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Revisiting the approaches to DNA damage detection in genetic toxicology: insights and regulatory implications

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Abstract

Genetic toxicology is crucial for evaluating the potential risks of chemicals and drugs to human health and the environment. The emergence of high-throughput technologies has transformed this field, providing more efficient, cost-effective, and ethically sound methods for genotoxicity testing. It utilizes advanced screening techniques, including automated in vitro assays and computational models to rapidly assess the genotoxic potential of thousands of compounds simultaneously. This review explores the transformation of traditional in vitro and in vivo methods into computational models for genotoxicity assessment. By leveraging advances in machine learning, artificial intelligence, and high-throughput screening, computational approaches are increasingly replacing conventional methods. Coupling conventional screening with artificial intelligence (AI) and machine learning (ML) models has significantly enhanced their predictive capabilities, enabling the identification of genotoxicity signatures tied to molecular structures and biological pathways. Regulatory agencies increasingly support such methodologies as humane alternatives to traditional animal models, provided they are validated and exhibit strong predictive power. Standardization efforts, including the establishment of common endpoints across testing approaches, are pivotal for enhancing comparability and fostering consensus in toxicological assessments. Initiatives like ToxCast exemplify the successful incorporation of HTS data into regulatory decision-making, demonstrating that well-interpreted in vitro results can align with in vivo outcomes. Innovations in testing methodologies, global data sharing, and real-time monitoring continue to refine the precision and personalization of risk assessments, promising a transformative impact on safety evaluations and regulatory frameworks.

Keywords Genetic toxicology, High throughput screening, Artificial intelligence, Multi-Omics technologies, In vitro testing

Introduction

Genetic toxicology is the study of the interactions between chemical agents and the genetic material of organisms, focusing on mutations, chromosomal aberrations, DNA damage, and the associated mechanisms [1]. It aims to assess the genetic harm caused

by chemicals that may induce acute or chronic toxicity. While foundational studies in the mid-20th century established key links between mutagenicity and carcinogenicity through assays like the Ames test, modern genetic toxicology has evolved significantly in response to increasing demands for rapid, accurate, and ethical testing [2]. By the 1990s, it became evident that in vitro assays alone were insufficient to fully predict human carcinogenicity, prompting a shift toward integrated strategies combining in vitro, in vivo, and in silico approaches. This integration laid the groundwork for the adoption of high-throughput screening (HTS), computational toxicology, and systems biology.

DNA damage plays a critical role in the onset and progression of various diseases, including cancer, neurodegenerative disorders, and developmental abnormalities. Understanding the mechanisms of genotoxicity is therefore essential for both disease prevention and therapeutic development. The growing recognition of these health implications coincided with new challenges in the field, particularly the vast number of substances requiring evaluation and the increasing complexity of biological systems. In the 2000s, this led to a transformation in toxicological testing approaches, emphasizing the integration of emerging technologies such as genomics, computational modeling, and high-throughput screening to improve efficiency and relevance in risk assessment. A radical overhaul of toxicology testing strategies was attempted to better meet the health and safety challenges of the 21st century [3]. Among these tools, quantitative structure-activity relationships (QSAR) gained prominence for predicting mutagenicity and carcinogenicity based on chemical structure (4). However, it was acknowledged that QSAR systems could not replace animal testing entirely due to its inability to fully replicate complex in vivo biological responses. The ongoing development of QSAR models and other predictive methods aimed to refine toxicological assessments, but their limitations underscored the need for continued research and improvements in testing protocols. The integration of quantitative dose-response analysis and risk assessment is gradually replacing qualitative hazard identification in applied genetic toxicology. Combining various assays enhances the sensitivity and specificity of genotoxicity testing, helping to predict their carcinogenic potential [5]. Present-day genetic toxicology includes the HTS of changes incurred in genetic material, including hereditary influence of such changes that may impact future generations [6].

Hence, genetic toxicology has transitioned from traditional assays to a predictive, high-throughput, and computationally advanced field. The integration of artificial intelligence (AI), big data, and multi-omics approaches enhances genetic risk assessment, reduces reliance on animal testing, and facilitates the development of safer pharmaceuticals and chemicals. This transformation improves public health protection while promoting ethical and efficient toxicological evaluations. AI and machine learning (ML) streamline genotoxicity testing by automating data interpretation and risk prediction, minimizing the need for conventional in vivo studies. AI-driven analysis of next-generation sequencing (NGS) and microarray data enables the detection of genetic damage signatures, while automated image processing in comet and micronucleus assays enhances accuracy in identifying DNA damage e and chromosomal aberrations. Deep learning models improve sensitivity and specificity by recognizing subtle gene expression changes linked to toxicity.

Regulatory agencies, including the FDA, EPA, and OECD, leverage AI for automated risk assessments, prioritization of chemicals, and toxicity classification, expediting

decision-making in pharmacology and environmental safety. Additionally, natural language processing (NLP) aids in extracting toxicological insights from scientific literature, refining data curation processes. Personalized toxicology is also advancing through AI-driven analysis of genetic variability, enabling the prediction of individual susceptibility to genotoxic agents and supporting precision medicine initiatives. Big data plays a fundamental role in consolidating toxicological, genomic, and epidemiological information, improving risk assessment frameworks, and fostering reproducibility in scientific research. Standardized data-sharing frameworks facilitate global collaboration among researchers and regulatory bodies, ensuring more reliable and comprehensive toxicological evaluations. As methodologies continue to evolve, the field of genetic toxicology increasingly prioritizes alternative testing strategies, further reducing the ethical and scientific reliance on animal models.

The purpose of this review is to provide a comprehensive overview of the evolution and current state of genetic toxicology, with a specific focus on the significant advancements enabled by HTS and data driven computational models. Such innovations have transformed the field by facilitating the rapid, cost-effective, and ethically responsible assessment of the genotoxic potential of chemicals and drugs. Furthermore, the review will examine the regulatory implications of these advancements.

Methodology

We conducted a comprehensive search for studies published between the 1990s and 2023 in the following literature databases: PubMed, Google scholar and Scopus. To ensure comprehensive literature retrieval, use a mix of keywords and Boolean operators (AND, OR) relevant to genetic toxicology, high-throughput screening, and computational methods were used. The core keywords included 'genetic toxicology', 'DNA damage and diseases', 'In vitro techniques in genetic toxicology', 'In vivo techniques in genetic toxicity', 'DNA damage detection', 'high-throughput screening (HTS), 'machine learning in toxicology, 'artificial intelligence in toxicology', 'AI in toxicology', 'machine learning in toxicology', 'ML in toxicology', 'in silico toxicology', 'genotoxicity assessment', 'bioinformatics in toxicology', 'regulatory toxicology', 'computational toxicology', 'omics-based toxicology', 'toxicogenomics', 'predictive toxicology', 'data-driven toxicology', 'environmental toxicology', 'big data in toxicology', 'toxicity prediction models'. In addition, we explored online sources for relevant conferences and organizations related to the topic of 'Advances in genetic toxicology in the high throughput era'.

The inclusion criteria for literature selection comprised peer-reviewed studies applying AI, ML, or computational methods in genetic toxicology, research on high-throughput screening for DNA damage detection, and articles integrating omics data for toxicity prediction. The search was specifically aimed at identifying studies that utilized 'testing for genetic toxicology methodologies'. Additionally, studies discussing regulatory implications of computational toxicology and reviews/meta-analyses covering bioinformatics and data mining approaches in toxicity assessment were included. Exclusion criteria included studies that did not incorporate AI, machine learning, or computational analysis, experimental studies without a bioinformatics component, duplicate articles, and publications in languages other than English.

DNA damage and diseases

Genetic toxicology, which investigates the effects of chemical and physical agents on the genetic processes of living cells and hereditary material (DNA), plays a crucial role in understanding diseases associated with exposure to hazards. Exposure can induce genetic instability, leading to a range of disorders, including cancers [7], genetic disorders, and epigenetically mediated transgenerational effects [8]. An important contribution of genetic toxicology has been in studying cancer. More than 90% of known human chemical carcinogens are genotoxic according to the International Agency for Research on Cancer (IARC) [9]. Exposure to environmental factors like UV radiation or aflatoxins can cause characteristic mutations in KRAS and TP53 genes, causing skin and lung cancer [10]. Hematopoietic cancers like acute myeloid leukemia (AML) and myelodysplastic syndromes (MDS) [11] have also genotoxic roots. Genetic toxicology not only aids in discovering root cause of many diseases but has also facilitated the development of targeted therapies, improving cancer diagnosis and treatment by identifying the genetic alterations responsible for tumor formation [12]. Understanding mutations in genes like BRCA1/BRCA2 or TP53 has led to precision oncology approaches that personalize treatment for patients. Moreover, genetic toxicology research has advanced our understanding of epigenetic modifications, such as DNA methylation and histone acetylation, which play a critical role in tumor development and progression. Mutations in genes involved in DNA methylation (e.g., DNMT1, DNMT3A) are frequently observed in colorectal cancers. The interplay between genetic mutations and environmental factors, such as dietary components [13]. This information has paved the way for the development of epigenetic therapies, including DNA methylation inhibitors, that target reversible genetic changes contributing to cancer. Beyond cancer, it has been instrumental in studying benign tumors, noncancerous growths often caused by environmental chemicals, genetics, or other factors. It has helped understand the genotoxic effects of chemicals and informs strategies to mitigate their impact on human health.

Genetic toxicology also plays a crucial role in studying hereditary abnormalities. Disorders such as cystic fibrosis, phenylketonuria, and Tay-Sachs disease are caused by gene mutations [14] and genetic toxicology investigates how chemical and physical agents influence DNA, potentially triggering genetic mutations linked to these conditions. Exposure to mutagenic agents can increase the risk of mutations that cause genetic disorders [1], emphasizing the importance of genetic toxicology in prevention. The field also plays a vital role in developmental toxicity, where exposure to genotoxic agents during critical stages of development can lead to congenital abnormalities and long-term health issues. Additionally, genetic toxicology has contributed to the study of epigenetic mechanisms, such as DNA methylation [15]. Epigenetic changes do not alter the DNA sequence itself but modify how genes are expressed, influencing cellular functions and overall health [16]. Research in genetic toxicology has shown that exposure to certain genotoxic agents can disrupt these epigenetic processes, leading to adverse health outcomes. Exposure to environmental toxins, such as heavy metals or endocrine-disrupting chemicals, can alter DNA methylation patterns in ways that not only affect the exposed individual but may also impact their offspring and subsequent generations [17]. These transgenerational effects have been linked to a range of health conditions, including developmental disorders, metabolic diseases, and increased susceptibility to cancer. The insights gained from genetic toxicology have also informed the development of therapies

that target epigenetic mechanisms. Drugs such as DNA methylation inhibitors and histone deacetylase inhibitors are now being used to treat cancers and other diseases where epigenetic dysregulation plays a key role [18].

Genetic toxicology has also been instrumental in identifying environmental toxins that damage DNA, leading to the development of regulations that limit exposure to these harmful substances and prevent adverse health effects. Hence, genetic toxicology provides invaluable tools for disease prevention, therapeutic development, and regulatory safety assessments, shaping how we approach the impact of environmental, occupational, and genetic factors on human health.

Techniques for assessment

In vitro and in vivo techniques in genetic toxicology testing

Genotoxicity assessment is a critical component of safety evaluations, aiming to prevent drugs and other substances from posing risks to human health. Both genotoxicity and mutagenicity tests play an essential role in industrial and regulatory health assessments. Since no single test can capture all relevant genotoxic endpoints, a combination of in vivo and in vitro testing methods is recommended to provide a comprehensive evaluation. These tests offer early insights into the potential harmful effects of chemicals across various domains, including pharmaceuticals, cosmetics, agrochemicals, industrial compounds, food additives, natural toxins, and nanomaterials. By identifying genotoxic risks at an early stage, they help mitigate harm, ensuring the safety of new compounds while safeguarding public health and the environment. Genetic toxicology tests are conducted following strict regulatory guidelines set by organizations like OECD (Organization for Economic Co-operation and Development), ICH (International Council for Harmonisation), and EPA (Environmental Protection Agency). These guidelines ensure that the tests are reliable, reproducible, and adhere to Good Laboratory Practice (GLP) standards.

In vitro tests are conducted in controlled laboratory environments, often using isolated cells or subcellular components. These methods are widely used for initial genotoxicity screening due to their cost-effectiveness, efficiency, and ethical advantage of avoiding animal use. Common in vitro assays include Ames test [19], chromosome aberration test [20], micronucleus [21] and comet assay. These are also known as short-term tests (STTs) and have been in use for decades. In vivo tests are performed in living organisms, such as rodents, and are crucial for confirming genotoxic effects observed in vitro. They provide insights into how genotoxic agents behave in a complex biological system, accounting for absorption, metabolism, and repair processes. Figure 1 highlights some of the most commonly employed methods for assessing genotoxic chemicals [22].

The Ames assay, developed in 1975, is a rapid, sensitive, and cost-effective method for assessing the mutagenicity of substances. It uses histidine-auxotrophic *Salmonella typhimurium* strains, which require histidine from the environment to grow. Mutagenic substances can revert these strains to a prototrophic state, allowing growth on histidine-free media. The assay detects a wide range of genotoxic carcinogens and mutation types, including frame shifts and base substitutions. However, its limited specificity and the challenge of interpreting positive results, along with the differences between microorganisms and mammals in genetic complexity and DNA repair systems, pose limitations to its use in drug development.

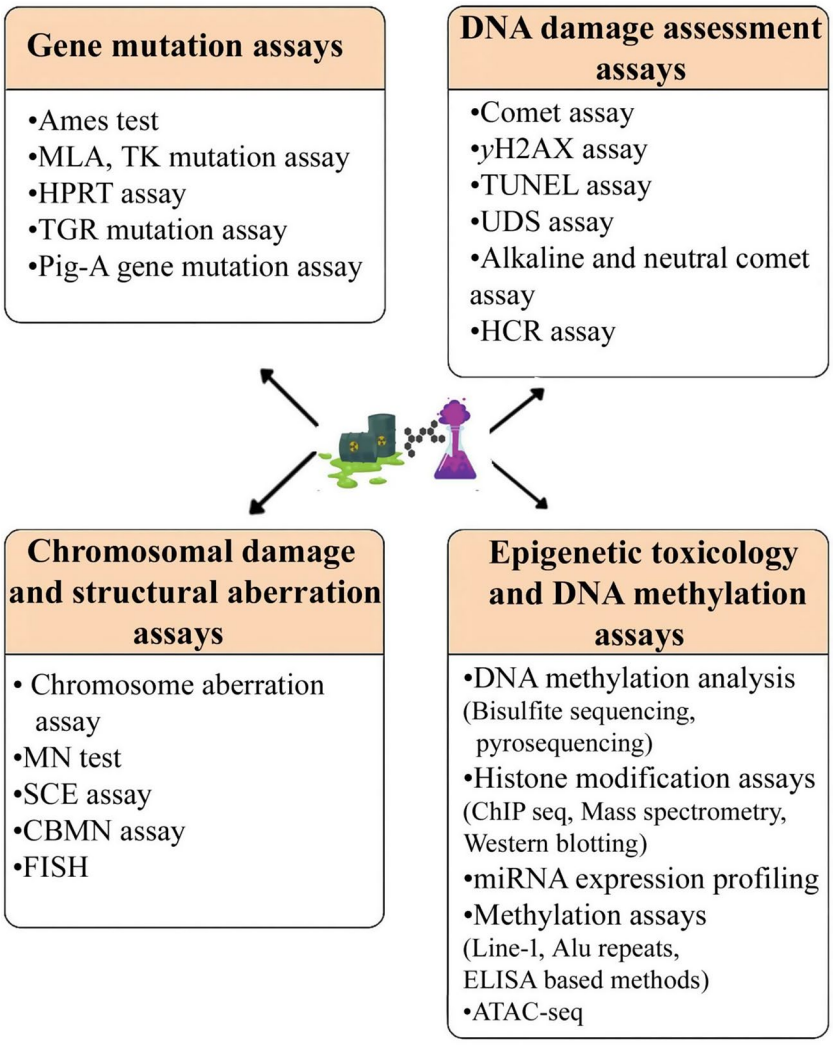

Fig. 1 List of various genetic and epigenetic toxicology testing methods with specificity to type of DNA alteration. MLA: Mouse lymphoma assay; HPRT: Hypoxanthine guanine phosphoribosyl transferase; TK: thymidine kinase gene mutation assay, TGR: Transgenic rodent mutation assay; Pig-A: phosphatidylinositol glycan class A (PIG-A) gene use for detecting somatic cell gene mutations; y-H2AX: phosphorylated form of histone H2AX serving as marker of double strand breaks; UDS: Unscheduled DNA Synthesis; TUNEL: Terminal deoxynucleotidyl transferase dUTP Nick-End Labeling; HCR: Host-cell reactivation; MN: micronucleus test; SCE: Sister Chromatid Exchange; CBMN: Cytokinesis-Block Micronucleus; FISH: Fluorescence In Situ Hybridization; ChIP: Chromatin Immunoprecipitation Sequencing; miRNA: microRNA; Line-1: Long Interspersed Nuclear Element-1; ATAC: Assay for Transposase-Accessible Chromatin

The comet assay and micronucleus assay (MNA) are two widely used genotoxicity detection techniques due to their simplicity, sensitivity, and ability to detect DNA strand breaks or chromosomal loss. Combining both assays has proven effective in elucidating the mechanisms of genotoxic compounds, as they each detect DNA damage at different levels. The comet assay, introduced in 1984, detects DNA strand breaks by visualizing comet tail-like structures formed by fragmented DNA migrating toward the anode during electrophoresis. The alkaline comet assay, a more sensitive variant, detects a range of DNA damage, including double-strand breaks and alkali-labile sites, making it particularly useful for identifying genotoxic agents. Its advantages include flexibility, sensitivity, low cell requirements, quick execution, and low cost.

The micronucleus assay, a well-established and reliable test, detects chromosomal damage by identifying micronuclei in the cytoplasm of erythrocytes. These micronuclei, discovered over 100 years ago, indicate chromosomal loss or disruption. MNA can assess both clastogenic (chromosome-breaking) and aneugenic (affecting chromosome number) effects. The chromosomal aberration assay further evaluates structural abnormalities in chromosomes using mammalian cell lines, such as Chinese Hamster Ovary (CHO) cells, to identify agents that cause chromosomal damage. Additionally, sister chromatid exchange (SCE) and cytokinesis-block micronucleus (CBMN) assays provide further resolution in detecting chromosomal alterations and cell cycle effects, while fluorescence in situ hybridization (FISH) enables the detection of specific chromosomal abnormalities. Beyond chromosomal damage, gene mutation assays contribute to genotoxicity assessment by detecting mutations at the gene level. The Ames test identifies point mutations in bacteria, while the mouse lymphoma assay (MLA) and hypoxanthine-guanine phosphoribosyl transferase (HPRT) assay detect gene mutations in mammalian cells. The transgenic rodent (TGR) mutation assay evaluates mutations in vivo in both somatic and germline cells, and the Pig-a assay enables in vivo detection of mutations in blood cells. DNA damage and repair assays provide insight into genotoxic events and cellular responses. These include the γH2AX assay for detecting DNA double-strand breaks, TUNEL assay for apoptosis-induced fragmentation, and unscheduled DNA synthesis (UDS) assay for assessing DNA repair. Host-cell reactivation assays evaluate cellular repair capability, while the use of both alkaline and neutral comet assays distinguishes between single- and double-strand breaks.

Transgenic animal models have recently been constructed and proven to be powerful, organ-specific, short-term mutagenicity assays to examine the many processes involved in spontaneous or induced mutations [23, 24] (Fig. 2). They allow researchers to observe the effects of genotoxic agents in a controlled environment, focusing on organ-specific responses. Transgenic rodents, such as those carrying specific reporter genes (e.g., lacZ, gpt), provide sensitive assays for detecting mutations and chromosomal aberrations. These models can reveal subtle genetic changes that might not be detectable in traditional assays, enhancing the sensitivity of genotoxicity testing.

Furthermore, as next-generation sequencing technology has advanced rapidly, new methods in genetic toxicology have grown up to directly examine genetic materials at the genome-wide level with single nucleotide precision [25]. Currently, multiple endpoint genetic tests like HepaRG are employed to assess genotoxicity. The platform incorporates various assays, including the Comet assay and micronucleus tests, to evaluate DNA damage and chromosomal abnormalities. It also employs transcriptomics to understand the mode of action for genotoxic and nongenotoxic compounds. 3D models mimicking tissues are also state of the art in assessing chemical hazards and have shown improved relevance over traditional 2D cultures, as they provide more realistic cell-cell and cell-matrix interactions, enhancing the predictability of genotoxic effects. Unlike traditional 2D monolayer cultures, 3D models mimic tissue-like architecture, offering enhanced cell-cell and cell-matrix interactions. These models more accurately replicate physiological responses, improving the predictability of genotoxic effects by allowing better assessment of DNA damage, repair mechanisms, and chemical penetration gradients. Examples include spheroids, organoids, and 3D bioprinted tissues, which

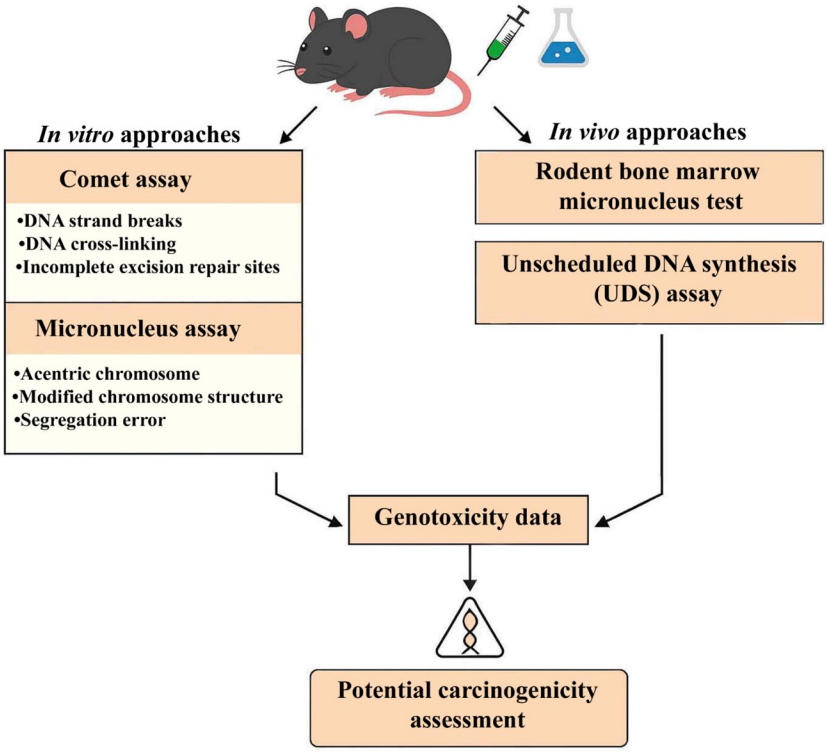

Fig. 2 Representation of in vitro and in vivo rodent genotoxicity assays for assessment of carcinogenicity

are increasingly used for genotoxicity testing due to their improved relevance over conventional 2D cultures.

In recent years, the integration of epigenetic endpoints has added a critical layer to genotoxicity testing. Various analytical approaches now enable the assessment of modifications in DNA, histones, non-coding RNAs, and chromatin structure, providing insight into gene regulation changes induced by genotoxic agents. These tools contribute to a more holistic evaluation of cellular responses, complementing traditional assays and improving the precision of hazard identification and risk assessment.

High throughput techniques in testing

The advent of high-throughput technologies has brought about a paradigm shift in genetic toxicology, moving away from traditional methods like animal testing towards more cost-effective, ethical approaches such as in vitro and in silico testing. This transition has generated vast amounts of "big data," enabling high-throughput screening and more efficient analysis. High throughput techniques utilize advanced tools and platforms to screen large numbers of compounds through comet, micronucleus and various other assays (γ-H2AX assay, bisulfite sequencing, transcriptomics, microarray analysis, imaging etc.) to identify genotoxic properties, mutations and DNA damage. Traditional bacterial assays, such as the SOS Chromotest have been adapted to facilitate rapid screening of genotoxic agents (Table 1). NGS and microarrays enable comprehensive genome-wide mutation analysis and gene expression profiling, enhancing sensitivity and resolution in detecting DNA damage. Techniques such as flow cytometry and high-content imaging provide quantitative, real-time assessments of genotoxic effects at the cellular level. Furthermore, mass spectrometry and omics-based approaches allow deeper molecular

Table 1 Key high-throughput technologies to assist the study of genetic toxicology

Technology	Application	Advantages	Disadvantages	Reference(s)
SOS Chromotest	Bacterial colorimetric assay indicating DNA damage or genotoxicity and easily adapted for HTS	Simplicity, high sensitivity, faster results compared to Ames test	Limited applicability to certain types of genotoxic agents	[26]
Next-Generation Sequencing (NGS)	Genome-wide mutation analysis, DNA damage detection	High sensitivity, comprehensive coverage	Difficulty detecting low-abundant somatic mutations, high cost, complex data analysis	[27–29]
Microarrays	Gene expression profiling, DNA damage response pathways	High throughput, multiplexed analysis	Limited to pre-defined gene sets	[30, 31]
Quantitative HTS	Screening compounds for genotoxicity and concentration-response profiling	Rapid testing of thousands of compounds, reduced false negatives, and cost-effectiveness	Requires robust informatics for data analysis; variability in potency estimates across profiles	[32]
Omics Technologies (Genomics, Transcriptomics, Proteomics)	Comprehensive study of genetic material and cellular responses	Enables holistic understanding of toxicity mechanisms; rapid genome sequencing	Complexity in data interpretation; requires integration across multiple datasets	[3]
Flow Cytometry	Cell cycle analysis, DNA damage detection	Rapid, quantitative analysis	Requires specific staining and instrumentation	[33–35]
Mass Spectrometry	Protein analysis, metabolite profiling, SNP genotyping, epigenotype analysis, and allele quantification	High sensitivity, specificity	Complex sample preparation, specialized instrumentation	[36, 37]
Imaging	Cellular morphology, DNA damage visualization	Visual confirmation, high-content analysis	Requires image analysis software, potential for subjective interpretation	(38–40)
High-throughput computational models	Predictive modeling, data integration	Rapid analysis, identification of potential hazards	Model accuracy depends on data quality and model complexity	[41, 42]

insights into toxicological mechanisms by integrating genomic, transcriptomic, and proteomic responses.

Micronucleus assays can be performed using automated imaging and flow cytometry systems, allowing for the analysis of thousands of cells in multi-well plates. A recent study has highlighted the use of an automated micronucleus assay in CHO-K1 cells, which demonstrated a predictivity of 91% with a sensitivity of 94% and specificity of 85% [43]. This high-throughput approach not only enhances the speed of genotoxicity testing but also reduces variability associated with manual scoring methods. Researchers have also adapted comet assays to screen large libraries of compounds for their potential genotoxic effects, thus accelerating the identification of harmful substances [44]. Innovations such as Quantitative High-Throughput Screening (qHTS) [45], transcriptomic biomarkers like TGx-DDI [46], and automated assays like CometChip® [47] are enhancing efficiency, accuracy, and relevance in toxicity assessments. qHTS tests compounds at multiple concentrations, generating concentration-response curves that provide detailed insights into the biological effects of chemicals. It has become popular for its ability to reduce false positives and negatives, thereby improving data reliability. The TGx-DDI biomarker is a transcriptomic tool designed to assess DNA damage-inducing agents in human cells. It has been integrated into a high-throughput testing framework

using the Nanostring nCounter system, allowing for efficient multiplexed screening [46]. The TGx-DDI Plexset assay enhances screening efficiency by eliminating steps such as concentration determination and RNA extraction, thus streamlining the process while maintaining specificity and sensitivity. The CometChip® assay enables high-throughput evaluation of DNA damage through a modified comet assay and TempO-Seq® that can process multiple samples simultaneously [48]. Combining the CometChip® assay with the TGx-DDI biomarker provides a highly accurate and efficient means of identifying DNA-damaging agents, demonstrating 100% accuracy in HepaRG™ cell cultures.

The γ-H2AX assay is specifically designed to detect DNA double-strand breaks, which are critical lesions associated with genomic instability and cancer [49]. This assay employs high-content imaging or flow cytometry with antibodies that specifically bind to phosphorylated H2AX, a marker indicative of double strand breaks. The automation of this assay has enabled researchers to conduct extensive screenings efficiently [50–52]. High-content imaging systems can also analyze DNA damage, cell cycle effects, and apoptosis, providing comprehensive data on cellular responses to various treatments [53]. ToxTracker system, a mouse stem cell-based reporter assay, and Litron Laboratories' MultiFlow multiplexed genotoxicity assessment method, leverage biomarkers and machine learning to classify genotoxic compounds. Hence, the integration of high-throughput techniques into genetic toxicology testing is revolutionizing how researchers assess the genotoxic potential of chemicals.

Programs like ToxCast™ by the U.S. Environmental Protection Agency (EPA) utilize HTS to profile toxicity across a wide range of chemical classes, providing insights into potential genotoxicity and carcinogenicity. Recent developments in three-dimensional (3D) tissue models for genotoxicity testing have also been promising, as they offer more human-relevant alternatives to traditional two-dimensional (2D) cell cultures. These models, which include skin, liver, and airway tissues, allow for more accurate predictions of genotoxic effects, especially for substances applied via dermal or inhalation routes. The 3D skin models have been successfully adapted for micronucleus and comet assays, providing a valuable tool for dermally applied chemicals, including those in cosmetics.

Computational methods

Since Ashby and Tennant's introduction of structural alerts for mutagenicity and carcinogenicity, computational systems have made significant strides in predicting genotoxicity endpoints [4]. These include mutations in cells of the organisms along with chromosome aberrations. Computational programs offer substantial benefits in quick screening and low resource requirements. They also help identify potentially genotoxic compounds early in the drug discovery process, assist in regulatory submissions, and reduce reliance on animal testing. Among them, QSAR models, expert rule-based systems, ML and deep learning (DL) models dominate the landscape. Techniques like multiple linear regression, partial least squares, and ML algorithms are used to build predictive models based on molecular descriptors [54, 55].

QSAR models analyze chemical descriptors (such as molecular weight, lipophilicity (logP), polar surface area (PSA), and various electronic properties (e.g., HOMO/LUMO energies)) ranked by their correlation with an observed endpoint, such as mutagenicity [56]. QSAR models can achieve external validation accuracies between 70% and 90%, depending on the dataset and specific model used [57]. Fisher et al. recently reviewed

that over 50% of tissue-specific QSAR approaches adhere to three OECD guidelines, yet only 5% meet al.l screening criteria [58]. The most frequently unmet criterion was the mechanistic interpretation of the model, corresponding to OECD criterion five. Popular QSAR platforms include Toxtree, VEGA, OECD QSAR toolbox [59]. Among emerging tools, RASAR stands out by combining read-across (unsupervised) with QSAR (supervised) techniques, enabling the rapid computation of similarity-based descriptors for q-RASAR model development [60]. The MolCompass framework, adhering to the low-code/no-code (LCNC) paradigm, leverages a pre-trained parametric t-SNE model for visualizing chemical space and assessing QSAR/QSPR models [61]. Another noteworthy platform, QSARtuna, integrates modern machine learning techniques with model uncertainty quantification, enhancing the reliability of molecule property prediction [62]. A recent addition is the Easy-MODA tool, which addresses the challenges of documenting complex simulation workflows, streamlining compliance with FAIR principles and improving the usability of MODA guidelines in materials modeling and nanotoxicity evaluation [63]. Coupling of deep learning with QSAR has been able to achieve AUC-ROC scores above 0.90, indicating high predictive performance for genotoxicity [64].

Expert systems, developed by human experts, use cause-and-effect relationships between structural moieties and mutagenic activity, providing specific mechanisms for testing and validation [65]. These systems facilitate the integration of new rules as knowledge evolves. Hybrid systems combine the strengths of both rule based expert systems and QSAR approaches, leveraging fragment-based descriptors and statistical methods alongside expert rule systems to refine predictive capabilities. Examples of such systems are DEREK Nexus and HazardExpert, which offer rules and templates for predicting genotoxicity based on chemical structures [66].

AI-driven models can uncover mechanistic pathways such as oxidative stress and chromosomal aberrations linked to chemical exposure by analyzing multi-omics data. ML and DL methods have recently become powerful tools for predicting genotoxicity. These approaches go beyond traditional QSAR by leveraging large datasets and learning from complex patterns in molecular structures. Supervised learning algorithms like support vector machines (SVM), random forest (RF), and k-nearest neighbor (KNN) can be used to classify chemicals as genotoxic or non-genotoxic based on their molecular features [67]. Yang et al. have reported accuracies of 80.5% and 83.4% for SVM and RF based genotoxicity prediction [68]. Models such as Pubchem_SVM and MACCS_RF have shown particularly reliable predictive abilities, making them useful for initial screenings of potential genotoxic compounds [67]. AI models also facilitate biomarker discovery by identifying gene expression changes and DNA methylation patterns associated with clastogen exposure, enabling early detection of carcinogenic potential. These biomarkers are validated using large-scale omics datasets from exposed populations, improving the precision of toxicity assessments. ML models, such as multi-task deep neural networks (MTDNNs), simultaneously analyze in vitro, in vivo, and clinical toxicity data, achieving superior predictive performance compared to single-task models. For example, MTDNNs utilizing SMILES embeddings improved clinical toxicity prediction AUC-ROC scores by 10–15% over traditional methods. Morgan fingerprints also enhance AI models to analyze chemical structures. Hybrid models incorporating physicochemical descriptors with bidirectional gated recurrent unit (BiGRU) neural networks have outperformed earlier approaches like DeepTox, achieving an average AUC-ROC

of 0.95 across 12 Tox21 endpoints. In addition, deep learning architectures, including random forests and support vector machines (SVMs), remain effective for quantitative QSAR modeling, but MTDNNs excel in handling high-dimensional multi-omics data. Transfer learning further improves toxicity predictions by adapting models trained on in vivo or in vitro data to predict clinical outcomes, thereby reducing reliance on costly human trials. Enhancing explainability, the Contrastive Explanations Method (CEM) identifies toxicity-inducing molecular substructures, improving model transparency. Recent advances in AI and ML have moved beyond binary toxicity classifications to predict the intensity of chemical effects through dose-response modeling. Integrating transcriptomic data with chemical descriptors further enhances prediction accuracy, particularly in hepatotoxicity and carcinogenicity studies.

Chemical exposure can lead to changes in gene expression and by analyzing transcriptomic data using computational methods as edgeR [69], potential biomarkers for genotoxicity can be identified [70]. Tools like Ingenuity Pathway Analysis (IPA) [71] and Reactome [72] can help understand the biological pathways that might be disrupted by a genotoxic substance. The adverse outome pathway (AOP) framework represents a systematic way to predict how molecular-level events lead to adverse health outcomes, including genotoxicity [73, 74]. Computational approaches using large datasets like Open TG-GATEs and DrugMatrix help generate hypotheses for AOP curation [75]. Information from computational models can prioritize laboratory testing based on anticipated risks, expanding chemical space and enabling hypothesis-driven SAR evaluations.

Automation and robotics have revolutionized genetic toxicology by enabling high-throughput screening of vast chemical libraries [44]. The integration of robotic systems, detectors, and software to manage the entire process allows for rapid analysis of chemical compounds, including the assessment of their affinity to biological structures, which is often linked to toxicity. Brinkmann and Eisentraeger have successfully automated the umu-test, a genotoxicity assay performed according to ISO 13,829 standards, using the RoboSeq® 4204 SE robotic platform [76]. Tools such as the Tox21 robotic screening system [77] are fully automated. It can test10,000 compounds in triplicate within a week. This effort aims to rapidly establish chemical signatures capable of predicting rapid in vivo toxicity in both humans and rodents.

Implications of high throughput assessment for regulatory frameworks

Regulatory agencies require comprehensive genotoxicity data as part of the approval process for new chemicals [78]. Regulatory testing for genetic damage has traditionally focused on identifying mutations and chromosomal damage, typically to assess carcinogenic risk. However, a shift toward quantitative risk assessment is occurring, emphasizing a broader understanding of genomic damage and its connection to various adverse health outcomes. The proposed next-generation testing strategy incorporates flexible, comprehensive approaches to measure genomic damage, allowing for a more nuanced risk evaluation that links genetic effects to human health risks, in line with modern risk assessment frameworks like RISK21 [79].

Defining contexts for toxicogenomic predictive models and validating their biological plausibility, reliability, and statistical performance are vital for regulatory adoption [80]. HTS has now become an integral part of modern toxicological assessments [81],

with regulatory agencies such as the U.S. Environmental Protection Agency (EPA) and the European Chemicals Agency (ECHA) incorporating these methods into their risk assessment frameworks [82].

Regulatory frameworks emphasize the importance of data quality to ensure robustness and reproducibility, integration of HTS data with traditional toxicological findings for comprehensive evaluations. Historically, discrepancies between in vitro and in vivo results posed challenges for regulatory agencies, prompting the adoption of a weight-of-evidence (WoE) approach [83]. This approach incorporates multiple data sources, including historical data and advanced methodologies such as Integrated Approaches to Testing and Assessment (IATA) and In Vitro to In Vivo Extrapolation (IVIVE), to bridge the gap between laboratory findings and real-world human health outcomes.

Regulatory frameworks have also evolved to integrate computational methods into genotoxicity assessments [84–86]. Key guidelines from organizations such as the Organisation for Economic Co-operation and Development (OECD), International Council for Harmonisation (ICH), and U.S. FDA provide a structured approach for this integration. OECD has established five validation principles for QSAR model integration, including defined endpoints, unambiguous algorithms, and mechanistic interpretation [87]. The ICH M7 guideline recognizes QSAR models as valid tools for predicting mutagenicity, requiring rule-based and statistical QSAR methodologies for evaluating genotoxic risks associated with pharmaceutical impurities [88, 89]. The European Union REACH regulation encourages the use of QSAR models to predict genotoxicity and reduce reliance on animal testing [90–92]. Similarly, the FDA supports computational methods in initial risk assessments as part of a tiered approach, emphasizing their utility in early screenings [88, 93].

The integration of new approach methodologies into regulatory frameworks is driven by the need to reduce animal testing and enhance mechanistic understanding of genotoxicity. High-throughput assays are revolutionizing chemical safety evaluations by providing more efficient, ethical, and data-rich alternatives, thereby expediting the chemical approval process while enhancing the quality of data and mechanistic insights into genotoxic effects. Despite their potential, challenges remain in the validation, standardization, and regulatory acceptance of new approach methodologies within established frameworks, requiring ongoing innovation to fully leverage these technologies for improved genotoxicity assessments and better protection of public and environmental health.

Limitations

Genetic toxicology methods face significant limitations that impact their reliability and regulatory applications. Traditional in vitro assays, such as the Ames test and micronucleus test, often produce misleading results due to excessive dosing or the lack of human-like metabolic pathways in rodent-derived cells. These models fail to replicate the complexity of human metabolism and tissue interactions, making it difficult to assess organ-specific effects or chronic low-dose exposures. Moreover, in vitro systems are primarily designed to detect DNA damage but do not effectively capture non-DNA-reactive genotoxic mechanisms, such as aneugenic effects or epigenetic modifications.

Similarly, in vivo genetic toxicology methods, including transgenic rodent assays, are constrained by ethical concerns, high costs, and limited sensitivity to cumulative

or low-dose genotoxicity. These assays prioritize acute toxicity over chronic exposure effects, which may lead to an underestimation of long-term genotoxic risk. Additionally, in vivo studies often fail to assess the combined effects of chemical mixtures, posing challenges for regulatory risk assessment. Carcinogenicity studies, which play a crucial role in regulatory decision-making, are often unavailable for non-pharmaceutical chemicals, leading to reliance on conservative assumptions such as the Threshold of Toxicological Concern (TTC) approach. Furthermore, in vivo assays struggle to differentiate between DNA-reactive and non-DNA-reactive genotoxicants, complicating hazard characterization.

A major limitation across both in vitro and in vivo methods is the challenge of extrapolating dose-response relationships to real-world exposure scenarios. Non-linear effects, such as threshold mechanisms observed in aneugens, complicate accurate predictions of genotoxicity. Additionally, neither approach adequately predicts the toxicity of chemical mixtures, failing to account for synergistic or antagonistic interactions between compounds. Regulatory fragmentation further impedes progress, as guidelines such as those from the OECD and ICH prioritize standardized endpoints but lack harmonization for emerging techniques, including 3D tissue models and AI-driven mode-of-action (MoA) analysis.

HTS methods, while valuable for rapid hazard identification, also face several challenges. HTS assays, such as the comet and micronucleus tests, often suffer from assay interference due to nanomaterials or chemical properties like fluorescence and aggregation, leading to false positives or negatives. Additionally, many HTS platforms rely on simplified cell-free systems, limiting their ability to detect metabolic complexity and organ-specific effects. Data variability remains a challenge in HTS, with results differing across platforms, necessitating orthogonal validation to ensure reliability. Standard HTS assays often exclude cytotoxic concentrations to avoid confounding effects, which may lead to the underestimation of low-dose genotoxicity signals. Furthermore, HTS models frequently lack considerations for pharmacokinetic (PK) and metabolic properties, reducing their predictive power for in vivo toxicity. Integrating ADMET (absorption, distribution, metabolism, excretion, and toxicity) parameters into HTS workflows remains an ongoing challenge.

Computational models in genetic toxicology also present limitations due to data quality issues, biological complexity, and challenges in modeling dose-response relationships. While QSAR and SAR models enhance chemical toxicity predictions, they often generate false positives and negatives due to incomplete mechanistic understanding. Tools such as Derek for Windows and MC4PC have limited predictive capabilities for noncovalent DNA interactions, which are significant contributors to genotoxicity but are underrepresented in current modeling approaches. Although regulatory agencies have adopted computational tools such as QSAR Toolbox and OPERA, standardization of computational toxicology protocols remains incomplete. Many models classify compounds as genotoxic or non-genotoxic without quantifying dose-dependent effects, which limits their applicability in risk assessment.

Despite advancements in AI-driven genetic toxicology, challenges persist. Data inconsistencies across toxicity databases necessitate rigorous benchmarking, and model performance varies depending on endpoints. For example, decision trees perform well for Tox21 targets, while multi-task deep neural networks (MTDNNs) are more suited for

clinical toxicity prediction. However, current models frequently prioritize binary classification over dose-response modeling, requiring the development of probabilistic frameworks to predict toxicity intensity more accurately. Regulatory adoption of AI/ML-based predictions is further hindered by the lack of standardized validation protocols.

To address these challenges, integrating multi-omics data, refining in vitro models with human-derived cells, and combining computational models with mechanistic toxicology insights are essential. Ongoing efforts aim to improve data quality, enhance model interpretability, and integrate high-throughput screening assays to train AI models effectively. These advancements will enable safer chemical prioritization, reduce reliance on animal testing, and accelerate regulatory decision-making in genetic toxicology. Standardization efforts and collaborative approaches between researchers, regulatory agencies, and industry stakeholders remain crucial for improving the accuracy and applicability of genetic toxicology assessments.

Future perspectives

The future of genetic toxicology testing is marked by significant advancements in technology, regulatory frameworks, and research methodologies, driven by the need for more efficient, accurate, and ethical approaches to assessing genotoxic risks. According to a report by the SkyQuest Technology, the global genetic toxicology testing market is projected to grow substantially, with a compound annual growth rate (CAGR) of > 10% through 2030 [94].

High-throughput genetic toxicology is undergoing transformative advancements, driven by innovations in technology, methodologies, and a commitment to ethical and human-relevant testing. AI and machine learning ML are revolutionizing data analysis, enabling the efficient processing of large datasets to identify patterns and predict genotoxicity with unprecedented accuracy. These tools enhance the precision of toxicity predictions by leveraging molecular structures and biological pathways, reducing the reliance on extensive in vitro and in vivo testing. The adoption of multi-omics technologies, including genomics, proteomics, metabolomics, and transcriptomics, provides a comprehensive understanding of chemical impacts on cellular systems, enabling the discovery of early biomarkers and improving the robustness of predictive models. Human-based testing models, such as 3D cell cultures, organ-on-a-chip systems, and human-induced pluripotent stem cells (iPSCs), are replacing traditional methods by offering more accurate depictions of human biology and facilitating the identification of tissue-specific genotoxic effects. The field also benefits from the creation of global toxicology databases that aggregate diverse data sources, enhance reproducibility, and establish standardized protocols, while collaborative initiatives like Tox21 and ECVAM exemplify the power of shared resources in advancing alternative testing methods. Furthermore, genetic toxicology is becoming more personalized by incorporating genetic data to identify susceptibility to genotoxic effects, enabling tailored safety evaluations for at-risk groups. Systems biology approaches are gaining traction as they integrate data from multiple sources to map toxicity pathways comprehensively. This holistic perspective helps elucidate how chemicals interact with cellular response networks and cause genotoxic effects. By combining systems biology with computational modeling, researchers can better predict long-term health outcomes associated with genotoxic exposures. Three-dimensional tissue models and organoids represent a significant advancement

in replicating human-like physiological conditions in vitro. These models improve the relevance of genotoxicity testing by mimicking tissue-specific responses and metabolic processes more accurately than traditional 2D cultures. The rise of personalized medicine is shaping the future of genetic toxicology by emphasizing precision healthcare tailored to individual genetic profiles. This shift requires more nuanced testing approaches that account for inter-individual variability in genetic susceptibility to genotoxic agents. Advances in toxicogenomics are expected to play a critical role in identifying biomarkers for personalized risk assessment.

Computational approaches, such as QSAR models, continue to evolve, streamlining the screening process and integrating with experimental data for cost-effective assessments. With increasing regulatory acceptance of in vitro and in silico methods, the shift away from animal testing aligns with ethical concerns while meeting safety standards. The exploration of environmental and epigenetic factors, coupled with comprehensive risk assessment models, is broadening the scope of genetic toxicology to include long-term health impacts and transgenerational effects. Emerging innovations, such as real-time monitoring through sensor technologies, promise to enhance risk management in occupational and environmental settings. Together, these advancements, supported by global data-sharing efforts and harmonized regulatory practices, position high-throughput genetic toxicology to meet modern scientific and societal challenges with efficiency, precision, and ethical rigor.

Conclusion

The field of genetic toxicology is essential for understanding the interactions between chemical agents and DNA, focusing on identifying and analyzing genetic damage caused by various substances. It has evolved from traditional methods to advanced high-throughput technologies, leading to improvements in chemical risk inference and associated disease understanding. The field is crucial for predicting long-term effects, assessing drug hazards, and designing strategies for hazard prevention. Non-animal testing approaches and HTS, are vital for maximizing the potential of genetic toxicology. The integration of robotics, ML and computational approaches allow HTS for chemical safety and genotoxicity assessment. Together, these technological advancements are shifting the focus of genetic toxicology toward more cost-effective, predictive, and efficient assessments. As regulatory bodies increasingly incorporate these innovations into chemical safety evaluations, the potential for reducing animal testing while improving the accuracy of risk assessments becomes more achievable, marking a significant step forward in both regulatory science and public health protection.

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Use of AI in writing
"The authors declare that they have not use AI-generated work in this manuscript. Trinka and ChatGPT was used to improve the structure, syntax, language and coherence in writing".

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