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Original Research Article

INTEGRATING TOXICOGENOMICS AND EPIGENETICS FOR NEXT-GENERATION TOXICITY RISK ASSESSMENT

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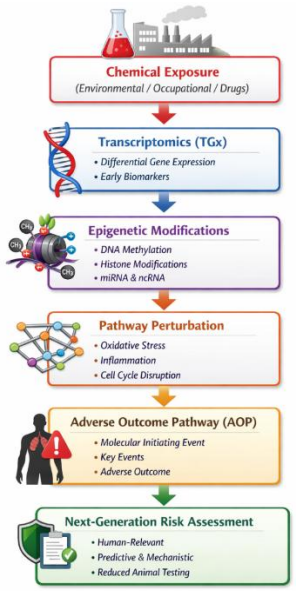
Abstract:

Modern toxicology is currently in process of fundamental change from observational toxicity testing to molecular-level evaluations of the effects of a chemical. Toxicogenomics and epigenetics have individually built on our knowledge of how toxicants alter biological systems via gene expression modifications and heritable regulatory pathways. Despite these advances, current risk assessment frameworks remain limited in their ability to interpret complex molecular responses, particularly associated with low-dose exposure or chemical mixtures, and the presence of long-term or latent health effects. Absence of an integrated molecular approach constrains the accurate prediction of toxicity to human health. We reviewed the prospect of synergistic benefit of integration of the toxicogenomic and epigenetic knowledge in the next-generation toxicity risk evaluation through mechanistic knowledge of chemical-induced biological perturbations. Existing experimental and computational reports were systematically reviewed, specifically transcriptome analysis, epigenetic alterations including DNA methylation and non-coding RNA regulation and their inclusion in systems toxicology and adverse outcome pathway analysis. There is evidence that integrated molecular signatures can enhance early recognition of toxic effects, clarify modes of action, and sensitivity to chronic and transgenerational toxicity risks. These designs also facilitate animal testing alternatives and enhance regulatory considerations. The fusion of toxicogenomics and epigenetics has presented a strong mechanism-driven basis for the future analysis of toxicity risk, leading the way to a more predictive, ethical, and human-appropriate chemical safety evaluation in the future.

Keywords:

Toxicogenomics, Epigenetic regulation, Mechanism-based toxicity, Next-generation risk assessment, Systems toxicology

Diagrammatic Abstract:



1. Introduction

Toxicologists have experienced a paradigm shift away from conventional apical endpoint-based diagnoses into molecular and systems-level methodologies that try to account for toxicity by its underlying biological mechanisms rather than by observed phenotypic outcomes alone (Waters, 2003; Waters and Fostel, 2004; NRC, 2007; Seal et al., 2025; Wang et al., 2025). The classical toxicology was primarily concerned with endpoints, such as mortality, organ pathology or reproductive failure, and while informative they reveal limited information on either acute or subtle biological processes prior to toxic effects (Krewski et al., 2014; EFSA et al., 2025; Sokolowski et al., 2025). This shift has been fueled by advances in molecular biology, computational modelling, and high-throughput technologies that enable toxicity to be investigated on a gene and pathway basis. Animal toxicity testing, a major pillar of standard risk assessment, is becoming less and less suitable for addressing modern regulatory and ethical needs (Kreutz et al., 2024; Tarazona et al., 2025). In animal studies, the extrapolation of data to human health risk suffers from species-specific differences in toxicokinetics and toxicodynamics, contributing to uncertainty and the potential misclassification of hazards (Hartung, 2009; Paustenbach et al., 2025). Not to mention that animal studies are resource intensive, time consuming and unsuitable for the evaluation of the extensive number of existing and emerging chemicals that need safety analysis (Adler et al., 2011). More specifically, threshold-based approaches like the no-observed-adverse-effect level (NOAEL) and lowest-observed-adverse-effect level (LOAEL) significantly reduce predictive capacities, because they are based on discrete experimental doses and provide no biological variability or mechanistic information (Crump, 2011). High-dose extrapolation from animal models could further obfuscate molecular effects at low doses relevant to those of interest in the environment (especially in endocrine-active or developmentally sensitive chemicals) (Vandenberg et al., 2012).

Toxicogenomics has gained a lot of traction through large-scale analysis of chemical exposure-induced gene expression changes. Technologies like high-throughput transcriptomic platforms, such as microarrays and RNA sequencing, offer sensitive techniques to detect early molecular perturbations before overt toxicity becomes apparent (Waters & Fostel, 2004). Toxicogenomic profiles have been successfully employed to classify chemicals by their shared modes of action, characterise salient toxicity pathways, and facilitate prioritization and screening of chemicals (Thomas et al., 2013; Alnasser et al., 2025). More importantly, transcriptomic data enable dose–response associations to be examined at the pathway level, rather than classical apical endpoints, which presents increased resolution (Merrick et al., 2015). But gene expression changes are temporary and context-restricted, and can be attributed to adaptation and response rather than to irreversible adverse effects (Krewski et al., 2014). The epigenetics approach adds a regulatory element that resolves some of the shortcomings in toxicogenomics. Genome modification under epigenetic regulation by DNA methylation, histone modifications and non-coding RNA regulation governs gene expression without modifying the underlying DNA sequence, and is especially sensitive to environmental stimuli (Feil & Fraga, 2012; Vaschetto et al., 2024). Unlike transcriptomic changes, epigenetic modifications may remain long after exposure has stopped, effectively serving as a molecular record of environmental stressors, termed as environmental memory (Baccarelli & Bollati, 2009). Recent evidence suggests that toxicant-induced epigenetic changes are central in developmental

toxicity, carcinogenesis, neurotoxicity and metabolic disorders (Head et al., 2012). Additionally, certain epigenetic changes can be passed down through generations, raising questions regarding long-term and widespread transgenerational health outcomes that traditional risk assessment paradigms cannot account for (Skinner, 2014; Alum et al., 2024).

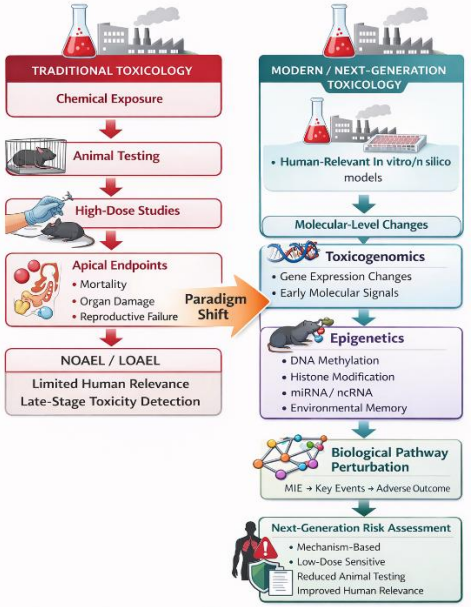


Fig.1. Paradigm Shift in Toxicology: From Traditional Apical Endpoints to Mechanism-Based Next-Generation Risk Assessment.

Although toxicogenomics and epigenetics have strengths in and of themselves, we can only partly characterize toxicity through them. Transcriptomic data offer high temporal sensitivity, but limited insight into the durability of biological effects, while their epigenetic counterpart records long-term regulatory changes but may lack immediate functional context (Ruprecht et al., 2024; Udeh-Momoh et al., 2025). Combining these complementary data sources allows for a fuller appreciation of both initial molecular initiating events and sustained regulatory alterations underlying adverse outcomes (Sturla et al., 2018; Johansson 2023). This kind of integration is especially needed to test for low-dose exposures, chemical mixtures, and chronic or latent toxic effects that are not well considered with conventional testing paradigms (Escher et al., 2017; Browne et al., 2024). We review the potential for blending toxicogenomic and epigenetic data to improve mechanism- and predictive-informed toxicity risk assessment (Fig. 1).

2. Methodology of Literature Review

2.1 Literature Search Strategy

A systematic and comprehensive literature search was performed to identify relevant studies dealing with the integration of toxicogenomics and epigenetics in toxicity risk assessment. The search included major scientific databases, including PubMed, Scopus, Web of Science, and Embase, so that the potential breadth and scope of research on the subject in terms of biomedical, toxicological, and interdisciplinary studies was well-selected (Moher et al., 2009). Controlled vocabulary and free-text terms alongside Boolean operators were applied in the searches and results in order to enhance sensitivity and specificity. Major search strings were "toxicogenomics AND epigenetics", "omics-based risk assessment" and "adverse outcome pathway AND epigenetic regulation" (Gutleb et al., 2025). Further relevant papers were identified by tracking the backward and forward citation of a few selected publications in order to decrease the potential for omission of relevant studies (Greenhalgh & Peacock, 2005; Hirt et al., 2023; Gusenbauer, 2024). To ensure accurate interpretation of findings only articles published in English were considered.

2.2 Inclusion Criteria

Studies were included if they provided mechanistic insight into toxicant-induced molecular responses using toxicogenomic, epigenetic, or integrated multi-omics approaches. Both experimental and computational studies were considered, encompassing human epidemiological data, animal models, and in vitro systems, reflecting the diversity of contemporary toxicological research (Sturla et al., 2018). Eligible studies were required to involve high-throughput transcriptomic analyses, epigenetic profiling, or integrative frameworks linking molecular alterations to biological pathways or adverse outcomes (Parikh and Shah, 2024). Particular emphasis was placed on studies explicitly discussing relevance to toxicity mechanisms, dose–response relationships, or regulatory risk assessment.

2.3 Exclusion Criteria

Studies were excluded if they were purely descriptive, lacked mechanistic interpretation, or did not link molecular changes to toxicological outcomes. In order to ensure scientific rigor and reliability, non-peer-reviewed sources (conference abstracts, editorials, commentaries, and preprints) were excluded (Higgins et al., 2022). We also excluded articles that were strictly clinical epigenetics or genomics and did not have toxicological relevance.

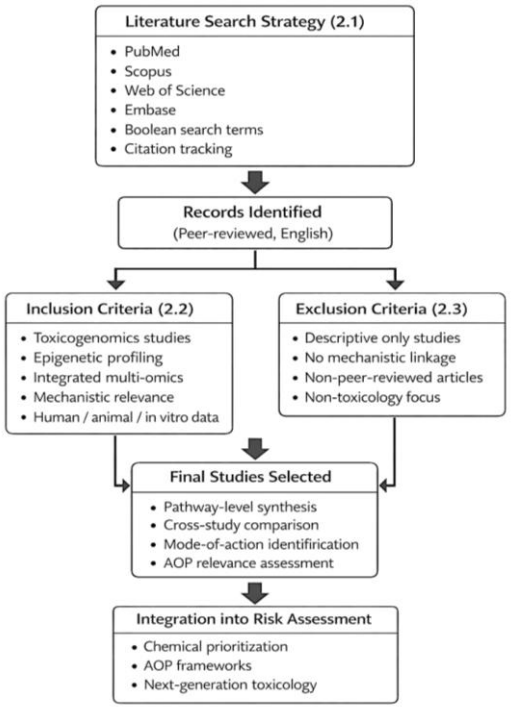


Fig.2. Workflow of literature search, study selection and data analysis.

2.4 Data Analysis Approach

Selected studies were qualitatively synthesized using a pathway-level analysis approach (Fig.2), focusing on the convergence of molecular signals across different experimental systems rather than specific changes at the gene level (Krewski et al., 2014). Across-study comparisons were made to detect recurring molecular pathways, epigenetic patterns, and modes of action of specific classes of toxicants. Finally, investigations were assessed in terms of their regulatory relevance, including their applicability to adverse outcome pathway (AOP) frameworks, chemical prioritization, and next-generation risk assessment paradigms (OECD, 2018).

3. Toxicogenomics in Toxicity Risk Assessment

3.1 Definition and Scope

Toxicogenomics refers to the application of genome-scale technologies to elucidate how chemical exposures alter biological systems at the molecular level, thereby contributing to toxicity and disease risk (Waters & Fostel, 2004). Although the field encompasses multiple omics layers—including transcriptomics, proteomics, and metabolomics—its primary focus in regulatory toxicology has been on transcriptomics, owing to its sensitivity and scalability (Thomas et al., 2013). Gene expression signatures generated following chemical exposure provide insight into perturbed biological pathways and cellular stress responses, enabling mechanistic interpretation beyond traditional apical endpoints (Merrick et al., 2015). These signatures can be quantitatively linked to dose and time, offering a molecular framework for understanding early events preceding overt toxicity (Krewski et al., 2014).

3.2 Key Technologies

Rise of high-throughput technologies led to the rapid uptake of toxicogenomics in toxicity assessment. RNA sequencing (RNA-Seq) stands out as the primary platform offering high sensitivity, wide dynamic range, and capability of detection of novel transcripts and alternative splicing events induced by toxicants (Wang et al., 2009). Earlier microarray-based platforms continue to be useful for comparative and legacy datasets, especially in large-scale screening programs where standardized reference data exist (Waters et al., 2010). More recently, single-cell transcriptomics has emerged as a powerful tool to resolve cell-type–specific toxic responses that are masked in bulk tissue analyses, thereby improving mechanistic resolution in complex tissues such as liver and brain (Trapnell, 2015). Such technologies enable multiscale analysis of toxicant-induced gene expression changes across biological systems.

3.3 Applications in Toxicity Risk Assessment

In toxicogenomics, early prediction of toxicity is one of the key applications, as transcriptomic perturbations often precede phenotypic or pathological changes detectable by conventional methods (Thomas et al., 2013). Pathway-based gene expression profiles have been used to distinguish adaptive stress responses from those leading to adverse outcomes, supporting more informed hazard identification (Merrick et al., 2015). Toxicogenomic data are crucial for mode-of-action (MoA) identification by linking chemical exposure to specific molecular initiating events and downstream signaling pathways (Andersen et al., 2018). This mechanistic perspective enables chemical grouping and read-across strategies, diminishing the need for extensive animal testing. Moreover, transcriptomic profiling has been integrated into chemical prioritization frameworks like high-throughput screening programs, which prioritize substances based on biological potency and pathway perturbation rather than solely on exposure levels (Kavlock et al., 2012).

3.4 Limitations and Challenges

Despite its utility, toxicogenomics still poses a number of limitations that hinder its standalone application in measuring risk, even though it possesses many of its strengths. Gene expression responses are dynamic (and therefore sensitive) and temporally variable, and transcriptional changes in gene expression do not always lead to lasting toxicity, and are often transient (Krewski et al., 2014). Differentiation of adaptive cell responses and truly harmful aberrations continues to be a tremendous conundrum, especially in low-dose or short-term exposure settings (Escher et al., 2017). Moreover, transcriptomic changes usually lack a heritable context and thus account for only limited information about long-term biological memory, or delayed health outcome (Feil & Fraga, 2012). This limitation is especially salient in the context of chronic exposures and in developmental windows of susceptibility, where persistent regulatory changes may occur in the absence of sustained gene expression

differences. As such, if toxicogenomics is not coupled with other, complementary layers of molecular composition, it risks over- or under-reporting risks. Altogether, toxicogenomics has revolutionized toxicity risk estimation through mechanism-based, pathway-level treatment approach. Its predictive power, however, is enhanced when integrated with regulatory constructs such as epigenetics for temporal persistence and long-term biological effects. This realization supports the continued importance placed on integrative, multi-omics strategies in next-generation toxicity risk assessment.

4. Epigenetics in Toxicology

4.1 Epigenetic Mechanisms

Despite its utility, toxicogenomics still poses a number of limitations that hinder its standalone application in measuring risk, even though it possesses many of its strengths. Gene expression responses are dynamic (and therefore sensitive) and temporally variable, and transcriptional changes in gene expression do not always lead to lasting toxicity, and are often transient (Krewski et al., 2014). Differentiation of adaptive cell responses and truly harmful aberrations continues to be a tremendous conundrum, especially in low-dose or short-term exposure settings (Escher et al., 2017). Moreover, transcriptomic changes usually lack a heritable context and thus account for only limited information about long-term biological memory, or delayed health outcome (Feil & Fraga, 2012). This limitation is especially salient in the context of chronic exposures and in developmental windows of susceptibility, where persistent regulatory changes may occur in the absence of sustained gene expression differences. As such, if toxicogenomics is not coupled with other, complementary layers of molecular composition, it risks over- or under-reporting risks. Altogether, toxicogenomics has revolutionized toxicity risk estimation through mechanism-based, pathway-level treatment approach. Its predictive power, however, is enhanced when integrated with regulatory constructs such as epigenetics for temporal persistence and long-term biological effects. This realization supports the continued importance placed on integrative, multi-omics strategies in next-generation toxicity risk assessment.

4.2 Environmental Epigenetics

Environmental epigenetics is about studying how external stressors (e.g., chemicals, pollutants, and dietary factors) can trigger epigenetic changes that affect health outcomes throughout life (Feil & Fraga, 2012). A distinguishing characteristic of epigenetic regulation consists of its sensitivity to low-dose exposures, which are likely to induce marked biological effects irrespective of phenotype-uncompromised toxicity (Vandenberg et al., 2012). Such sensitivity can be observed in particular at key windows of development, where epigenetic programming is critical to organogenesis and physiological setpoints (Heindel et al., 2015). The developmental origins of health and disease (DOHaD) framework emphasizes the role of early-life epigenetic changes in predisposing individuals to a number of chronic diseases as they get older, including cancer, cardiovascular disorders, and neurodevelopmental abnormalities (Gluckman et al., 2008). In addition, there is growing evidence showing that some epigenetic changes driven by exposure to toxicants can escape the mechanisms of epigenetic reprogramming and be transmitted between generations, with long-term health implications for populations (Skinner, 2014).

4.3 Epigenetic Biomarkers

Environmental exposure-induced epigenetic alterations provide potential for biomarker discovery in toxicology and risk assessment. Persistent epigenetic marks, including stable changes in DNA methylation, can act as molecular records of past exposure, even in the absence of detectable chemical (Baccarelli et al., 2019). These exposure biomarkers are especially useful for measuring chronic or intermittent exposures that are hard to measure using standard monitoring methods. Moreover, epigenetic profiles have also been linked to altered disease susceptibility and offered early evidence of increased risk even before becoming clinically apparent (Ladd-Acosta & Fallin, 2016; Zhu et al., 2025). To illustrate, exposure-associated miRNA signatures have been associated with inflammatory responses, metabolic dysfunction, and tumorigenesis, making them useful as highly sensitive and mechanistically informative biomarkers (Hou et al., 2011). However, translation of epigenetic biomarkers to regulatory practice continues to be limited by population and experimental-system variability (Fig.3.).

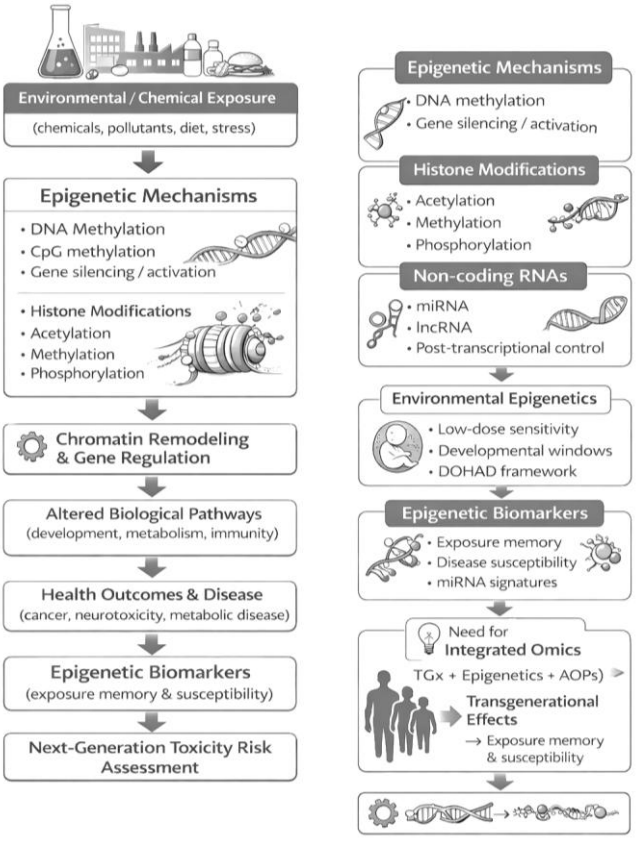


Fig.3. Epigenetic mechanism in toxicology: From exposure to risk relevance.

4.4 Challenges and Limitations

Despite being a relevant mechanistic resource, using epigenetic data in toxicology is not without obstacles. Epigenetic responses are often so tissue- and cell-type-specific that extrapolation from accessible tissues, such as blood, to target organs of toxicity is challenging (Lokk et al., 2014; Meier et al., 2025). It is challenging to demonstrate causality between epigenetic changes and adverse effects, as observed changes may represent secondary effects or adaptive responses rather than drivers of toxicity (Jones, 2012). In addition, the absence of standardized experimental protocols, data normalization methods, and reference epigenomes constrains cross-study comparability and regulatory acceptance (Sturla et al., 2018). Overcoming these challenges would require harmonized methodologies, longitudinal study designs, and integration with complementary omics data for enhanced interpretation of mechanisms. Overall, epigenetics offers essential insight into the persistence, timing, and inheritance of toxicant-induced effects, complementing transcriptomic approaches, and further emphasizes the necessity for integrated molecular frameworks in next-generation toxicity risk assessment.

5. Integration of Toxicogenomics and Epigenetics

5.1 Rationale for Integration

Combining toxicogenomics and epigenetics overcomes inherent limitations of toxicogenomics and epigenetics used in isolation to evaluate toxicity risks. Toxicogenomics (TGx) predominantly encapsulates immediate and dynamic transcriptional responses following chemical exposure, representing early-stage cellular stress signals, pathways activated by chemical exposure, and adaptive responses (Waters & Fostel, 2004; Morais et al., 2025). But these gene changes are generally short-lived and will not persist once exposure ceases, which may limit their capability to predict long-term or latent toxicity (Krewski et al., 2014). Epigenetic mechanisms, on the other hand, provide sustained regulatory control, capturing long-term biological memory of exposure based on stable but reversible changes (such as DNA methylation and chromatin remodeling, Feil & Fraga, 2012). Incorporating these layers facilitates a connection between early molecular initiating events and long-lasting regulatory alterations and optimizes the interpretation of mechanisms at different time scales that correlate with chronic disease etiology and delayed adverse events (Sturla et al., 2018).

5.2 Integrated Molecular Signatures

Molecular signatures derived from combined transcriptomic and epigenetic data yield greater resolution and specificity for elucidating toxicity pathways and modes of action. As a result of a unified analysis of gene co-expression patterns and DNA methylation changes, differentiation can be made between adaptive transcriptional fluctuations and stable regulatory disruptions suggesting adverse effects (Thomas et al., 2013; Fishman and Tauber, 2025). More recently, concordant promoter hypermethylation and gene downregulation bolster causal inference regarding transcriptional repression following toxicant exposure (Jones, 2012). Additionally, miRNA–mRNA regulatory networks provide critical insight into post-transcriptional control mechanisms that fine-tune toxicant responses, particularly under low-dose or repeated exposure conditions (Hou et al., 2011; Li et al., 2025). The combination of these different genetic signatures enables them to detect biologically relevant perturbations more accurately, and this has the potential to improve both the predictability and translatability of biomarkers (Baccarelli et al., 2019).

5.3 Systems Toxicology Framework

The use of toxicogenomic and epigenetic information is best achieved through the systems toxicology approach that conceptualizes toxicity as network-level disturbances, and not as individual molecular events (Sturla et al., 2018; Pan et al., 2025). Network biology methods map connections between genes, epigenetic regulators, proteins, and metabolites, to locate critical nodes and hubs for toxic responses (Barabási et al., 2011). A pathway perturbation analysis allows pooling of multi-omics signals into biologically interpretable pathways and permits comparisons across studies, species, and exposure scenarios (Krewski et al., 2014). In addition, causal inference approaches, particularly Bayesian networks and computational modeling, contribute to the differentiation between correlation and causation by tying molecular initiating events to downstream key events and adverse outcomes through AOP frameworks (OECD, 2018). This systems-level convergence enhances weight-of-evidence evaluation and reconciles molecular data with regulatory decision-making requirements.

5.4 Case Studies Illustrating Integration

In the evaluation of endocrine-disrupting chemicals, integrated toxicogenomic–epigenetic approaches have been especially insightful, whereby low-dose exposures induce subtle transcriptomic changes accompanied by persistent epigenetic reprogramming during sensitive developmental windows (Vandenberg et al., 2012). In carcinogenicity assessment, combined analysis of studies indicates that early gene expression alterations in DNA repair and cell cycle pathways are often reinforced by long-lasting epigenetic silencing of tumor suppressor genes, contributing to cancer progression (Herceg & Vaissière, 2011). Likewise, neurotoxicant studies show that transient transcriptional changes in neural signaling pathways may coincide with enduring epigenetic modifications affecting synaptic plasticity and neurodevelopment, thereby explaining delayed or progressive neurological dysfunction (Liu et al., 2016; Singh and Mishra, 2024). These examples exemplify how integration improves mechanistic understanding and predictive power at various toxicological endpoints.

So, the summary is, combining toxicogenomics with epigenetics offers a multidimensional view of toxicity with focus on immediate cellular and long-term regulatory effects. Such convergence is conducive to an ability to conduct a strong and mechanism-oriented approach for toxicity risk analysis, especially for low dosage, in combination, and for long-term exposures which are not easily testable by conventional testing paradigms.

6. Integration with Adverse Outcome Pathways (AOPs)

6.1 Molecular Initiating Events (MIEs)

Adverse Outcome Pathways (AOPs) introduce a conceptualised approach for identifying associations among exposure to chemicals and adverse health events which is organized along a chain of causally related biological events that start with a Molecular Initiating Event (MIE) (Ankley et al., 2010). MIEs are the first detectable interaction of a toxicant with a biological system, typically at a molecular level due to receptor binding, enzyme inhibition, or direct interaction with DNA or chromatin (Villeneuve et al., 2014). Toxicogenomic data help identify MIEs through the identification of early changes in gene expression that are linked to particular signaling pathways, stress responses and/or receptor-mediated signaling pathway (Thomas et al., 2013; Stierum et al., 2025). Epigenetic alterations, including DNA methylation changes and histone modifications, may function as MIEs when they directly perturb the transcriptional regulatory system for transcriptional control post-exposure (Feil & Fraga, 2012). The combination of transcriptomic and epigenetic triggers further fortifies MIE characterization with the availability of information for the simultaneous capture of both rapid and slow functional responses and persistent regulation-induced regulatory interruptions to triggering toxicity pathways(Fig.4) (Sturla et al., 2018).

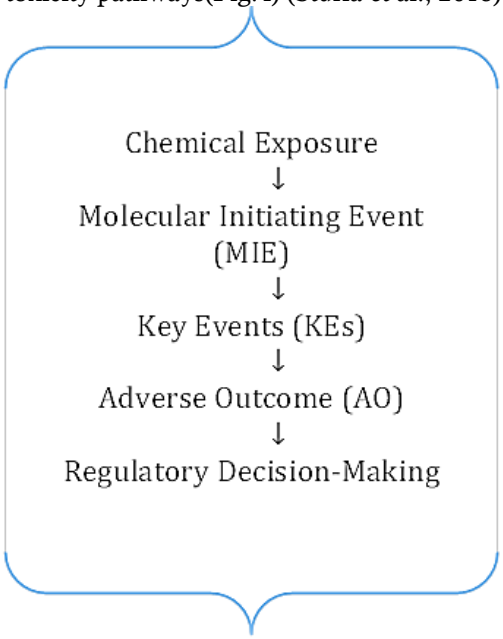


Fig.4. Integration of Toxicogenomics & Epigenetics with AOPs

6.2 Key Events (KEs)

These AOPs evolve from the MIE to intermediate KEs describing the essential and measurable changes at increasing levels of biological organization, ranging from cellular dysfunction to tissue and organ-level effects (Ankley et al., 2010). Incorporating toxicogenomic and epigenetic information offers strong evidence for the detection of KEs through the concomitant identification of molecular perturbations with downstream cellular pathways including oxidative stress, apoptosis, altered cell proliferation, and inflammatory signaling (Krewski et al., 2014; Liu et al., 2025). For instance, concerted gene expression changes in cell-cycle regulators and continual epigenetic silencing of tumor suppressor genes are convincing evidence of KEs which are known to be linked to carcinogenesis (Herceg & Vaissière, 2011). At the tissue level, epigenetic reprogramming during development possibly underlies altered organ structure or function, and delayed adverse outcomes cannot be explained quickly via short-term transcriptomic responses (Heindel et al., 2015). This multifactorial integration enhances causal linkages between KEs and also reduces pathway trajectory uncertainty and increases biological plausibility.

6.3 Regulatory Relevance and Weight-of-Evidence

For instance, the Organisation for Economic Co-operation and Development (OECD) has formally adopted the AOP framework as a tool for chemical hazard identification, risk assessment, and regulatory decision-making (OECD, 2018). One of the key issues of regulatory toxicology is the need to provide ample weight-of-evidence to underpin decisions based on non-animal, molecular data (Villeneuve et al., 2014; Stoykova, 2025). Thus, incorporating both toxicogenomic and epigenetic information promotes weight-of-evidence by maintaining mechanistic uniformity at various levels of biological organization, meeting essential OECD AOP evaluation criteria in relation to biological plausibility, empirical support and essentiality of key events (OECD, 2018). Moreover, molecular data compatible with AOPs permits chemical grouping, read-across, and prioritization strategies – reducing dependence on extensive animal testing whilst also promoting confidence in the use of molecular data (Kavlock et al., 2012; Deepika et al., 2025). In a regulatory environment in which these kinds of next-generation risk assessment paradigms have become more prevalent, the incorporation of multi-omics data into the AOP framework provides a transparent, reproducible and human-relevant basis for toxicity assessment. In conclusion, the integration of toxicogenomic and epigenetic information into the AOP paradigm provides stringent means of identifying molecular initiating events, increases the causative relationships between key events, and increases regulatory weight-of-evidence. This convergence facilitates mechanism-driven predictive toxicity risk assessment and reconciles molecular toxicology with international regulatory expectations.

7. Regulatory and Risk Assessment Implications

7.1 Limitations of Traditional Risk Assessment

Conventional chemical risk assessment has historically relied on high-dose animal testing to identify adverse apical endpoints, which are then extrapolated to estimate human safety at lower exposure levels (National Research Council [NRC], 2007). While this approach has provided a foundational framework for hazard identification, it is increasingly recognized as insufficient for addressing the complexity of modern environmental exposures (Krewski et al., 2014). High-dose testing may activate toxicity pathways that are not relevant at environmentally realistic concentrations, leading to inaccurate risk characterization, particularly for endocrine-active and non-monotonic dose–response chemicals (Vandenberg et al., 2012). Animal-to-human extrapolation further introduces uncertainty due to species-specific differences in toxicokinetics, receptor biology, and gene regulation, which can result in both over- and under-protection of human health (Hartung, 2009). Additionally, traditional approaches are poorly equipped to assess mixture toxicity, chronic low-dose exposure, and delayed or latent health outcomes, as they largely focus on overt toxicity rather than early molecular perturbations (Escher et al., 2017). These limitations underscore the need for more human-relevant, mechanism-based strategies that move beyond empirical dose thresholds.

7.2 Advantages of Integrated Omics Approaches

Integrated omics approaches that utilize toxicogenomics, epigenetics, and other molecular datasets have a number of advantages over traditional risk assessment paradigms. First, omics-based data is more relevant for humans, owing to the potential of using human-derived in vitro systems and epidemiological datasets to minimize interspecies extrapolation (Sturla et al., 2018; Yang et al., 2023). Transcriptomic and epigenetic signatures represent conserved biological pathways closely associated with human disease mechanisms that lend greater translational confidence (Thomas et al., 2013; Paraskar et al., 2025). Second, integrated omics provides mechanistic transparency by connecting the exposure to chemicals with molecular initiating events, downstream pathway perturbations, and adverse outcomes through structured approaches such as Adverse Outcome Pathways (AOPs) (OECD, 2018; Wiklund, 2025). This mechanistic understanding supports regulators in differentiating adaptive responses from truly adverse effects (Krewski et al., 2014), which has been a significant source of uncertainty in classical toxicological testing. Third, multi-omics data can be used to mitigate animal testing, consistent with the 3Rs principles (replacement, reduction, refinement) through high-throughput screening techniques, chemical prioritization, and read-across strategies (Adler et al., 2011). These advantages combined position integrated omics as a cornerstone of next-generation risk assessment.

7.3 Application in Regulatory Toxicology

Integrated omics have shown a growing role in regulatory toxicology, particularly in chemical screening and in prioritization programs. High-throughput transcriptomic and epigenetic profiling platforms serve to rank chemicals by biological potency and pathway perturbation, allowing regulatory authorities to concentrate budgets on the most worrisome substances (Kavlock et al., 2012; Alnasser, 2025; Alnasser, 2025). Omics-derived points of departure, which may include benchmark dose estimates from pathway activation, are being developed as alternative safety thresholds to the traditional NOAEL ones (Crump, 2011; Thomas et al., 2013). These molecular points of departure represent early biological effects more accurately and alleviate uncertainty of arbitrary dose selection. On the policy level, integrated omics data inform evidence-based decision-making through enhancing the weight-of-evidence assessments, complementing transparent, reproducible risk assessment (OECD, 2018; Alnasser, 2025). The increasingly comprehensive use of omics-informed approaches is also increasingly accepted in the regulatory space by the regulatory authorities, who have been emphasizing mechanistic data and non-animal methods to assess chemical safety in the guidance documents (NRC, 2007). Although there are certain issues of standardization and validation that require refinement, utilization of integrated omics in regulatory toxicology signifies a meaningful transition towards predictive, human-relevant, and ethically responsible chemical risk assessment. In short, integrated omics approaches to eliminate challenges in standard risk evaluations improve regulatory decision-making by providing better relevance, mechanistic comprehension and efficiency in addressing hazards. These developments are key to the progression of next-generation toxicity risk management frameworks.

8. Computational and AI-Based Integration

12.1 Bioinformatics Tools

Bioinformatics techniques are central to the integration of toxicogenomic and epigenetic datasets as they facilitate multi-omics data fusion to yield biologically meaningful interpretations of complex molecular signals (Hasin et al., 2017). Integration strategies combine transcriptomic, epigenomic, proteomic, and metabolomic layers to identify convergent pathways perturbed by chemical exposure, rather than isolated molecular changes (Sturla et al., 2018; Liu, 2024; Stierum et al., 2025). Gene regulatory networks and protein–protein interaction networks, such as these, can be used to model gene, epigenetic regulator, and signaling pathway interactions to find key driver nodes and toxicity hubs (Barabási et al., 2011). These systems-level analyses provide broader mechanistic insight and aid in aligning with adverse outcome pathway (AOP) frameworks by linking molecular perturbations to higher-order biological effects (Krewski et al., 2014).

8.2 Machine Learning Approaches

Machine learning (ML) techniques have proven to be

efficient for extracting predictive patterns from high-dimensional omics datasets. Supervised ML methods of random forests and support vector machines have previously been used to develop toxicity prediction models for differentiating chemicals based on molecular signatures rather than apical endpoints (Thomas et al., 2019). Such unsupervised methods allow pattern recognition, uncover hidden groups of chemicals with common modes of action and facilitate chemical grouping and read-across strategies (Liu et al., 2019; Stierum et al., 2025). Crucially, the ML-based integration of both transcriptomic and epigenetic features boosts sensitivity to low-dose and mixture effects, overcoming major limitations of classical risk assessment (Sturla et al., 2018).

8.3 Digital Toxicology

Digital toxicology connects bioinformatics, machine learning, and computational modeling to inform data-driven assessment of risk and advanced predictive toxicology platforms in silico (Hartung et al., 2017). These platforms allow for fast screening, prioritization, mechanism-based hazard assessment, and less reliance on animal testing (Yang et al., 2025). As regulatory agencies increasingly adopt next-generation risk assessment frameworks, digital toxicology represents scalable, transparent, and human-relevant tools for future chemical safety evaluation.

9. Challenges and Knowledge Gaps

Despite extensive progress in toxicogenomics and epigenetics, the most common problems that prohibit their common use in toxicity risk assessment are as follows. Data standardization remains a major challenge; the experimental design, platform, normalization methods and analytical pipelines vary widely which complicates differences in studies and the meta-analysis of omics data sets (Hasin et al., 2017; Brooks et al., 2024). The absence of standardized and universally accepted reference data and reporting standards also diminishes the regulatory trust in integrated omics evidence (Sturla et al., 2018; Brancato et al., 2024). A similar short-term gap arises because many studies report exposure time points exclusively or for a short sequence of months that do not capture the dynamic molecular trajectories linking early responses to long-term adverse outcomes (Krewski et al., 2014; Lloyd and Saglani, 2023). This restriction is particularly important for epigenetic changes, as their modifications often occur long after exposure. Additional concern arises from cross-species extrapolation, as variability in gene regulation, epigenetic programming, and developmental timing complicates extrapolations from animal or in vitro models to the human health risk (Hartung, 2009; Chou et al., 2025). Lastly, there is limited degree of acceptance by regulators of integrated omics data as a consequence of issues with reproducibility, validation and interpretability in current risk assessment frameworks (OECD, 2018; Sheng et al., 2025). Tackling these knowledge gaps will necessitate coordinated research methodologies, longitudinal study designs, enhanced human-relevant models and sustained collaboration between researchers and regulators to facilitate wider adoption of next-generation, mechanism-based risk assessment paradigms.

10. Future Perspectives

Future advances in toxicity risk assessment will be driven by emerging molecular technologies and regulatory innovation. Single-cell multi-omics approaches will enable resolution of cell-type–specific toxic responses, uncovering heterogeneity that is masked in bulk analyses and improving mechanistic precision (Sturla et al., 2018; Le et al., 2025; Baysoy et al., 2023). Spatial epigenomics (Table.1) will further enhance understanding by linking epigenetic alterations to their anatomical context within tissues, clarifying structure–function relationships following chemical exposure (Larsson et al., 2021; Walton et al., 2023). Integration of molecular profiles with individual genetic and exposure data will facilitate personalized toxicology, allowing susceptibility-based risk assessment and more accurate protection of vulnerable populations (Thomas et al., 2019; Alemu et al., 2025). Finally, global regulatory harmonization of omics data standards and AOP-informed frameworks will be essential to ensure consistent, transparent, and science-based chemical safety evaluation across international jurisdictions (OECD, 2018).

Table.1. Epigenetic Modifications Induced by Major Toxicant Classes

S. No	Toxicant Class	Representative Toxicant	Epigenetic Modification	Biological Effect	Reference
1	Heavy metals	Arsenic	Global DNA hypomethylation	Carcinogenesis	Reichard & Puga, 2010
2	Heavy metals	Cadmium	DNA hypermethylation	Cancer promotion	Takiguchi et al., 2003
3	Heavy metals	Lead	Altered DNA methylation	Neurodevelopmental toxicity	Senut et al., 2012
4	Heavy metals	Mercury	miRNA dysregulation	Neurotoxicity	Baccarelli & Bollati, 2009
5	Air pollutants	PM2.5	DNA methylation changes	Cardiopulmonary disease	Baccarelli et al., 2009
6	Air pollutants	Diesel exhaust	Histone acetylation	Airway inflammation	Clifford et al., 2017

7	Pesticides	Organophosphates	DNA methylation alterations	Neurotoxicity	Slotkin et al., 2008
8	Pesticides	DDT	Histone modification changes	Endocrine disruption	Skinner et al., 2013
9	Pesticides	Atrazine	DNA methylation reprogramming	Reproductive toxicity	Wirbisky et al., 2015
10	Endocrine disruptors	Bisphenol A	DNA hypomethylation	Developmental defects	Dolinoy et al., 2007
11	Endocrine disruptors	Phthalates	miRNA changes	Reproductive disorders	Zhang et al., 2016
12	Industrial chemicals	Dioxins (TCDD)	Histone acetylation	Immunotoxicity	Singh et al., 2011
13	Industrial chemicals	PCBs	DNA methylation changes	Neurobehavioral effects	Rusiecki et al., 2008
14	PAHs	Benzo[a]pyrene	DNA methylation & miRNA disruption	Carcinogenesis	Sadikovic et al., 2014
15	Nanomaterials	Silver nanoparticles	DNA methylation & histone changes	Cytotoxicity	Mytych et al., 2017
16	Nanomaterials	Carbon nanotubes	miRNA dysregulation	Pulmonary toxicity	Ghosh et al., 2017
17	Pharmaceuticals	Valproic acid	HDAC inhibition	Teratogenicity	Phiel et al., 2001
18	Pharmaceuticals	Chemotherapeutic agents	DNA methylation changes	Epigenetic toxicity	Sharma et al., 2010
19	Food contaminants	Aflatoxin B1	DNA hypermethylation	Hepatocellular carcinoma	Herceg & Paliwal, 2011
20	Lifestyle toxicants	Tobacco smoke	DNA methylation & miRNA changes	Cancer, CVD	Breitling et al., 2011

11. Conclusion

The combining of toxicogenomics and epigenetics is a groundbreaking step forward in contemporary toxicology as it adds mechanistic richness to traditional apical endpoint–based assessments. Toxicogenomics data reveal early pathway-dependent transcriptional response to chemical exposure; epigenetic information helps illustrate chronic regulatory responses encoding biological memory and long-term susceptibility. Co-presence of these complementing molecular layers enables enhanced characterization of mechanisms of toxic effects temporally and biologically. Importantly, this integrative approach improves accuracy tremendously as it connects molecular initiating events to key downstream events and adverse outcomes, which are systematically included into frameworks such as Adverse Outcome Pathways (AOPs), reducing uncertainty with the use of high-dose and empirical measurements of risk (Krewski et al., 2014; OECD, 2018). Crucially, with human-relevant omics data, this represents a move towards ethical and human-centered toxicology, helping not only to decrease testing in animals but also to bring them into line with the principles of next-generation risk assessment (National Research Council, 2007).

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