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Genetic toxicology and genetically active environmental factors

Abstract: Anthropogenic impact on the environment, including, but not limited to water and soil contamination with oil and xenobiotics, causes cytotoxic, mutagenic and carcinogenic effects, and nowadays is considered the main negative factor of genetic alterations in living populations. In examinations of mutation processes in populations, inhabiting at ecologically unfavorable areas, high frequency of dominant mutations that alter normal growth inside uterus, causes development glitches in newborns, or even stillbirth. Such data is conditioned by direct dependence between the intensity of environmental pollution and impairment of ecologically effected genetic situation. Assessment of genotoxicity can be provided through specific tests, indicating degree of mutagenic effect and probability of its manifestation. Nevertheless, even living organisms, such as plants, suffering from environmental pollution, can produce chemical compounds that induce genotoxicity. Medicinal substances isolated from these plants are not exception as well. An attempt to provide analysis of genotoxicity implementation and importance of investigations performed in this field is presented in this paper.

Key words: genotoxicity, mutagenic effect, carcinogenicity, chemical compounds, negative factors, regulatory documents.

Introduction

Genetic toxicology is the scientific discipline dealing with mutagenic effects of chemical, physical and biological agents, resulting in DNA damage. Progress in this field of science comes in close connection with the development of various techniques for visualization of genetic material impairment (eg. the Ames assay, Comet assay, or single cell gel electrophoresis, and micronucleus assay), mechanisms laying in the basis of those (eg. chromosome aberrations and single nucleotide polymorphisms) and means for repair of such lesions (eg. photoreactivation, base and nucleotide excision repair) in cells of eukaryotic and prokaryotic organisms.

From the ecological point of view, one should pay close attention to the previous studies, related to the influence of toxicological agents on physiology and life span of ecosystem participants, such as animals and plants. For instance, when analyzing the data from the study of oil pollution on plant morphology and cytogenetic characteristics we can detect the accumulation of strong mutagens and carcinogens in plants as clear indicator of the negative impact of oil. A genotoxic agent may cause DNA and chromosome

damage. Such alteration in a germ cell may further lead to an inheritable mutated trait, affecting genotype of all individuals in a given population. On the other hand, DNA damage in somatic cells is one of the main factors that trigger malignance and cancer. Some carcinogens and mutagens go through metabolic pathway for activation until reactive species that can interact with DNA forming DNA adducts are detected in cells and tissues by different techniques. Such methods include micronucleus tests, Ames test, biotransformation, dominant lethal tests, reverse mutation assay, sister chromatid exchange test, specific locus test and others. These assays play an important role in predicting potential of compounds to cause genotoxicity and carcinogenicity, as well as revealing the nature and effect of damage. Cytogenetic methods, such as anaphase-telophase chromosome aberration assay, were developed for rapid screening of chemicals and environmental samples (water, soil, air, and waste). Conventional cytogenetics using regular chromosome analysis remains a simple and popular technique for visualization of the human karyotype. Implementation of cytogenetic analyses, at least at diagnosis, is mandatory for analyzing the outcomes of many clinical trials, and it can also be

used to stratify patients for different types of therapy. However, we have molecular methods of damaged DNA recovery as well; to include: direct repair, base excision repair, nucleotide excision repair, mismatch repair, single/double strand break repair. If talking about other related areas, we may take as an example nuclear medicine using ionizing radiation as an important clinical tool for both medical diagnosis and therapy. The use of radiopharmaceuticals in diagnostic imaging has brought a significant contribution to the field of health sciences [1].

Development of genetic toxicology as science

Even before the biochemical bases of heredity were understood, the field of genetic toxicology began its development with the early investigators observing the possibility of heritable mutations due to the action of physical and chemical agents. Muller was the first to report the role of radiation in producing heritable changes in a living organism, while Auerbach was the first to report the ability of chemicals to cause mutations. Based on these early investigations of induced alterations in genetically heritable traits the field of science was created nowadays known as genetic toxicology. Genetic toxicology testing is required for all classes of chemicals and drugs in order to reveal their pharmacodynamics and pharmacokinetic effect, negative side effects, analyzing the probability of positive result during treatment. Since the 1980s, there has been an increase in our knowledge of the mechanisms leading to genetic toxicity as well as in our experience with the use of the tests on genetic toxicity. Our interpretation of test results has evolved, comprising our identification of the critical steps, strengths and weaknesses of the different tests. Moreover, it has become clear that tests detecting the types of genetic damage, which can be transmitted (gene mutations, structural chromosome damage and numerical chromosomal abnormalities) in mammalian cells, should be considered as the most relevant for the evaluation of the mutation inducing potential of certain chemicals.

Genetic toxicology for many years has explored the mechanisms of heredity with tools applied to study the nucleic acids structure, DNA repair and recombination, the role of mutation at the individual level. The study of mutagenesis has proved significantly important in many areas including environmental monitoring with notable ecological aspects. This field involves studies of air, water, soil and sediments pollution as result of industry development leading to accumulation of mutagenic and carcinogenic substances in cells of living organisms. In the

final stage, we have substantially altered genotype, manifesting mutation in phenotypical characteristics. Different test systems include high diversity of both eukaryotes and prokaryotes, as well as bacteriophages, viruses and mammalian cells in culture. Endpoints that have been used to measure genotoxicity comprise DNA adducts, DNA strand breakage, changes in chromosome number or structure, DNA repair, and cell transformation to malignant phenotypes. The rapidly increasing number of researchers and amount of published material in genetic toxicology through the 1960s led to the formation of professional societies (to name a few Genetic Toxicology Association, US Environmental Protection Agency, Organization for Economic Cooperation and Development, European Environmental Mutagen Society, Mutagenicity and Experimental Pathology Society of Australasia, Genotoxicity and Environmental Mutagen Association as well as several European organizations for the development of alternative genetic toxicology methods) and information resources focused on genetic toxicology.

Application of computational toxicology to safety testing within a regulatory setting and *in silico* genotoxicity screening approaches are some of the current means for reducing the need for animal testing and human clinical trials. Computer modeling, molecular biology systems and/or adverse outcome pathway approaches can provide more accurate toxicity predictions, whether high-content study data, pluripotent stem cells or new scientific disciplines, such as epigenetics and adductomics, could be integrated into the risk assessment process. With close collaboration between industry, academia and regulators next generation predictive models and high-content screening have the potential to transform genetic toxicology testing in the 21st century [2].

One of the recent major events with more than 160 sessions took place in San Antonio, TX, USA in March 2018 with the topics ranging from ecotoxicology and exposure assessment to epidemiology and human population evaluation, and from immunotoxicity to pesticide neurotoxicology (<http://www.toxicology.org/events/am/AM2018>).

High spectrum of the negative factors

International agency for research on cancer estimated that more than 90% of classified chemical compounds may nowadays be considered as carcinogenic as they simultaneously induce tumors at multiple sites in rodent species. Modern genotoxicity studies allow simple, rapid, and inexpensive risk identification via assessing genetic lesion caused by

chemical induction. Important step that must be followed include reliable and accurate measuring of previously existed and newly appeared chemicals for toxicological and mutagenic properties, genetic potential more efficiently, cost-effectively, and with lesser reliance on animal models. Computational prediction of genotoxicity and carcinogenicity on the base of physicochemical nature, biological aspects has proven of value, while using in the framework of research, also may be applied to chemicals that are not currently synthesized. The Ames bacterial mutagenicity assay has stood the test of time and has gained strong consensus as the assay of choice for prediction of mutagenicity and carcinogenicity. The assay detects 90% of known human carcinogens, most of which are trans-species rodent carcinogens [3]. Among other well-known human carcinogens are benzene and its principal metabolites, phenol, catechol and hydroquinone. Chromosomal aberrations in cultured cells are triggered by catechol, lesser by hydroquinone, and to a marginal extent by phenol at concentration of only 100 μ M. Aneuploidy in the near diploid range of cells is significantly induced by benzene and catechol [4].

Risk factors affecting background rates of micronuclei and chromosomal aberration formation include both endogenous factors and those due to methodological variation were evaluated. A number of host risk factors, namely age, gender, smoking habit, folate, vitamin B and hormonal status need to be identified for assessing probability of their impact on background levels of genotoxicity biomarkers. Evaluation of these factors has to be considered in genotoxicity biomonitoring studies, as well as weak or insufficient evidence including alcohol consumption, disease conditions and infections, physical exercise, body mass index and genotype [5]. Some negative factors may influence the authenticity of data resulted in research. Negative factors, as reactive oxygen species, ultraviolet and ionizing radiation, nucleoside analogues, topoisomerase inhibitors, protein synthesis inhibitors and others may contribute to the false or skewed results, even with different defense mechanisms present in animals, and are one of the biggest issues in animal testing.

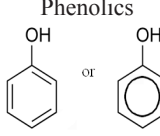
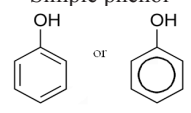
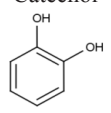
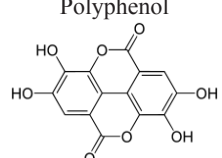
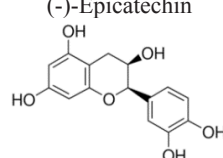
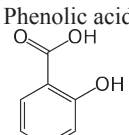
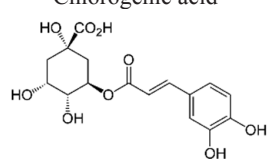
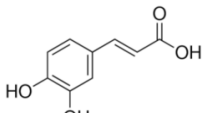
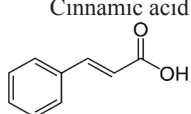
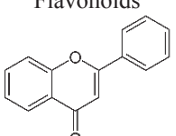
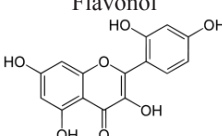
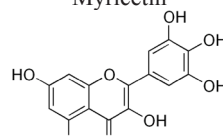
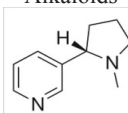
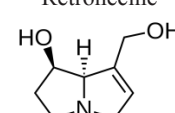
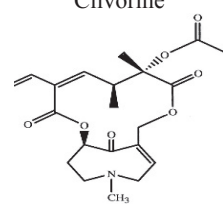
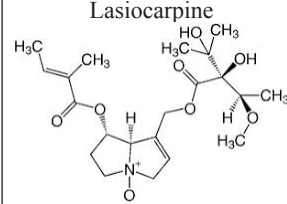
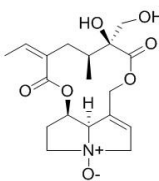
There is a vast variety of phytochemicals, known as secondary plant metabolites, which possess different biological activities, such as antioxidant, antimicrobial effects, modulation of hormone metabolism and detoxification enzymes, stimulation of the immune system, decrease in platelet aggregation and anticancer properties. Phytochemicals are non-essential nutrients; nevertheless, they have ability to prevent or fight against some common diseases.

Many of these benefits suggest a possible role of phytochemicals in prevention and treatment of diseases. Secondary constituents are the remaining plant chemicals, such as alkaloids, terpenes, flavonoids, lignans, plant steroids, curcumines, saponins, phenolics, flavonoids and glucosides. Phytochemicals may reduce the risk of coronary heart disease by preventing the oxidation of low density lipoprotein, cholesterol, reducing its synthesis and absorption, normalizing blood pressure and clotting, and improving arterial elasticity [6]. They may detoxify substances that cause cancer. They appear to neutralize free radicals, inhibit enzymes that activate carcinogens, and activate enzymes that detoxify carcinogens. Among other physiological activities of such biologically active compounds are alkylation, used for construction of carbon skeleton as well as for protection of functional groups, and intercalation, insertion between flatness of DNA nitrogen bases, alternating its structure.

As can be seen from the Table 1 most of the mentioned phytochemicals, such as chlorogenic acid, isatin, caffeic acid have genotoxic effects and cause DNA damage.

When providing examination of genotoxicity, various specific screening methods must be applied, such for instance as electrochemiluminescent arrays aimed at sensing DNA damage to identify genotoxic chemistry related to reactive metabolites [16]. These arrays feature DNA/enzyme films that form reactive metabolites of test chemicals that can subsequently react with DNA, thus enabling prediction of genotoxic chemical reactions. They are used for determining chemical toxicity of new drug that is why good in preclinical researches. Yeast DNA repair reporter, also GreenScreen assay is cost-effective method, developed to perform pre-regulatory screening. It provides a higher throughput and a lower compound consumption than existing eukaryotic genotoxicity assays and is sensitive to a broad spectrum of mutagens and, importantly, clastogens. One more technique, named ToxTracker assay is a mechanism based on mouse embryonic stem cells that uses GFP-tagged biomarkers for detection of DNA damage, oxidative stress and general cellular stress upon exposure. It identifies dangerous properties and mechanisms of elements, such as metal oxides, silver nanoparticles, and non-metallic materials (diesel, carbon nanotubes and quartz). BlueScreen™ HC is a precise and rapid *in vitro* human cell-based assay, estimating genotoxicity and cytotoxicity of compounds and mixtures. This method detects substances that can cause damage in genetic material, especially DNA [17].

Table 1 – Classification of biologically active substances and their mutagenic activity *in vitro*

Class	Subclass	Name	Genotoxicity
Phenolics 	Simple phenol 	Catechol 	Not mutagenic in the Ames test, but is co-mutagen with benzopyrene. Mutagenic in comet assay on human lymphocytes [7].
	Polyphenol 	(-)-Epicatechin 	(-)-Epicatechin significantly diminished the oxidative DNA damage induced by etoposide, in comparison to etoposide alone. It effectively protected bone marrow cells of rats against oxidative DNA damage induced by etoposide [8].
	Phenolic acid 	Chlorogenic acid 	Both chlorogenic acid and caffeic acid induced single strand breaks in DNA in acellular test systems that favored formation of oxygen radicals, particularly in the presence of transition metals (co-mutagens). Not mutagenic in standard bacterial mutagenicity assays [9].
		Caffeic acid 	
		Cinnamic acid 	
	Flavonoids 	Flavonol 	Myricetin 
Alkaloids  Alkaloid: Nicotine	Retronecine 	Clivorine 	Mutagenic, (±)-6, 7-dihydro-7-hydroxy-1-hydroxymethyl-5H-pyrrolizine (DHP)-derived DNA adducts were formed <i>in vitro</i> [13].
		Lasiocarpine 	DHP-derived DNA adducts were formed [14].
		Isatidin 	<i>In vitro</i> comet assay detected DNA strand break [15].

Genetic toxicology, old and new: *in vivo* vs. *in vitro*

Many *in vitro* and *in vivo* tests for genotoxicity have been developed that, with a range of endpoints, detect DNA damage or its biological consequences in prokaryotic (e.g. bacterial) or eukaryotic (e.g. mammalian, avian or yeast) cells.

During the process of using *in vitro* genotoxicity testing it is necessary to include tests in both bacterial and mammalian cells, and be able to detect gene mutations, chromosome damage and aneuploidy. This method may be conducted via combination of the Ames test and the *in vitro* micronucleus test, consequently in the result observing both chromosomal aberrations and aneuploidy [18]. There have been a number of recent advances in the area of genetic toxicity testing that would reduce animal usage and still provide the necessary information for an assessment of the genotoxic potential of substances. Certain genotoxicity studies, including the micronucleus and comet assays, can be effectively incorporated into routine toxicology

studies. The integration of the cytogenetic tests into repeated dose toxicity studies can be used to satisfy the *in vivo* cytogenetic data requirement. The evaluation of micronuclei in peripheral blood or bone marrow cells covers the evaluation of structural and numerical chromosomal aberrations. The integration of the mammalian bone marrow and the rodent erythrocyte micronucleus assays is technically feasible and is a scientifically acceptable alternative to conducting independent *in vivo* cytogenetic assays. The assessment of genotoxicity represents an essential component of the safety assessment of all types of substances. Several *in vitro* tests are available at different stages of development and acceptance, yet they are not considered at present sufficient to fully replace animal tests needed to evaluate the safety of substances. For an overall improvement of the traditional genotoxicity testing paradigm, several recent activities have taken place. These include the improvement of existing tests, the development of novel tests, as well as the establishment and exploration of approaches to optimize *in vitro* testing accuracy.

Table 2 – *In vitro* assays with *in vivo* follow-up studies measuring comparable endpoints [19]

Basic Test	<i>In vitro</i>	<i>In vivo</i>
Gene mutation endpoint	Ames (or other bacterial) Mouse lymphoma Chinese hamster ovary (hypoxanthine-guanine phosphoribosyltransferase test) Yeast forward or reverse mutation Direct chemical/DNA interaction	Mouse somatic cell coat color (spot) assay <i>Drosophila melanogaster</i> sex-linked recessive lethal test Mouse specific-locus assay or suitable dominant mutation assay (germ cell) Clastogenicity
Chromosome aberration endpoint	<i>In vitro</i> cytogenetic analysis in various cell lines Chromosomal aberrations Micronucleus test Sister chromatid exchange test	Rodent micronucleus Rodent bone marrow metaphase analysis Dominant lethal assay (germ cell) Heritable translocation (germ cell) Germ cell chromosome aberrations
Techniques for identifying genotoxic chemicals	Bromodeoxyuridine (or other) is injected prior to metaphase arrest; sister chromatids exchange is evaluated in M2 cells Cell culture treated with ³ H-thymidine (or other radioisotope) is evaluated by auto radiographic method Cell culture is placed into selective and non-selective (or other medium); further stimulation by growth factors is provided	Natural metaphase stop and assessing of chromosomal lesions Exposing of animals <i>in vivo</i> , perfusing target organs (liver) and collecting cells (hepatocytes) Collecting material by surgical methods; fixing and staining for microscopic examination For each substance tested in <i>in vivo</i> studies it is recommended performing homogeneity and stability testing including analytical method validation/evaluation in the vehicle used
Mammalian cell assay	Lymphoma assay Chinese hamster ovary mutation assay Unscheduled DNA synthesis assay Chromosome aberrations Sister chromosome exchange Cell transformation	Dominant lethal assay Cytogenetic analysis Micronucleus assay Heritable translocation assay Specific locus assay DNA adduct formation

Continuation of table 2

Basic Test	<i>In vitro</i>	<i>In vivo</i>
Most frequently used systems	Metabolically proficient mammalian cell systems	Mammalian models (eg. rats, mice, zebra fish, Chinese hamster)
Most frequently used methods	<i>In vitro</i> : cytogenetic evaluation of chromosomal damage with mammalian cells or mouse lymphoma assay <i>In vitro</i> adduct formation <i>In vitro</i> cell transformation	<i>In vivo</i> test for chromosomal damage using rodent hematopoietic cells DNA binding in selected target organs using radiolabeled chemical or ³² P-postlabeling Liver focus assay in rats

As can be seen from the Table 2 differences between *in vivo* and *in vitro* assays mostly include objects of investigations, scheme of experiment and screening methods. Dose-response modeling to relate the concentrations at which effects can be seen in the *in vitro* assays to anticipated human exposures requires the development of computational systems that model the molecular signals underlying genotoxicity pathways. In order to choose the most effective toxicity prediction model it is necessary to understand its strengths, limitations, scope of application and interpretation and customize these methods for each problem if necessary. It is however possible to follow those factors only if the data and processes to develop the model are transparent, applicability domains are well defined, the outputs

of the models are clearly explained, and models are simplified. One of the promising examples is the Tox-21c stresses replacing animal testing with human-relevant testing methods, either *in vitro* or *in silico*. With the increasing number and variety of alternative testing methods, it is necessary to apply strategies to intelligently combine and use this information for toxicity assessment and decision-making.

In perspective, computational methods are likely to expand to include models for special and new types of toxicity endpoints and chemicals, provide insight into toxicological pathways, combine and compare results from different models, customize models to meet users' expectations, and refine models as new data becomes available [20].

Table 3 – Genotoxicity testings *in vivo* in OECD Principles on Good Laboratory Practice*

Assay	End point	Guidelines OECD
Mammalian erythrocyte micronucleus test	Determination of chromosomal damages induced by testing chemicals, or erythroblasts mitotic apparatus due to formation of micronuclei in erythrocytes of bone marrow and peripheral vessels.	Test No. 474
Mammalian bone marrow chromosomal aberration test	Determination of chromosomal aberrations, induced by testing chemicals in cells of animal bone marrow	Test No. 475
Rodent dominant lethal test	Detecting of chromosomal aberrations in sexual cells due to number of implants and mortality of embryos in pregnant females	Test No. 478
Mammalian spermatogonial chromosomal aberration test	Determination of structural chromosomal aberrations in dividing spermatogonial epithelia of mammals	Test No. 483
Genetic toxicology: mouse spot test	Detecting of chemical impact on target cells in developing embryo, precisely melanoblasts by using mice special lines. Measuring is provided due to frequency of colored spots formation in wool.	Test No. 484
Genetic toxicology, mouse heritable translocation assay	Determination of translocation activity due to embryonic mortality and cytological aberration analysis in the stage of diakinesis (metaphase I) in primary spermatocytes.	Test No. 485
Unscheduled DNA synthesis test with mammalian liver cells <i>in vivo</i>	Measuring of labelled thymidine introducing during DNA synthesis (S phase).	Test No. 486
<i>In vivo</i> mammalian alkaline comet assay	Identification of DNA damage using electrophoresis in alkaline pH and recording of migrating DNA "tails" length.	Test No. 489
*Based on: OECD Guidelines for the Testing of Chemicals, Section 4: Health Effects, 2014-2016 (http://www.oecd-ilibrary.org/environment/oecd-guidelines-for-the-testing-of-chemicals-section-4-health-effects_20745788)		

As can be seen from the Table 3, laboratory animals are widely used in study of chemicals genotoxicity using different methods. However, not all of given tests are used in assessment of medicinal phytochemicals. According to recommendations of ICH Topic S2B “Genotoxicity: a standard battery for genotoxicity testing of pharmaceutical” guide, standards battery from three tests, including the Ames assay, *in vitro* test assessing chromosomal aberrations and *in vivo* test of bone marrow cells and erythrocytes of mammals is used. If the results from these three methods are reliable and negative, then chemical is considered as non-mutagenic. In the cases, when even one test gives positive result, then wide researches will be provided, for example dominant lethal mutation test on rodents [21]. Often there is a difficulty in assessment of the genotoxicity of many natural compounds. Some flavonoids become mutagens after metabolic transformations or depending on concentration or dose like when testing *Quercus sideroxylla* plant extract containing polyphenols [22]. Controversial data were obtained when testing catechins from green tea, when positive results were obtained in the test of chromosomal aberrations *in vitro* and negative – *in vivo* [23].

Such differences in genotoxicity testing of some flavonoids and polyphenols related with molecular structure, as well as influence of biological system features. Flavonoids and polyphenols are double- and triple-bounded compounds. They easily interact with various reactive oxygen forms and with radicals. At the same time, they are converted into more stable and less active form than the radical, what trigger their transformation into pro-oxidants [24]. As a result, they exhibit antimicrobial activity – inhibit the electron transport chain, synthesis of nucleic acids or damage bacterial DNA; consequently, it explains the genotoxicity of many flavonoids and polyphenols. With positive results of testing for genotoxicity, it is necessary to include additional research on animals. From our opinion, this may be *in vivo* test of mammalian alkaline comet assay, rather than a rodent dominant lethal test. This choice is explained by cell genetic apparatus damage mechanism and by the method of detecting DNA strands disruption, which is observed under the flavonoids activity [25].

Regulatory issues

Genotoxicity investigations are controlled by regulatory documents. Those include: Interstate standard (2013), Rules of Registration and Examination of drug plants for medical application (Module 4,

section 4.2.3.3 Genotoxicity), Uniform Requirements for general characteristics of drugs for medical applications (2015), Organization for Economic Cooperation and Development Principles on Good Laboratory Practice and others (1998) and others. Interstate standard is valid in countries: Belarus, Kazakhstan, Kyrgyzstan, Russia, Tajikistan, and Uzbekistan since 2013, comprising standards on genotoxicity, carcinogenicity studies and toxic effects on reproductive system (Part 3). Most of such documents reveal potential dangerous, taking into account influence of such factors, like degree of impact, mechanical and physical aspects.

The Organization for Economic Cooperation and Development is an intergovernmental organization in which representatives from 29 industrialized countries in North America, Europe and the Pacific, as well as the European Commission meet to coordinate and harmonize policies, discuss issues of mutual concern, and work together to respond to international problems. Regulatory papers issued by them contain principles of the Good Laboratory Practice (GLP) that should be applied to the non-clinical safety testing of test items contained in pharmaceutical products, pesticide products, cosmetic products, veterinary drugs as well as food additives, feed additives, and industrial chemicals in the laboratory, greenhouses and in the field. These test items are frequently synthetic chemicals, but may be of natural or biological origin and, in some circumstances, may be living organisms. The purpose of testing these items is to obtain data on their properties and/or their safety with respect to human health and/or the environment. GLP is a quality system concerned with the organizational process and the conditions under which non-clinical health and environmental safety studies are planned, performed, monitored, recorded, archived and reported, in Kazakhstan basic rules were adopted in 2006 (Standard 1613-2006, approved by the Order No. 392 of the Ministry of Healthcare and Social Development of the Republic of Kazakhstan from May 27, 2015).

Education and innovations

International in scope, with contributions from over 30 countries Information Resources in Toxicology (Academic Press, 2009) with Philip Wexler as Chief-Editor mentions MEDLINE/PubMed® (Entrez) and the NLM Gateway with eChemPortal and TOXNET among the most widely used Internet-based resources on genotoxicology. Initiatives such as the “Human Toxome Project” (humantoxome.

com) which aims to map the “Pathways of Toxicity” in man illustrate a trend that moves away from our current reliance on high-dose animal toxicity studies to a wide range of new tools such as functional genomics, proteomics, metabolomics, high data content screening, pharmacokinetic modeling, and systems biology to study the effects of chemicals on cells, tissues, and organisms in a rapid and cost-efficient manner. These technologies are also paving the way to improve the evaluation of health risks posed by chemicals found at low levels in the environment. These advances have led to a new sub-discipline of toxicology: “toxicogenomics”, which may be defined as “the study of the relationship between the structure and activity of the genome (the cellular complement of genes) and the adverse biological effects of exogenous agents”. This broad definition encompasses most of the variations in the current usage of this term, and in its broadest sense includes studies of the cellular products controlled by the genome (messenger RNAs, proteins, metabolites, etc.).

The new “global” methods of measuring families of cellular molecules, such as RNA, proteins, and intermediary metabolites have been termed “-omic” technologies, based on their ability to characterize all, or most, members of a family of molecules in a single analysis. With these new tools, we can now obtain complete assessments of the functional activity of biochemical pathways, and of the structural genetic (sequence) differences among individuals and species, that were previously unattainable. These powerful new methods of high-throughput and multi-endpoint analysis include gene expression arrays that will soon permit the simultaneous measurement of the expression of all human genes on a single “chip”.

Although nucleic acid microarray technologies have received much attention recently, other powerful new tools for global analysis of cellular constituents are already available and will also have a major impact on the field of toxicology. These include technologies for global analysis of proteins and peptides (proteomics), and of cellular metabolites (metabonomics). Among these advances are improvements in classical 2-dimensional gel electrophoresis, the introduction of multidimensional liquid chromatography, tandem mass spectrometry, and database searching technologies, and improved mass spectroscopic identification of protein sequences.

Many companies that employ toxicologists (such as pharmaceutical, chemical, food and automotive companies) provide postdoctoral training opportunities for individuals with doctoral degrees in toxicology or related disciplines. For instance, Col-

gate-Palmolive Postdoctoral Fellowship is directed specifically toward innovations in toxicology methodology involving alternatives to whole animal use in testing (<https://researchfunding.duke.edu/colgate-palmolive-postdoctoral-fellowship-award-vitro-toxicology>).

Fields of genotoxicity in Kazakhstan

Most of investigations concerning genotoxicity in Kazakhstan are related to either ecological issues or medical aspect, including, but not limited to the health effects of radon and uranium on the population of Kazakhstan [26], apoptotic and genotoxic effects of low-intensity ultrasound on healthy and leukemic human peripheral mononuclear blood cells [27], genotoxicity evaluation of drinking water and rates of population morbidity in the Northern Kazakhstan region [28], mutagenic effect of the rocket fuel component asymmetric dimethylhydrazine on rats of various ages [29], Glycophorin A somatic cell mutations in a population living in the proximity of the Semipalatinsk nuclear testing site [30].

One of the major factors affecting the environment and human health is the problem of contamination by radioactive elements. The World Health Organization has identified the chronic residential exposure to radon and its decay products as the second cause of lung cancer in healthy non-smokers. Enhanced levels of radon are observed in the Northern and Eastern regions of Kazakhstan due to the natural radiation sources and the long-term and large-scale mining of uranium that is why this direction is still important [26].

The Semipalatinsk nuclear testing site was the primary nuclear testing site for the Soviet Union. The prize for successful development of nuclear bomb was its terrible impact on local population, suffering in the result from numerous types of genetic anomalies and mutagenic sicknesses. The work published by the Radiation Research Society on somatic cell mutations in a population living in the proximity of the Semipalatinsk nuclear testing site and other related to this event investigations are considered as one of the most important of genotoxicity related studies in the history of Kazakhstan. Here Glycophorin A somatic mutation assay was performed to evaluate the magnitude of exposure to ionizing radiation among the human population living in the nearest areas to the nuclear testing site [30].

In the case of genotoxicity evaluation of drinking water and rates of population morbidity in Northern Kazakhstan region, assay of the drinking water in

the regional centers was carried out by a cytogenetic anaphase-telophase method using barley root tips, resulting on the water's compatibility to cause toxic and mutagenic effects. These effects are mostly congenital abnormalities (because of lesions of intrauterine development), including malignant neoplasms and further developing cancer, gastric and duodenal ulcers [31].

One more mentioned research on apoptotic and genotoxic effects of low-intensity ultrasound on healthy and leukemic human peripheral mononuclear blood cells is more related to medicine. It has been shown that ultrasound has a great potential for therapeutic applications, specifically for induction of apoptosis and cell death in malignant cells and also for drug delivery [32]. Positive for the use of the low-intensity ultrasound for damaging cancerous cells were obtained, but only when healthy cells do not largely undergo to this process. However, considering the long-term effects of ultrasound on DNA in healthy cells, therapeutic application of low-intensity ultrasound requires further experiments and analysis for exploring various ultrasound parameters and experimental conditions, including *in vivo* studies.

One more investigation that will be reviewed here is associated with therapeutics. Chemo-resistance is the main obstacle to the effectiveness of cancer therapies as it allows the cancer cells to survive the treatment and proliferate uncontrollably. Currently, no therapy has an efficacy of 100% since drug resistance limits the potency of both conventional chemotherapeutic and novel biological agents. Chemotherapy kills drug-sensitive cells, but resistant cells survive and become more aggressive and prone to metastasis due to the hypoxic conditions established by the therapy in the neoplastic mass [33]. This research reveals that microRNAs can represent an effective therapeutic strategy for overcoming the obstacle of chemo-resistance to anti-cancer drugs. However, there are still many challenges, such as their stability in body fluids and tissues, and ability to reach the target tissue that is why these problems require further study before microRNAs can be effectively used in humans.

The rapid development of nanotechnology, obtaining of nanomaterials with new, unique properties actualized the problem of their investigation. This problem is topical and is on the agenda of OECD. A special Testing Program of Manufactured Nanomaterials was developed in which the interaction of nanomaterials with DNA was noted as a separate item (<http://www.oecd.org/chemicalsafety/nanosafety/overview-testing-programme-manufactured-nano->

[materials.htm](#)). For instance, sulfur nanoparticles display broad activity against bacteria, fungi as well as insects, parasitizing on skin integuments, intensity of which depends on polymorphism, size and form of sulfur. At the same time, the relatively low toxicity of elemental sulfur for mammalian cells makes sulfur nanoparticles very promising for antimicrobial preparations based on them. There is also data on the antitumor activity of elemental sulfur. However, if the toxicity of precipitated microcrystalline sulfur is well studied, then its nanoform requires in-depth studies. It is known that the structure and arrangement of atoms or molecules in a crystal affect the biological activity of pharmaceutical substances. In addition to the polymorphism of the crystals, the particle sizes also affect the properties of the substance. It is shown that the size of the particles of sulfur, selenium, zinc, copper, and titanium depends on their bioavailability, activity and toxicity, and not. In all cases this dependence is linear. Acute oral toxicity of nanosulfur size of about 75 nm was studied in female's mice. LD₅₀ values were between 300–2000 mg/kg for females in mice. Toxic signs were manifested in the form of depression locomotor activity. The thoracic and abdominal cavities were meticulously examined. At necropsy and histology we revealed flatulence colon, dystrophic changes in the liver and kidneys. Hepatocytes are filled with small and medium-sized lipid droplets. These results indicate that nanosulfur more toxic than powdered sulfur. The micronuclear test showed no mutagenic properties of sulfur nanoparticles. The metabolic activation of sulfur nanoparticles with a microsomal rat liver fraction does not affect toxicity. It is assumed that the mechanism of cytotoxic action might be associated with the interaction of elemental sulfur with sulfhydryl groups of molecules inside the cell previously mentioned in several publications. This investigation was performed within the framework of the program-oriented financing from the Ministry of Education and Science Republic of Kazakhstan for 2015-2017 on the priority direction "Rational use of natural resources, processing of raw materials and products": "Development of new methods for the preparation of sulfur nanoparticles to create different functional appointment technologies" [34-36].

Conclusion

Even before the biochemical bases of heredity were understood, the field of genetic toxicology began its development with early investigators observing the possibility of heritable mutations as con-

sequence of physical and chemical agents' action. Nowadays we know a number of agents, which may result in genomic instabilities and/or epigenetic alterations translated into a variety of diseases. Therefore, finding new effective testing methods to identify and measure the genotoxicity of given agents is quite important. An attempt to provide analysis of genotoxicity studies implementation and importance of investigations performed in this field, as well as diversity of genotoxic agents and testing of their mutagenic and carcinogenic properties in various conditions is presented in this paper.

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