organ-on-a-chip systems and multi-organ microphysiological platforms, are rapidly evolving, we are still far from fully replicating whole-organism physiology *in vitro*. Systems toxicology approaches, which integrate data across multiple biological levels using computational modeling, aim to bridge this gap by predicting organism-level outcomes from molecular and cellular inputs. Despite progress, achieving sufficient accuracy and verifiability remains a major challenge.

There are also practical and educational constraints. Broad implementation of *in silico* toxicology requires specialized knowledge in fields such as informatics, statistics, and AI, as well as access to computational resources. Moreover, it is increasingly clear that progress in this domain will depend on the effective use of AI. Traditionally trained toxicologists may need additional instruction or collaboration with data scientists to fully leverage these new tools and competently use AI-based platforms. Standardization presents another challenge: numerous platforms, algorithms, software packages, and databases are available, but they do not always produce consistent results. Harmonized guidelines for the use of *in silico* methods, analogous to OECD test guidelines for *in vitro* and *in vivo* methods, are necessary to ensure comparability and regulatory acceptance.

Finally, no single method offers a universal solution. The strongest predictive power arises from integrating multiple complementary approaches within the framework of Integrated Approaches to Testing and Assessment (IATAs).[14] A combination of modern methods—e.g., an *in vitro* assay identifying a mechanism of action, an *in silico* model predicting systemic toxicity, and an exposure assessment—can provide more convincing evidence than any single method used in isolation. Importantly, consistent negative results from such an integrated, modern testing strategy should be considered strong evidence of safety rather than a justification for continuing animal testing indefinitely. Many industrial chemicals show no toxicity at relevant exposure levels, and increasing confidence in negative outcomes generated by alternative methods would allow unnecessary animal tests to be avoided. In other words, if a coherent suite of modern methods indicates that a substance is not harmful, we should trust that conclusion, thereby saving time, costs, and animal use, and allowing resources to be focused on substances that truly warrant concern.

The importance of in silico toxicology for regulatory and industrial applications

In silico methods are gradually moving from the realm of scientific research into practical use in both regulatory decision-making and industrial settings. Notably, even traditionally cautious regulatory agencies, such as the U.S. FDA, are increasingly willing to rely on evidence generated through alternative methods. A clear example is the International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use (ICH) M7 guideline on mutagenic impurities in pharmaceuticals,[15] which has effectively institutionalized the use of *in silico* approaches, in the form of two complementary QSAR assessments, as an acceptable substitute for the Ames test, a bacterial reverse-mutation assay commonly used to detect mutagenic potential.

From the industry perspective, the message is clear: companies recognize the cost savings and strategic advantages offered by *in silico* methods. Implementing computational predictions early in product development can significantly reduce the number of toxicological "surprises" at later stages, saving both time and capital. Moreover, *in silico* approaches support compliance with the 3R principles by enabling the reduction and replacement of animal testing where possible and by refining unavoidable *in vivo* studies through improved targeting. For example, early insights into potential target organs of toxicity make it possible to design more focused animal studies.

In the pharmaceutical sector, *in silico* toxicology is particularly valuable because it helps reduce the number of expensive, months-long preclinical experiments and allows earlier elimination of compounds with unfavorable ADMET profiles. Every drug candidate that fails during *in vivo* studies or, even worse, in clinical trials generates financial losses amounting to tens of millions of dollars. *In silico* models enable earlier filtering of high-risk compounds, thereby improving the overall efficiency of the R&D pipeline. This industry also benefits from the ability to simulate scenarios that would be impossible or unethical to test in animals, such as analyzing reactive metabolites, predicting drug–drug interactions, or identifying structural toxicophores responsible for adverse effects. As a result, *in silico* approaches are becoming not merely supportive tools but essential components of strategies aimed at reducing business risk and increasing the likelihood of clinical success.

The future of in silico toxicology: from vision to implementation

Toxicology at the turn of the 21st-century is rapidly moving toward a new paradigm in which *in silico*, *in vitro*, and mechanistic approaches will play a dominant role. The

arguments for this shift are compelling: on one hand, there is a growing number of substances requiring safety assessment, accompanied by rising costs and ethical concerns; on the other hand, scientific progress has provided tools that allow toxicity to be studied faster, at lower cost, and with far greater mechanistic insight. What is needed is a genuine "inversion" of traditional procedures, transferring the primary burden of testing from live animals to advanced *in vitro* models and computational simulations. Only a radical paradigm shift will allow us to meet the scientific, regulatory, and societal challenges of the coming decades. To fully implement this new paradigm, coordinated efforts are required across several key areas:

- **Further scientific development of alternative methods.** Continuous advancement of *in silico* models is essential, including deeper integration of deep learning approaches, hybrid models that combine chemical and biological descriptors, and progress in *in vitro* systems such as optimized cell lines, organoids, and microfluidic technologies.
- **Validation and standardization.** The toxicology community must establish modern validation frameworks for both *in silico* and *in vitro* approaches. Evidence-based validation, inspired by the principles of evidence-based medicine, is increasingly recommended for evaluating new methods. International organizations such as the OECD have begun publishing initial guidance, including the well-known OECD Principles for QSAR validation. Crucially, it must be demonstrated in practice that integrated alternative approaches can predict hazards with confidence levels comparable to those of traditional *in vivo* tests. Demonstrating such equivalence is essential for building trust among regulators and the public.
- **Integration of data and approaches.** No single method, whether *in vitro* or *in silico*, can capture the full complexity of biological systems. Therefore, the future lies in IATAs, which combine diverse types of data into coherent risk assessments. Formal weight-of-evidence frameworks should be further developed to guide the integration of outputs from multiple complementary methods. Equally important is the creation of informatics platforms capable of integrating large toxicological datasets, ensuring that big-data omics studies are accessible and genuinely useful for modeling and decision-making.
- **Education and cultural change in science.** Transitioning to this new paradigm requires preparing a new generation of toxicologists who are fluent in both the biological and data sciences. Academic and industrial communities must also move beyond their attachment to "the old and familiar" (such as routinely repeated rodent assays) and adopt a mindset open to innovation. Continuing to rely on legacy animal models without rigorous evaluation of their relevance is no longer justified. Instead, decisions should be guided by the principle of selecting the best available method for each scientific question, regardless of its historical status.

CONCLUSIONS

In silico toxicology has emerged as a fundamental component of modern chemical safety assessment, extending well beyond QSAR modeling to include advanced computational, mechanistic, and AI-driven approaches. Although its scientific value is increasingly supported by robust evidence, its full integration remains hindered by persistent reliance on traditional animal-based paradigms. Overcoming these barriers demands a decisive shift toward innovation-driven, interdisciplinary, and evidence-based frameworks, positioning *in silico* toxicology as a central pillar of 21st-century toxicology.

Conflicts of interest
The author declares no conflicts of interest.

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