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Review

SYSTEMS FOR GENETIC ASSESSMENT OF THE IMPACT OF ENVIRONMENTAL FACTORS

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One of the most important components of environmental protection is the development of hygiene standards aimed at shielding the human population from the adverse effects of environmental pollution. The European and American Chemical Societies have reported approximately 800,000 chemicals, with no available information on potential risks to human genetic health and negative environmental impact. Given the exponential increase in chemical compounds generated by humanity in various industries, the issue of effectivly identifying and accounting for various genetic and carcinogenic hazards is particularle relevant. The assessment of potential genotoxicity of environmental factors is an integral part of genetic safety assessment for both prokaryotic and eukaryotic organisms, including humans. The evaluation of the genetic activity of chemical compounds is a fundamentsl requirement for their comprehensive toxicological assessment. From the perspective of genetic and epigenetic mechanisms of influence, our review considers standard methods for detecting and assessing the potential genetic hazard associated with environmental factors. These methods are part of a standard, generally accepted test system battery. Additionally, the review covers some modern experimental methods that are not widely accepted today. A detailed analysis of approaches to the assessment of potential genetic mutagenic activity was carried out, presenting their main advantages and disadvantages. Taking into account the recommendations issued by the Organisation for Economic Co-operation and Development on testing hazardous chemical compounds that may affect human health, an attempt was made to find optimal approaches to solving the task of predicting genetic effects and their consequences for humans.

Keywords: genome; deoxyribonucleic acid damage; genotoxicity; carcinogenesis; mutagenesis; mutation test system.

Introduction

The early 20th century saw the rapid development of the natural sciences, particularly as part of the sixth technological paradigm, which relies on advances in modern computer technology, biotechnology, molecular biology, genetic engineering, artificial intelligence systems, nanotechnology, etc. In such circumstances, researchers are paying significant attention to life sciences at the molecular level, based on the development and implementation of modern methods for biopolymer sequencing, which allowed nucleotide sequencing of the human genome for the first time [1]. The progressive development of computational molecular biology has led to the formation of the main vector of development of modern medical sciences in view of a personalised and individualized approach. This approach is crucial for determining treatment strategies, which can be guided by the quantitative and qualitative composition of the coding and regulatory DNA regions of the human genome. In terms of the development of personalised medicine, the results of the pilot phase of the 1000 Genomes Project, which made it possible to study the nature of genetic mutations in different animal populations and establish links between the respective types of nucleotide substitutions and the manifestations of various genetic and oncological diseases, were quite important from the scientific point of view [2].

In July 2015, the Chemical Abstracts Service of the American Chemical Society announced the registration of the one hundred-millionth chemical substance to its chemical substance database. Just five years later, the number of registered chemical substances was on the verge of surpassing 200 million, with many of them lacking studies on their biological effects or being studied under significant limitations regarding their ultimate genotoxic effects. As of today, the trend of increasing the number of registered chemical compounds continues. By the beginning of 2020, information was available on more than approximately 100,000 types of chemical substances industrially produced and capable to adversely affect human health or affect the global environment [3]. A serious problem arises from the fact that newly developed chemical, physical, and biological agents of potential genotoxicity can induce changes in DNA, which in turn can lead to hereditary and somatic diseases [4, 5]. To ensure

the viability of the organism at different stages of ontogenesis and to preserve the reproductive function, the DNA molecule, which is the carrier of hereditary information, must exhibit genomic stability, and this can be ensured by excluding the influence of genetically active substances on the hereditary information [6, 7]. Furthermore, the functioning of the repair system, which is quite conservative in terms of evolution and molecular mechanisms, allows maintaining genomic stability by repairing damage caused at the level of the DNA molecule [6, 8]. Genetic material can be exposed to a number of internal and external factors that may cause damage [6]. The system for maintaining genome stability is quite effective given the large number of different damages that can occur over a fixed period of time at the level of a single DNA molecule in a single cell [8]. Damage to the genetic material associated with changes in the nucleotide sequences at the level of coding DNA regions is crucial for the synthesis of the final gene product [9, 10]. Such modifications, depending on the nature of their occurrence, can range from simple substitutions of heterocyclic bases to more complex ones, which are most often associated with single- or double-stranded DNA breaks [9]. According to scientific papers [6, 11], the approximate number of damages can be 70,000 per day per human cell. Errors during DNA replication, repair and recombination lead to point mutations [12] (including single nucleotide substitutions and insertions/deletions of heterocyclic bases), chromosomal aberrations and genomic mutations [13]. According to [14], the integrity of cellular DNA is under constant stress, which is associated with 30,000 recorded damages to genetic material per day.

DNA repair is crucial for preventing the accumulation of damage at the level of genetic material [6, 8, 13, 14]. Disruption of repair processes can lead to destabilisation of cellular metabolic homeostasis and may contribute to carcinogenesis through dysregulation of cell growth and apoptosis [15, 16]. Genomic instability is a universal feature of all cancers [15]. DNA damage is also crucial for the development of hereditary and non-genetic diseases [17–19].

In terms of studying the epigenetic mechanisms of environmental factors, special attention should be paid to the study of the peculiarities of the initiation of histone post-translational modifications, which do not change the qualitative and quantitative composition of DNA, but play a crucial role in the regulation of biologically important cellular processes, including transcription, replication and DNA repair [20–22]. This process is realised through the methylation, acetylation, ribosylation, ubiquitination and phosphorylation of serine and threonine, which occur at the level of amino acid residues shaping the primary amino acid sequence of nuclear proteins [20, 22]. Post-translational modifications of proteins by ubiquitination underlie intracellular signal transduction, activation and coordination of molecular mechanisms for maintaining the stability of genetic material [7]. The authors of [7] consider ubiquitination as a vital mechanism in the regulation of cellular homeostasis, which is implemented with the participation of a large number of enzymes and proteins [7].

DNA methylation is an important mechanism of chromatin conformation change lying at the heart of gene expression regulation and occurs in specific highly conserved sites – CpG islands located at the 5′ end of protein gene coding regions [15, 23]. For example, benzaprene, one of the first investigated and studied carcinogens from the polycyclic aromatic hydrocarbon family, can induce the onset of cancer through inhibition of methylation processes near the promoter regions of tumor suppressor genes [23, 24].

For the purpose of identifying possible causes of induced carcinogenesis, scientists pay considerable attention to the study of processes associated with DNA damage and repair in the case of oncogenic virus replication [25]. In such a situation, the process of malignancy of hereditary information can also be initiated by damage that promotes the viral DNA integration (e.g., human papillomavirus, Merkel polyomavirus, hepatitis B virus, etc.) into the human genome [26, 27].

Despite the fact that carcinogenesis is multistage [28, 29], one of the primary tasks for researchers is to obtain information about the peculiarities of the initiation of the malignancy process [30], which can be induced by environmental factors of chemical, physical or biological nature [4–6]. Oncological diseases are promoted by the aging process, which is primarily associated with systemic changes caused by the accumulation of errors at the level of genetic material [23, 31, 32]. According to scientific paper [33] experimentally proved the relationship between changes in the epigenetic profile and aging of unicellular and multicellular eukaryotic organisms.

The relevance of studying the molecular mechanisms of induced DNA damage in carcinogenesis and mutagenesis is obvious. The need for research in this area is confirmed by the experimental data obtained. According to [34], most mutagenic

agents or their metabolites exhibit potential carcinogenic properties through inducing DNA sequence changes in pro- and eukaryotic genomes. Experimental data on the detection and assessment of the potential genetic hazard from environmental factors indicate some discrepancies in their interpretation. In our opinion, this is due to the use of different methodological approaches for assessing genotoxic effects in laboratories. Therefore, there are publications stating that some genotoxic substances do not exhibit mutagenic properties. To date, chemical compounds that do not cause genetic changes but are genotoxic have been identified and studied [35], and this fact can be explained by the use of either insufficiently adequate research methods or imperfect methods of effect assessment by the authors. In addition, *in vitro* experiments conducted with prokaryotic and eukaryotic indicator cells show a fairly large number of false-positive and false-negative results, which indicates the need for additional research to improve standard methods for assessing genetic safety, as they have significant limitations. For example, for the Ames test, the main problem is the discrepancy observed when comparing the molecular organisation of hereditary information and repair systems of eukaryotic and prokaryotic organisms. One of the significant factors adversely affecting the *in vitro* specificity of standard methods belonging to the battery of test systems is the use of metabolically incompetent prokaryotic and eukaryotic cells. Metabolic activation by S9 fraction can partially compensate for the absence of phase I enzymes, while phase II metabolic enzymes catalysing the formation of DNA-reactive metabolites and participating in their biotransformation are not considered. The main problem of popular *in vivo* DNA comet assay is the variability of electrophoregrams, which is primarily due to different conditions of indicator cell cultivation. When conducting research to identify genotoxic substances, it is also necessary to consider not only their genetic mechanisms, but also epigenetic mechanisms of direct and indirect effects on the DNA molecule.

Based on the above, it can be argued that in the context of an exponential increase in the number of chemicals requiring genotoxic assessment, there is an urgent need to improve the efficiency and predictive ability of existing methods for toxicological testing [36, 37]. The purpose of the present review is a detailed literature analysis regarding existing methods for assessing the genotoxicity of environmental factors. Taking into account the ICH M7 "Evaluation and Control of DNA-Reactive (Mutagenic) Impurities in Pharmaceuticals to Limit Potential Carcinogenic Risk" [38] and the OECD guidelines for testing chemical compounds, a comparative analysis of approaches to genotoxicity assessment was carried out, showing their advantages and disadvantages. The necessity of applying modern *in silico* methods for assessing the genotoxic potential of environmental factors was shown.

1. Sources and Types of DNA Damage

The study of the peculiarities of interaction and the mechanisms of influence of various chemical and physical factors on genome stability is quite important from a scientific point of view [39]. Depending on the localisation of chemical factors inducing DNA damage, we can distinguish between endogenous (physiological and metabolic) and exogenous factors [40–43]. The DNA molecule can be affected by endogenous and exogenous factors through direct and indirect action. The direct mechanism of action is determined by the direct interaction of endogenous or exogenous factors with the DNA molecule, which leads to the breakdown of chemical bonds at the DNA level and initiates changes in its spatial structure [44, 45]. The mechanism of mediated action by exogenous and endogenous factors is realized through their metabolism and activation of intermediate products, whose interaction with DNA underlies its damage [46, 47]. While exogenous and endogenous factors have a great potential for modifying genetic information, the relative contribution of internal and external factors to the incidence of cancer remains uncertain [6].

1.1. Endogenous factors

Each cell of the human body can suffer a large amount of DNA damage per day, most of which is usually caused by cellular metabolic processes [11]. As for the effect of mutagens on any living cell, they (mutagens) are divided into "direct" ones, i.e. those whose effect is caused by the original chemical structure of the chemical substance and "indirect" ones, whose effect is caused by their intermediate metabolites. In this case, we can refer to "cellular metabolic processes". The mechanism of DNA damage by endogenous substances *is the basis for the appearance of mismatches in the substitution* of heterocyclic bases, inter- and intra-chain cross-linking, and the formation of an abnormal DNA structure [6, 39, 41]. Such adverse effects are associated with hydrolysis, oxidation, and alkylation reactions resulting from normal physiological

processes [6]. Biological macromolecules are extremely susceptible to spontaneous chemical reactions, primarily involving hydrolysis, which are responsible for the formation of apurinic/apyrimidinic sites where heterocyclic nitrogenous bases may be absent [40, 41, 48]. The induced deamination of nitrogenous bases of DNA nucleotides is also associated with hydrolysis reactions [39]. The speed of this process can be significantly increased by exposure to ultraviolet radiation, DNA intercalating agents, nitric acid and sodium bisulfite [49, 50–52].

A fairly large amount of DNA damage by endogenous chemicals occurs due to DNA's participation in hydrolytic and oxidative reactions with water and reactive oxygen species present in the cell [48]. Spontaneous mutations characteristic of all cells can originate from the chance misincorporation of nucleotides during DNA replication or from DNA lesions that arise between replication cycles and are not repaired correctly [53]. Despite the highly developed replication apparatus, errors in the incorporation of pyrimidine and purine heterocyclic bases occur with a frequency of 10^{-8} to 10^{-6} per cell per generation [15, 54–56].

The reactions of DNA with reactive intermediate of oxygen, at high concentrations, contribute to the development of hereditary and sporadic cancers [57]. Violation of the redox balance due to an increase in the concentration of active oxygen can lead to dysfunctions manifested in the form of damage to nucleic acids, protein molecules, lipids and membrane structures, which may be associated with the development of cardiovascular and neurodegenerative diseases [9, 41, 58]. At the same time, a reduced concentration of reactive oxygen species can induce chronic granulomatous disease and autoimmune disorders [42]. Reactive oxygen and nitrogen species are involved in the formation of more than 70 oxidised varieties of heterocyclic bases and acid sugars as part of modified DNA monomeric units and affect the stability of genetic information [59].

1.2. Exogenous factors

DNA damage can also be caused by environmental exposure to the genetic apparatus [16, 40]. Within the framework of the present review, special attention should be paid to the study of the impact of exogenous factors whose negative effect on the human genetic apparatus can be avoided, especially in a situation where the genotoxicity of a certain chemical compound has been proven by experimental methods or by modern predictive *in silico* models [41, 60]. Scientific papers [15, 40, 58, 61] provide information on the peculiarities of endogenous and exogenous DNA damage and highlight the functioning of the repair system. The basic exogenous factors of physical nature (environmental factors) that can induce processes of damage to genetic material include ionising, ultraviolet and infrared radiation, as well as chemical agents exhibiting genotoxicity properties [6, 40, 61]. Table 1 provides information on the basic endogenous and exogenous factors that can affect genetic stability, taking into account the specifics of damage and activated repair mechanisms.

Exogenous sources of damage to genetic stability, such as ionising radiation (X-rays), cosmic and ultraviolet radiation, and exposure to mutagenic chemicals, contribute to the accumulation of DNA damage every cell must counteract on a daily basis [6, 16].

Paper [62] investigated the effect of ionising radiation on the central nervous system of higher eukaryotic organisms. Radiation-induced damage to the central nervous system is associated with the development of oxidative stress, the accumulation of free radicals, which is the basis of molecular and cellular changes, including DNA damage, which can lead to disorders in the structure of neurons, synaptic plasticity, cause systemic inflammation and lead to neuronal death [62].

The direct effect of infrared radiation causes chemical changes in DNA, disrupts its structure, which may affect the replication process. This type of damage accounts for 30–40% of chemical modifications of DNA induced by infrared radiation [39]. The indirect impact of infrared radiation is associated with the processes of radiolysis of water molecules, which is a stimulating factor for the accumulation of free radicals in the cell directly involved in oxidative DNA damage [63, 64].

Ultraviolet radiation, as one of the environmental factors exerting constant pressure on the genomic integrity of the body, is one of the most common hazards to human health. Radiation with wavelengths from 280 to 315 nm is one of the most powerful physical agents that can induce various mutagenic and cytotoxic disorders [65]. Ultraviolet radiation can initiate the synthesis of cyclobutane pyrimidine dimers and pyrimidine-(6,4)-pyrimidine photoproducts with subsequent changes in the spatial structure of DNA and blocking of transcription and replication processes [16, 66].

The study of the impact of various physical and chemical agents on the human genetic apparatus is one of the priority areas of modern genetic toxicology. This interest is primarily related to the problem of inadequate assessment of genetic safety

Table 1: Endogenous and exogenous sources of DNA damage [17, 40, 58, 61]

Factors	Damage mechanism	Damage result	Repair mechanisms
Endogenous	Oxidation	Modification of heterocyclic nitrogenous bases	Nitrogenous base excision repair
	Alkylation	Methylation of heterocyclic nitrogenous bases	Direct repair Nitrogenous base excision repair Repair of mismatched nitrogenous base pairs
	Hydrolysis	Creation of apurinic sites	Nitrogenous base excision repair Post-replicative repair
		Cytosine deamination and uracil synthesis	Nitrogenous base excision repair
	DNA polymerase errors	Substitutions, insertions and deletions of heterocyclic nitrogen bases	Mismatch repair system
Exogenous	Ionizing radiation	Double-strand breaks	Non-homologous joining of the ends of DNA; Homologous recombination
		Single-strand breaks	Nitrogenous base excision repair
	Ultraviolet radiation	Cyclobutane pyrimidine dimer synthesis	Nucleotide excision repair
	Chemical compounds (aromatic amines, alkylating agents, natural toxins, chemotherapeutic agents)	Damage to heterocyclic nitrogenous bases; DNA adduct synthesis	Nucleotide excision repair Direct repair Mismatch repair system Repair of mismatched nitrogenous base pairs

of a large number of registered chemicals for the human population [3]. To date, the main obstacle to solving this problem is the lack of free access to information on more than 50,000 chemicals, as such information is considered confidential [67]. In addition, for a large number of chemicals, experimental toxicological data are limited [36, 68], making it impossible to use classical *in vitro* approaches to assess the genotoxicity of these chemicals [68]. In everyday life, the human hereditary apparatus is exposed to a large number of external DNA-damaging chemical agents [16, 41, 59]. Despite the fact that the number of chemicals affecting genetic stability is increasing every year, the mechanisms of DNA damage for the most common structural classes of exogenous agents such as aromatic amines, polycyclic aromatic carbohydrates, natural toxins, alkylating agents and chemotherapeutic agents are now well understood [16, 41].

Since the second half of the 21st century, there has been an active development of genetic toxicology as a science associated primarily with the discovery of the spatial structure of DNA and the development of modern sequencing methods [1, 2]. It is quite interesting that a large number of scientific papers have documented the results of studies of the mutagenic potential of aromatic amines [69, 70–72]. The results of the studies published in papers [73–76] show a clear connection between the belonging of chemical compounds to this class (aromatic amines) and their expressed mutagenic activity. Aromatic amines are by-products of tobacco combustion that pose a potential risk to human health and remain the most common cause of lung cancer deaths worldwide [15]. In addition, aromatic amines are among the main environmental pollutants, however they can be used as basic components in the production of cosmetics, dyes, plastics, food and pesticides [16, 40, 77]. The best-known and most scientifically studied aromatic amines are 2-aminofluorene and its acetylated derivative 2-acetylaminofluorene, which were used as insecticides until their carcinogenicity was proven [78]. Aminofluorenes are transformed into carcinogenic esters and sulfate alkylating agents with the participation of the cytochrome P450-dependent monooxygenase system, which can attack the 8th position of the guanine carbon at the

level of the DNA molecule [79]. If the resulting DNA adducts are not removed by nucleotide excision repair, this can lead to heterocyclic base substitutions and a subsequent reading frame shift [80]. In scientific works [81, 82], the peculiarities of the biological transformation of aromatic amines with the participation of prokaryotic organisms were investigated, which creates preconditions for maintaining a genetically safe environment.

Aromatic amines are widely used as intermediates in the synthesis of active ingredients in medicinal products. In this situation, the mutagenicity of impurities poses serious obstacles to genetic safety and to the prevention of the release of pharmaceutical products with potential genotoxic properties [83, 84]. For assessing the genetic effects of the basic component of a certain pharmaceutical drug, it is also necessary to take into account the fact that aromatic amines can be synthesised as metabolites by hydrolysis of active or auxiliary components of the drug containing amide bonds in their structure [85].

Polycyclic aromatic hydrocarbons (PAHs) are widely distributed in the environment and are among the main air pollutants [86]. As early as in 1983, the US Environmental Protection Agency reported sixteen polycyclic aromatic hydrocarbons as major environmental pollutants [86]. Polycyclic aromatic hydrocarbons are persistent polutants with toxicity, mutagenicity, carcinogenicity, and immunotoxicity properties for both prokaryotic and eukaryotic organisms [87]. The carcinogenicity of this class of chemical compounds, as well as of some aromatic amines, is associated with the enzymatic activity of the cytochrome P450-dependent monooxygenase system [88, 89]. The metabolism of polycyclic aromatic hydrocarbons (e.g. quinones) occurs through the synthesis of reactive intermediates that are not polar enough for excretion and may cause damage to cell membranes, proteins and DNA [40, 87, 90].

Natural toxins form a class of genotoxic and carcinogenic chemicals utilised by microorganisms or fungi in defence reactions [91]. Filamentous fungi belonging to the genus *Aspergillus* are the main cause of aflatoxin contamination of cereals and oilseeds, as well as dairy products [92]. *Aspergillus flavus*, which mainly synthesises B-afflatoxins B1 and B2, and *Aspergillus parasiticus*, which produces G-afflatoxins G1 and G2, are the aflatoxin producers [93, 94]. Aflatoxin B1, one of the most important factors in the incidence of hepatocellular carcinoma worldwide, has been classified as a carcinogen by the Food and Agriculture Organization of the United Nations [95]. Biotransformation of aflatoxin B1 involves the cytochrome P450-dependent monooxygenase system with the formation of a toxic and carcinogenic product, aflatoxin B1-8,9-epoxide, which can interact with the nitrogen in the 7th position of guanine with the formation of a DNA adduct [16, 40, 95]. Such a newly synthesized complex weakens the glycosyl bond and leads to DNA depurinization [40]. Another pathway of chemical transformation of aflatoxin B1 has also been studied, this pathway involves additional hydrolysis of the DNA adduct with the formation of aflatoxin-B1-formamidopyrimidine, which blocks DNA replication and has a high potential for inducing transversion-type mutations [40, 95].

In the context of global industrialisation and urbanisation, there is a significant increase in the number of chemicals that can be potential environmental pollutants. This trend creates new challenges for humanity and stimulates the scientific community to develop, streamline and improve the regulatory framework necessary to accompany the procedures at the stage of assessment, registration, control, permitting and prohibiting the use of chemicals used in various spheres of human life. International organisations and regulatory bodies have been registered all over the world dedicated to ensuring environmental protection and human health. Standards for the genetic evaluation of chemicals are usually achieved by taking into account the recommendations of the Organisation for Economic Co-operation and Development (OECD) [96, 97] and the international document ICH M7 "Assessment and control of DNA reactive (mutagenic) impurities in pharmaceuticals to limit potential carcinogenic risk - Scientific guideline" [87, 98, 99]. Considering the large number of methods of genetic assessment of environmental factors, it is necessary to draw conclusions about the genotoxicity of a chemical substance based on scientifically sound procedures, methods and international regulatory documents and recommendations adopted by the scientific community.

At the end of 2022, the European Chemicals Agency published information on approximately 800,000 chemicals for which no complete information on direct or indirect impact on the human genetic apparatus is available [61]. Despite the fact that maximum attention is paid to ensuring genetic and environmental safety worldwide, one of the main challenges of genetic toxicology is to solve the problem of obtaining genetic assessment for all chemical compounds, information on which is stored in modern databases such as ChemSpyder [100],

PubChem [101], SciFinder [102], and others. The need to develop new approaches and methods for genetic assessment of environmental factors is also discussed in scientific papers [60, 103–106]. In the present review, with the aim of finding possible ways for improving the basic methods and searching for new approaches to genotoxicity assessment, we will consider classical basic and modern methods for assessing the genetic safety of environmental factors for which there are no OECD recommendations for genotoxicity testing.

2. Genotoxicity Assessment Methods

A variety of *in vitro* and *in vivo* methods have been developed to test chemicals for potential genetic activity using eukaryotic and prokaryotic organisms. According to the OECD Guidelines for the Testing of Chemicals [107], there are more than 150 methods accepted by the scientific community and regulatory authorities that provide information on toxic and genotoxic potential. The OECD provides recommendations for 20 classical methods [108], most of them developed more than 30 years ago [35, 109] and currently do not allow for a fully adequate and objective answer to the question of whether a chemical is potentially genetically active. The OECD Guidelines for the Testing of Chemicals include 9 *in vitro* experimental models with laboratory animals and 11 test systems using prokaryotic and unicellular eukaryotic organisms and mammalian cell lines [107, 109]. In order to obtain a reliable assessment of chemicals in terms of their potential genotoxic properties, it is necessary to take into account the three main outcomes of DNA damage, which are primarily associated with the occurrence of gene mutations, chromosomal aberrations and aneuploidy [110]. To date, there is no short-term test system which would allow taking into account such damage to hereditary information simultaneously [111]. Therefore, a classical battery of short-term tests is used to comprehensively assess the ability of chemicals to cause damage to genetic material, given the three endpoints of damage [111–113]. Chemicals can be assessed as not showing genotoxic potential if a negative result is obtained for all DNA damage endpoints using *in vitro* methods [114]. Despite the fact that to date more than a hundred methods for assessing genotoxicity have been developed, preference is given to test systems where the standardized methodology is accepted by the scientific community and approved by the relevant guidelines of international organizations (for example, OECD, ECHA, UK-EMS, US-FDA, EFSA, etc.) [107, 109]. Table 2 contains information about the classical battery of short-term test systems recommended by the OECD as basic methods for genotoxicity assessment [107, 111, 112].

The authors of the scientific work [97] focus on three main methods related to the standard system battery of short-term tests represented by the Bacterial Reverse Mutation Test (TG471), *In Vitro* Mammalian Chromosomal Aberration Test (TG473) and *In Vivo* Mammalian Erythrocyte Micronucleus Test (TG474).

By April 2014, the standard battery of test systems for genotoxicity assessment additionally took into account tests TG472, TG477, TG479, TG480, TG481, TG482 and TG484, which, by de-

Table 2: Standard test system battery for genotoxicity assessment

Test no. (OECD)	Name of the test system	Features of the test	Links
TG471	Bacterial Reverse Mutation Test	*In vitro*	[115]
TG473	Mammalian cell chromosome aberration test	*In vitro*	[116]
TG474	Mammalian erythrocyte micronucleus test	*In vivo*	[117]
TG475	Mammalian bone marrow chromosomal aberration test	*In vivo*	[118]
TG476	*In vitro* mammalian cell gene mutation tests using the *Hprt* and *xprt* genes	*In vivo*	[119]
TG478	Dominant lethal test	*In vivo*	[120]
TG483	Mammalian spermatogonial chromosomal aberration test	*In vivo*	[121]
TG485	Mouse Heritable Translocation Assay	*In vivo*	[122]
TG486	Unscheduled DNA Synthesis (UDS) Test with Mammalian Liver Cells *in vivo*	*In vivo*	[123]
TG487	*In Vitro* Mammalian Cell Micronucleus Test	*In vitro*	[124]
TG48	*In Vivo* Mammalian Alkaline Comet Assay	*In vitro*	[125]

cision of the OECD Council, were removed from the list of recommended tests [108]. The European Food Safety Association (EFSA), based on a comprehensive assessment of the results of Unscheduled DNA Synthesis (UDS) Test with Mammalian Liver Cells *in vivo* (TG486), no longer recommends this method for assessing the genetic safety of environmental factors [110]. However, the OECD recommendation for the TG486 test system remains to date [107].

In 2012, the International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use (ICH), approved the guidance on S2(R1) Genotoxicity Testing and Data Interpretation for Pharmaceuticals intended for Human Use (ICH S2 (R1)), according to which two theoretically based application plans were presented for *in vitro* and *in vivo* batteries of standard test systems for genotoxicity determination [126]. The first approach [111, 112, 126], which was proposed for testing, takes into account the use of the following short-term tests: 1) Ames *Salmonella*-microsome assay (TG471) (bacterial reverse mutation test); 2) *In vitro* mammalian chromosomal aberration in bone marrow cells test (TG473); 3) *In vivo* test for detection of mammalian peripheral blood erythrocyte micronuclei (TG473). The second approach [111, 112, 126] is based on the use of such test systems as: 1) Bacterial Reverse Mutation Test (TG471); 2) *In vivo* test for detection of mammalian erythrocyte micronuclei (TG473); 3) Unscheduled DNA Synthesis (UDS) Test (TG486) or *In Vivo* Mammalian Alkaline Comet Assay (TG489). The most common methods for assessing genotoxic potential include Bacterial Reverse Mutation Test (TG471), *In Vitro* Mammalian Cell Micronucleus Test (TG487) and *In Vitro* Mammalian Chromosomal Aberration Test (TG473) [68].

2.1. Bacterial reverse mutation test system (TG 471)

The basic method for assessing the genetic safety of environmental factors is the reverse mutation test developed in the 1970s by the American molecular biologist Bruce Ames [127]. The bacterial reverse mutation test is a fast, inexpensive, and easy-to-perform method [110, 111]. The Ames test is used as the main *in vitro* method [128] for assessing mutagenic potential and is performed using bacterial strains *Salmonella typhimurium*, which are histidine auxotrophs [113, 127, 129–131]. Mutations in hisG and hisD genes at the level of histidine operon of *Salmonella typhimurium* strains are stimulating to inhibit the growth processes of tester strains on the nutrient medium free of histidine [68, 111]. Mutagenic chemicals are responsible for the transition from histidine auxotrophicity to prototrophicity, which occurs by inducing mutations that include replacing pairs of nucleotide bases and the reading frame shift [68, 110, 111, 127]. To assess the mutagenicity of the test chemicals, the number of revertant colonies cultured in the petri dish was measured compared to the control. The resulting estimation is based on the selection of revertants, not the frequency of mutations induced by environmental factors [110]. This approach is a classic semi-quantitative Ames method, which is used in almost all laboratories worldwide to test chemical compounds for their potential genetic activity. There is a modification of the Ames method, it is a quantitative method recording directly the mutation frequency that occur under the action of mutagens, however, it is not very popular among researchers due both to its complexity, and the problem of reproducibility of the experiment in different laboratories. Standard test strains of *Salmonella typhimurium*, in addition to point mutations at the level of histidine operons, may contain additional mutations causing increased sensitivity of the test organism to the action of chemical compounds, which improves the predictive ability of the test system [132]. For example, the structure of the outer lipopolysaccharide layer is disrupted by *rfa* mutation, which increases the cell wall permeability to potential environmental pollutants [110, 111]. In addition, increasing the sensitivity of the test system is achieved through the use of *Salmonella typhimurium* mutant strains where the gene *uvrB* is missing, the protein product of which is the main component of the multi-enzyme excisional repair system of nucleotides in bacteria [127, 133]. In order to increase the prognostic capabilities of the Ames test, the need for the introduction of the plasmid *pKM101* which determines the resistance of *Salmonella typhimurium strains* to antibiotics such as ampicillin and carbenicillin, was proved [127]. In addition, resistance factor (or R-factor) carries information about the genes *mucA* and *mucB*, which encode DNA polymerase V participating in translesion DNA synthesis [113]. The test system's sensitivity to xenobiotics with genotoxicity properties can be increased by DNA synthesis through damaged areas [113]. *Salmonella typhimurium* standard strains such as TA97, TA98, TA100, and TA102 contain the plasmid pKM101 [113, 132]. In *Salmonella typhimurium* strains TA1538, TA1535, and TA1537 the resistance factor is absent. Table 3 provides information on the genotypes of the main

Table 3: Genotypes of mutant of *Salmonella typhimurium test strains*

Strains *Salmonella typhimurium*	Reverse mutation type	Mutations at the level of histidine operon	Additional mutations		Presence of the plasmid pKM101	Links
			rfa	*uvr*		
TA98	Reading frame shift	D3052	+	+	+	[134]
TA1538	Reading frame shift	D3052	+	+	–	[135]
TA100	Base substitution	G46	+	+	+	[136]
TA1535	Base substitution	G46	+	+	–	[137]
TA1537	Reading frame shift	C3076	+	+	–	[138]
TA102	Transitions/Transversions	G428	+	+	+	[139]

mutant *Salmonella typhimurium* strains, which will be used to assess the genetic safety of environmental factors.

In accordance with the recommendations of the Organization for Economic Cooperation and Development, five strains of *Salmonella typhimurium* (TA1535, TA1537, TA98, TA100, TA102) are used to assess mutagenic potential, the use of which proves their predictive value in terms of identification of genotoxic substances [115]. Substitutions occurring at the level of heterocyclic base pairs G and C can be detected using strains TA100 and TA1535 [115, 132, 136]. Strains TA98 and TA1537 allow us to identify mutations by the type of reading frame shift [115, 132, 134, 137]. In [138], the *Salmonella typhimurium* TA102 test system was investigated, which allows us to obtain a genotoxic assessment of mutagens interacting with nucleotides A or C [115, 132, 138]. Quite interesting is the fact that the detection of some mutagens is possible only with the use of the TA102 test strain, while other strains recommended for OECD testing give a negative result [138].

The bacterial reverse mutation test uses the cells of prokaryotic organisms as a test system that differ significantly from mammalian cells, especially in such indicators as uptake and metabolism. Quite significant differences are also observed in terms of molecular mechanisms of repair. In order to decompensate the difference in the metabolic characteristics of eukaryotic and prokaryotic organisms, it was proposed to use an exogenous source of metabolic activation for mutagenicity testing [115, 139]. The most common system used for metabolic activation is S9 fraction obtained from liver hepatocyte cells of laboratory animals [114, 140]. Although the metabolic activation system does not allow us to fully consider the features of metabolism of the above eukaryotic organisms, including humans, the prognostic potential for such a test system increases and the range of chemical compounds that manifest their activity is significantly expanded when using the metabolic activation system, especially in the case of studying potential carcinogens that can be intermediate products of reactions involving the monooxygenase P450 enzyme system [132]. The main obstacle to obtaining reliable results of *in vitro* mutagenicity testing using the Ames test is highly specific for metabolic activation, which depends on a person's age, gender, genotype, etc. [141]. The use of a HepaRG cell model for mutagenicity testing which demonstrates significant activity of P450 monooxygenase enzymes partially solved the problem of insufficient consideration of the basic pathways of xenobiotic biotransformation in the human body with *in vitro* test systems [68, 142].

2.2. *In Vitro* mammalian interphase cell micronucleus test ayatem (TG487)

In vitro micronucleus test system has been used to assess cytogenetic damage to inherited material over the past few decades [143, 144] and is one of the main methods related to the short-term battery of genotoxicity tests [107, 111, 112]. The micronucleus identification method is used to assess the aneugenic and clastogenic potential of the studied chemical based on its ability to induce micronuclei in rodent bone marrow cells or peripheral blood lymphocytes [143, 144]. Micronuclei are identified as fragments of DNA with a nuclear membrane that are not connected to the spindle apparatus and are not subject to migration during cell division [145]. Micronuclei represent lesions occuring during mitotic division of indicator cells that are inherited and passed on to daughter cells, whereas chromosomal aberrations in cells at the metaphase stage of mitosis are not inherited [124]. Whole chromosomes or fragments of them that cannot be included in the main nucleus after the division can be identified in micronuclei [146]. The frequency

with which micronuclei in interphase cells will be identified is used to quantify DNA damage [111, 146]. In order to neutralize the influence of the formed separated abnormal genetic microstructures arising under the influence of genotoxic substances, the count of micronuclei occurs after one mitotic division. This procedure can be achieved by blocking the division of indicator cells with cytochalazine [147]. In [148, 149], an optimized approach for detecting micronuclei without using mitotic division blockers is proposed, but in the case of studying genotoxic substances that slowly induce micronuclei due to their effect on cytostasis, this approach often leads to erroneous results. Scanning microscopy and flow cytometry are currently used to automate micronucleus analysis and improve testing performance [150]. In [146], the authors propose to use a deep learning model based on a convolutional neural network to quantify genotoxicity and cytotoxicity based on a micronucleus test. The trend of active application of machine learning algorithms and the introduction of effective methods for assessing genotoxicity *in silico* can be traced in scientific works [85, 105, 151] and deserves special attention from scientists.

2.3. *In vitro* test system for mammalian chromosomal aberration (TG473)

The impact of most of the environmental contaminants studied on the genetic apparatus of eukaryotic cells often manifests itself as chromosomal damage [152]. Double-stranded DNA breaks, which are the main cause of structural chromosomal aberrations, can be induced by direct or indirect exposure to genotoxic substances due to DNA replication or repair errors [153]. *In vitro* chromosomal aberration testing enables us to identify chemicals that influence the hereditary information of cultured mammalian cells by rearranging chromosomes [108, 154]. The short-term test system for detecting chromosomal aberrations in mammalian bone marrow cells is recommended by the OECD as the basic method and belongs to the standard battery of non-genotoxic tests [97, 107, 111, 112]. Among chromosomal rearrangements, chromosomal and chromatid aberrations are distinguished [154]. The first ones are associated with changes in the number and structure of chromosomes. Chromatid-type aberrations cover only one chromatid of the eukaryotic chromosome [111]. Despite the fact that aneugens are inducers of changes in the ploidy of eukaryotic cell nuclei, the detection of polyploidy in chromosomal aberrations tests does not allow us to objectively assess the aneugenic potential for such chemicals. Aneuploidy established by the test system may show the cytotoxic potential of a chemical having a destabilizing effect onto the cell cycle regulation system [155]. When studying the influence of environmental factors on changes in nuclear ploidy, the OECD recommends using *In Vitro* mammalian cell micronucleus test [124].

Various cell lines of eukaryotic organisms, including humans, are used for testing. To study chemically-induced chromosomal mutations, scientists in the historical context focused heavily on Chinese hamster lung and ovary cell lines [156]. However, studies have shown that such cell lines are genetically unstable and are characterized by a high rate of spontaneous aberrations, which can negatively affect the final test result [157]. Chromosomal aberrations caused by physical and chemical factors can currently be detected with a modern method using primary diploid cultures of lymphocytes from peripheral human blood, which are stimulated to divide by the mitogen phytohemagglutinin [158]. In [159], the effect of ionizing radiation using human peripheral blood lymphocytes is studied. Chinese hamster fibroblast cell lines are quite popular in various laboratories [160]. Studies of chromosomal aberrations in peripheral blood lymphocytes have been used for decades to monitor healthy individuals exposed to chemicals that are potential mutagens or carcinogens [161].

Obtaining an objective assessment of genotoxic effects by *in vitro* chromosomal aberration detection requires simulating mammalian metabolism. Such a process is achieved through the addition of a mixture of S9 enzymes and allows the identification of environmental factors of indirect effect on the hereditary material [141]. Damage to hereditary material cannot be caused by the interaction of the studied environmental factor with the DNA molecule due to the incomplete modeling of conditions *in vivo* by the exogenous metabolic activation system. In such a situation, False positive results can be caused by pH changes [162] and interactions with environmental chemical compounds [163], which result in the synthesis of intermediates that are not taken into account in testing. The method of detecting chromosomal aberrations in mammalian cells involves treating tissue culture with the test substance both in the absence and presence of enzymes of the metabolic activation system, since interaction with genetic material often occurs after metabolic activation [111]. The culture of mammalian cell line cultures or primary human cell cultures is done before adding the test substance in an *in vitro* test. Treatment of cells with DNA-dama-

ging agents can lead to the appearance of non-renewable damage in both strands of DNA, which can result in the rupture of chromosomes. To estimate the clastogenic effect, the percentage of cells with structural aberrations is the primary parameter. Microscopically examining metaphase preparations in which the genetic material is in the most condensed state with the clearest structure is used to perform a detailed analysis of induced chromosomal lesions [116, 164]. Cell cycle arrest at the metaphase stage is achieved by using mitosis blockers [164]. The mechanisms of action of such popular blockers as colcemide and colchicine have been studied in scientific papers [165, 166].

2.4. *In vivo* mammalian erythrocyte micronucleus test system (TG474)

The process of establishing metabolic pathways and assessing xenobiotic toxicity is a technically complex and time-consuming process, it requires the use of both *in vitro* cell systems and *in vivo* animal models before conducting human clinical trials [142]. While animal tests are still the primary standard method for assessing the genetic safety of xenobiotics, scientists are increasingly focusing on the concept of '3R', guided by principles designed to reduce, improve, and replace animal models when testing for genotoxicity [167, 168]. In such a situation, the *in vivo* genotoxic potential is assessed further only after a positive result has been obtained with the *in vitro* basic test systems [112].

In vivo micronucleus analysis using rodent bone marrow hematopoietic cells or peripheral blood cells is widely used to assess the clastogenicity and aneugenicity of environmental factors or individual chemicals. The micronucleus identification method, which belongs to the standard battery of short-term genotoxicity tests [97, 107, 111, 112], is one of the most popular and reliable and allows us to obtain a more or less objective assessment of the induction of genotoxic effects by environmental factors in the form of chromosomal aberrations and damage to the mitotic apparatus of mammalian erythroblasts [117, 169]. Micronuclei test can be used to identify substances that cause chromosomal aberrations and xenobiotics that cause aneuploidy because they are formed from whole chromosomes and fragments of chromosomes that fall into the nuclei of daughter cells during mitosis [111]. The *in vivo* test system for detecting rodent erythrocyte micronuclei ranks first among the short-term test systems recommended by OECD [117, 170]. Taking into consideration factors of *in vivo* metabolism, pharmacokinetics, and DNA repair processes can be achieved through micronucleus analysis, but these factors may differ for different types of organisms, tissues, and investigational endpoints of DNA damage [117].

The number of immature polychromatic erythrocytes found in the bone marrow or peripheral blood of experimental animals with micronuclei induced by environmental factors can be counted during an *in vivo* micronucleus test [111]. Comparing the frequency of occurrence of micronuclei in the control experiment gives an estimate of possible genotoxic effects on the human body [171]. Differentiation of bone marrow erythroblast into an immature erythrocyte (reticulocyte) is associated with enucleation of the basic nucleus [111, 117]. The use of such cells in testing for genotoxicity has certain advantages that greatly simplify the complex process of identifying micronuclei and visualizing them [111, 117]. With the development of information technologies, automated systems have been introduced to identify and quantify micronuclei, which minimizes the influence of the human factor when conducting manual calculations on images obtained using light microscopy [172, 117]. Automation and optimization of research using microkernel *in vivo* the test is achieved on the basis of flow [173] and scanning [174] cytoflourimetry and image analysis systems [175, 176].

2.5. *In vivo* comet assay (TG489)

In 2016, the Organization for Economic Cooperation and Development published recommendations for genotoxicity testing using *in vivo* mammalian alkaline comet assay [126]. Two application plans were proposed by the International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use, which considers the classic standard battery of short-term tests for determination of the genotoxic potential of environmental factors [177]. According to the first plan, two *in vitro* methods and one *in vivo* method are used for testing. To assess the genetic effects that can be induced by factors of physical and chemical and biological nature according to the second plan, two *in vivo* methods and one *in vitro* method are used [177, 178]. Among *in vitro* methhids, bacterial reverse mutation test is mandatory for both the first and second testing plans [177, 178]. For *in vivo* genotoxicity tests, a micronucleus test with bone marrow erythrocytes [175] and comet assay with hepatocytes [179, 180] are used.

Over the past few decades, comet assay has been the most widely used experimental method for assessing DNA damage in eukaryotic cells [180, 181].

Among the basic *in vivo* methods for detecting genotoxicity related to the standard battery of test systems, comet and micronucleus assays are quite popular, mainly because of their simplicity, sensitivity and versatility [35]. According to scientific papers [36, 177, 182, 183], the predictive potential of genotoxicity assessment is improved by combining two methods. *In vivo* DNA comet assay provides assessment of genetic effects using in various fields of science, such as molecular epidemiology, pharmacology, genetic toxicology, oncology, and also used in basic research to study the mechanisms of DNA damage and repair [184]. In addition, *in vivo* DNA comet assay is also used to assess the level of genetic damage in the development of human diseases such as essential hypertension [185], chronic kidney disease [186] and type 2 diabetes [187].

In vivo DNA comet assay is based on the gel electrophoresis method, which allows assessing damage to the hereditary material of eukaryotic cells, taking into account the level of migration activity of chromosomal DNA in the agarose gel. At the same time, negatively charged fragments of single-stranded DNA are moved from the cathode to the anode, which resembles the shape of a comet [125]. During electrophoresis, DNA fragments containing breaks lose their helical structure, which affects gel electrophoretic mobility [125, 188]. In an alkaline environment, at the value of pH > 13 comet DNA assay makes it possible to detect single- and double-stranded breaks that can be caused by genotoxic environmental factors [125]. The size, shape, and amount of DNA within a "comet" is crucial for determining the level of DNA damage [125, 184, 188, 189]. Increase in testing performance using the DNA-comet assay is achieved through the introduction of automated analytical tools, among which OpenComet [190], HiComet [191], CometChip [192], CometAnalyzer [193] are quite popular, which allows minimizing human influence at the decision-making stage to assess the genotoxic potential of a certain environmental factor [194].

3. Modern methods for assessing the genotoxicity of environmental factors

Over the past 50 years, various *in vivo* and *in vitro* methods have been developed for obtaining an objective assessment of the genetic safety of environmental factors, some of them have been adopted by the scientific community, followed by approval by relevant guidelines of international organizations (such as OECD, ECHA, UK-EMS, US-FDA, EFSA, etc.) [107, 109]. To date *in vivo* and *in vitro* standardized methods are the basis for genetic testing in the European Union [109]. The scientific work [195] presents a classical testing plan, according to which, an assessment with the use of a basic battery of test systems is performed for the detection of genetic effects that can be induced by chemicals used in cosmetic products, genotoxic environmental agents, biocides and basic ingredients of pharmaceutical preparations. An assessment of genetic safety for humans can be obtained on the basis of the step-by-step principle, which at the initial stage of testing takes into consideration the use of a basic battery of *in vitro* tests, according to which, in some cases, *in vivo* testing is carried out. An exception to the rule is testing for genotoxicity of active substances of cosmetic products, for which *in vivo* tests are prohibited in EU countries [167, 195, 196]. According to [107, 111–113], most of the methods included in standard battery test systems were developed more than 30 years ago. In addition, according to papers [197, 198], *in vitro* experiments conducted with prokaryotic and eukaryotic indicator cells show a fairly large number of false-positive results, which indicates the need for additional research to improve standard methods for assessing genetic safety, as they have significant limitations. For example, for the Ames test, the main problem is the discrepancy observed when comparing the molecular organisation of hereditary information and repair systems of eukaryotic and prokaryotic organisms. The main problem of popular *in vivo* DNA comet assay is the variability of electrophoregrams, which is primarily due to different conditions of indicator cell cultivation [35]. The search for effective new and optimization of classical methods for assessing genotoxicity is extremely important. A significant paradigm shift in genotoxicity testing was observed after Sanger discovered a method for determining the nucleotide sequence of DNA in 1977, followed by the active development of bioinformatics. The need to revise and transform the basic methods for assessing the genetic safety of environmental factors, taking into account the achievements of bioinformatics, systems biology and computational toxicology, is traced in scientific papers [199, 200]. In addition, the active integration of machine learning algorithms into genetic toxicology deserves special attention, which gives hope for solving the main problem of genetic toxicology associated with the lack of information about the genotoxic potential of a large number of chemical compounds existing in the environment [201]. It should be noted that to date,

modern methods and approaches for genetic testing have been developed, they have a sufficiently large percentage of sensitivity and specificity, but no OECD recommendations were issued for them [109]. The increase in prognostic reliability (taking into account the sensitivity and specificity of the method) and the performance of modern test systems is achieved through the search for new molecular pathways for the genotoxic effects of environmental factors [195, 199], the development of new indicator cells and the improvement procedures for their cultivation [109, 111].

One of the significant factors adversely affecting the *in vitro* specificity of standard methods belonging to the battery of test systems is the use of metabolically incompetent prokaryotic and eukaryotic cells. Metabolic activation by S9 fraction can partially compensate for the absence of phase I enzymes, while phase II metabolic enzymes catalysing the formation of DNA-reactive metabolites and participating in their biotransformation are not considered [115, 138, 141]. GGenotoxic metabolites may be formed after one or more stages of phase I and/or phase II metabolism. Therefore, *in vitro* genotoxicity testing requires cell models that are metabolically competent. To date, there has been some progress in solving the problem of using metabolically incompetent indicator eukaryotic cells for genotoxicity investigation with the use of *in vitro* classical methods. Cellular lines of human hepatocellular carcinomas such as HepaRG [202] and HepG2 [203] are used for genotoxicity prediction. HepG2 cell line shows markedly low metabolic activity, while the HepRG cell line with the best metabolic potential in phases I and II is considered a cell line for which there is no need for metabolic activation [203]. In the study of the genotoxic effect of pyrolizidine alkaloids, the hepatoma cell lines Huh6 and HepG2 are quite effective [204]. Using mutant Tk6 cell lines represents an approach *in vitro*, which allows for a more comprehensive genotoxicity analysis in comparison with traditional classical methods, reducing the number of false results. The scientific work [205] highlights the issues of assessing the genotoxic potential using the human B-Lymphoblast cell line TK6, taking into account two endpoints of DNA damage, which demonstrates the obvious advantages of this approach in comparison with *in vitro* using standard battery test systems. Some mutant cell lines TK6 show a special phenotypic behavior, showing either increased sensitivity or tolerance to a certain mutagen, which makes it possible to use this approach not only for assessing genetic effects, but also in conducting fundamental research on DNA repair mechanisms [111].

One of the most dangerous DNA damage is double-stranded breaks, which can be initiated by exogenous environmental factors of a physical, chemical or biological nature. Repair of such damaged DNA regions can cause oncogenic rearrangements [6]. In response to double-stranded breaks, phosphorylation enzymes of serine-threonine ATM, ATR, and DNA-PKc kinases are activated [206, 207]. In the scientific work [208], after a detailed proteomic analysis, about 900 phosphorylation sites were identified, covering more than 700 proteins. An important substrate of ATM, ATR, and DNA-PKc is H2AX histone proteins, which are converted to yH2AX after phosphorylation of serine at position 139 [209]. Quite interesting and not standard, as for evaluating the final genetic effects is the approach that provides for assessment of genotoxic effects by determining the degree of manifestation of H2AX phosphorylation processes in response to exposure to genotoxic agents. This procedure is performed with the use of such methods as flow cytometry, immuno-fluorescence microscopy, and Western Blot immunoassay [210, 211] the main cell lines of this method are human B-lymphoblasts HepG2 and TK6 [207].

ToxTracker is a promising tool for assessing genotoxic effects, taking into account non-standard points of DNA damage. "ToxTracker" testing system based on mammalian stem cells makes it possible to detect the activation of specific cellular signaling pathways that make it possible to carry out genotoxic profiling of environmental factors, taking into account their dosed effect. ToxTracker uses a panel containing six green fluorescent reporter proteins, one for each cell line, to evaluate the ability of genotoxic agents to react with genetic material. The result of this interaction may be blocking of DNA replication, induction of oxidative stress, activation of reactions mediated by denaturation of proteins or general reactions of cellular stress, dependent on the transcription factor R5, which serves as a suppressor of tumor growth [212, 213]. The ToxTracker system allows determining the genotoxic potential of the test compound in one test, taking into account various endpoints of DNA damage [213].

The test system for assessing the genotoxicity of environmental factors based on the detection of mutations of the Pig-a gene deserves special attention. Despite the fact that *in vivo* Pig-A assay demonstrated its prospects for assessing the end point of DNA damage in the form of mutations, back

in 1999 [214], the OECD recommendation was received by the methodology only in mid-2022 [215]. The Pig-a gene encodes the catalytic subunit of N-acetylglucosamine transferase involved in the early synthesis of glycosylphosphatidylinositol [216, 217], which binds protein markers on the surface of homopoietic cells of humans and laboratory mammals (for example, the product of the CD59 gene) [218]. Of all the genes associated with glycosylphosphatidylinositol, the Pig-a gene only is located on the X chromosome [216]. Accordingly, the phenotype characterized by the absence of glycosylphosphatidylinositol will be informative in terms of the presence of mutations at the level of the coding region of the Pig-a gene. In [219], when studying the genotoxic potential of twenty-four chemicals, it was experimentally proved that the Pig-a mutation detection test system is more sensitive when used as indicator cells of reticulocytes. The Pig-a test system with rat peripheral blood cells, in terms of sensitivity to mutagen detection, was significantly inferior to the reticulocyte system [219]. In order to study chemotherapy and radiation therapy schemes in cancer patients, scientific papers [220, 221] consider the possibility of expanding the Pig-a test system in rodents to Pig-A using human erythrocytes.

Scientific papers [222, 223] highlight the issues of assessing the genotoxicity of environmental factors using a promising new model that uses the method of micronucleus analysis with fertilized chicken eggs and erythrocytes. The main advantages of the method include the ability to assess genotoxic effects at the *in vitro* model level, taking into account the parameters of ADME, which are decisive from the point of view of the bioavailability of the chemical compound and associated with its adsorption, distribution, metabolism, release and toxicity. Thus, in accordance with the basic principles of the "3R" concept [168, 169], it becomes possible to obtain an estimate of the genotoxic potential of a certain environmental factor without the additional use of *in vivo* test systems.

In response to the exponential increase in the amount of genotoxic chemicals produced by humanity, the scientific community is becoming more active in finding new approaches to assessing the genetic safety of environmental factors. A significant paradigm shift in genotoxicity testing was observed after the introduction of modern methods for biological sequencing. The development of modern next-generation sequencing (NGS) technologies, followed by the development of a new technology (ecNGS) that allows correcting errors in obtaining reads of DNA fragments, has demonstrated rather good results in detecting somatic mutations induced by environmental factors that have a rather low frequency of occurrence. The scientific paper [224] highlights the basic principles of duplex consensus sequencing, which allows assessing the mutational potential of xenobiotic effects on the human genetic apparatus. The technique makes it possible to identify sequencing artifacts derived from a library preparation at the amplification stage, by comparing the frequency of occurrence of nucleotides at a certain position of a large number of copies of DNA fragments. Mutations caused by environmental factors, according to the consensus duplex sequencing method, will be present in most amplified DNA fragments [224, 225]. The advantage of this method is obtaining information about the genotoxic potential of xenobiotics with a certain localization of damage at the DNA level and their qualitative characteristics. Next-generation sequencing technology based on the approach that allows identifying misread nucleotides provides detailed characterization of induced damage to genetic material at the single nucleotide level, which provides completely new opportunities for solving the problem of complex assessment of mutagenic effects of environmental factors, taking into account the dose-dependent genetic effect [226, 227].

The classical scheme for assessing the genotoxic potential of environmental factors involves the use of a standard battery of *in vitro* and *in vivo* test systems, which have significant disadvantages in terms of time and cost of experimental studies [130, 228, 229]. Furthermore, according to the basic principles of the "3R" concept, it is necessary to reduce the number of studies *with* laboratory animals. In the context of an increase in the number of chemicals that can exhibit genotoxic properties, scientists pay special attention to *in silico* methods that can act as alternative approaches for genetic assessment of environmental factors. The approval of the scientific guideline "ICH M7 Assessment and control of DNA reactive (mutagenic) impurities in pharmaceuticals to limit potential carcinogenic risk" is a defining event that has stimulated the implementation of modern *in silico* models used for obtaining an objective assessment of the mutagenic activity of environmental factors [3, 87, 105, 230] and toxic effects that can be induced by xenobiotics [231, 232]. Computational Toxicology using Qsar (Quantitative Structure-Activity Relationship) *in silico* predictive models in combination with machine learning algorithms and apparatus of mathematical statistics allow us to obtain information about the mutagenic

potential, even in a situation where there are no experimental data on genotoxicity for a particular chemical compound [38]. The use of QSAR *in silico* models are a promising approach for solving regression and binary classification problems for a set of chemicals with unknown genotoxic and toxic properties. The predictive power of such models is based on a set of molecular descriptors that represent the physicochemical, spatial, structural, and electronic properties of a particular xenobiotic under study [111, 233]. The need for research using QSAR models for Computational Toxicology problems is evident in recently published studies [233–236].

Conclusions

In the context of global industrialisation and urbanisation, there is a significant increase in the number of xenobiotics that can be potential environmental pollutants. For a large number of such chemicals, there is no genotoxic assessment, which creates significant obstacles to the study of complex processes associated with the development of hereditary and oncological diseases. Today, the problem of effective identification and consideration of various factors of genetic and carcinogenic danger needs to be solved. Standard toxicology paradigm for conducting genotoxicity testing using a classical battery of *in vitro* and *in vivo* test systems accepted by the scientific community need to update and expand the list of effective and more productive methods, especially taking into account the "3R" concept, which is guided by principles aimed at reducing, improving and replacing animal models in genotoxicity tests. But despite attempts around the world to reduce the number of tests *in vivo* on animals, unfortunately, to date, *in vitro* test systems do not provide complete information about the genotoxic potential, taking into account the three endpoints of DNA damage.

The problems of modern toxicology can be solved through the integration of sciences which were formed and developed in the end of the 20th century. In this context, achievements in bioinformatics and computer science deserve attention. Taking advantage of modern computational QSAR models of toxicology in combination with machine learning algorithms and highly productive next-generation sequencing technologies can be considered as the main vector of development of modern computational toxicology. When forming a new concept of testing for genotoxicity, it is necessary to pay attention not only to solving the problem of binary clysification for potential genotoxic chemical compounds, but also to take into consideration the dose-dependent effect of xenobiotics on the human hereditary apparatus.

Interests disclosure

The authors declare no conflict of interest prior to disclosure.

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СИСТЕМИ ГЕНЕТИЧНОЇ ОЦІНКИ ВПЛИВУ ФАКТОРІВ НАВКОЛИШНЬОГО СЕРЕДОВИЩА

Однією з важливих складових частин охорони довкілля є розроблення гігієнічних нормативів, що дають змогу захистити популяцію людини від несприятливого впливу забруднення природи шкідливими речовинами. Європейським і американським хімічними товариствами були представлені приблизно 800 тис. хімічних речовин, для яких на сьогодні відсутня інформація щодо потенційних ризиків для генетичної складової здоров'я людини та негативного впливу на довкілля. В умовах експоненційного збільшення хімічних сполук, що генерує людство в різних сферах виробництва, особливо актуальною є проблема ефективного виявлення та обліку різноманітних факторів генетичної та канцерогенної небезпеки. Оцінка потенційної генотоксичності факторів навколишнього середовища є невід'ємною складовою оцінки генетичної безпеки з урахуванням як прокаріотичних, так і еукаріотичних організмів включно з людиною. Оцінка генетичної активності хімічних сполук є базовою вимогою для проведення їх всебічної токсикологічної оцінки. У представленому огляді, з точки зору генетичних та епігенетичних механізмів впливу, розглянуто стандартні методи виявлення й оцінки потенціальної генетичної небезпеки факторів довікілля, що відносяться до стандартної, загальноприйнятої батареї тест-систем, а також деякі сучасні експериментальні методи, що не є на сьогодні масово визнаними. Проведено детальний аналіз підходів щодо оцінки потенційної генетичної мутагенної активності. Показані їхні основні переваги та недоліки. З урахуванням рекомендацій Організації економічного співробітництва та розвитку щодо проведення тестування небезпечних хімічних сполук, які можуть впливати на здоров'я людини, зроблено спробу пошуку оптимальних підходів для вирішення задачі прогнозування генетичних ефектів та їхніх наслідків для людини.

Ключові слова: геном; пошкодження дезоксирибонуклеїнової кислоти; генотоксичність; канцерогенез; мутагенез; тест-система мутацій.