

Intraspecific Sensitivity to Toxicants – a Methodological Problem of Bioassay: Review

Anna Olkova^{1*}

¹ Vyatka State University, Moskovskaya Str. 36, Kirov, 610000, Kirov Region, Russia

* Corresponding author's e-mail: morgan-abend@mail.ru

ABSTRACT

The article discusses the scientific studies that reveal the mechanisms of intraspecific differences in responses of organisms to chemical exposure. The factors of individual sensitivity to toxicants are represented by genetic differences between individuals of the same species and genetically unconditioned factors. The genetic factors are mutations, including under-researched mitochondrial DNA mutations and genomic drift, genetic polymorphism, and gender-related differences. Age, conditions of keeping and state of organisms, seasonal variations of body functions, and biotic interactions are considered as the factors that are not related to the genetic apparatus. The article considers a number of works, in which the effects of the combined action of external factors on sensitivity of organisms in model groups are studied.

Keywords: bioassay, environmental pollution, intraspecific sensitivity of organisms, genetic factors, genetically unconditioned factors

INTRODUCTION

Today, bioassay is a demanded environmentally oriented group of methods for determining the toxic properties of various substances: water of various origins, bottom sediments, soil, waste, new substances and materials, waste and even air [Hansen et al. 2007; Kokkali and Van Delft 2014]. The bioassay methodology is developing next to the needs of industrial activities, environmental protection, pharmacology and other related industries. The problems of complex multicomponent samples bioassay [Terekhova 2011], selection of the most sensitive test-organisms and their informative reactions [Josko and Oleszczuk 2014; Olkova and Berezin 2019], effective combinations of methods based on the principle of a “battery of bioassays” [Slabbert and Venter 1999; Zovko et al. 2015], statistical support of bioassay [Dette and O’Brien 2004] are solved.

Carrying out tests using living organisms entails a number of difficulties and problems that affect the objectivity of the obtained results. Among

the problems of bioassay, a special position is occupied by the standardization of test conditions and test-cultures. Bioassay under tightly controlled conditions using standard test-cultures improves the accuracy and reproducibility of toxicological assessments. There are the following difficulties in bioassay standardization: stock and supply of standard materials, storage stability of the standards, criterion of acceptance [Asano 2006], selection of cultivation water, variety and quality of test-cultures, standardization of test conditions [Olkova et al. 2018; Terekhova et al. 2018]. These issues can be solved through development of international bioassay protocols and introduction of additional criteria for the quality of test-cultures [Olkova 2021]. However, the aspect as the intraspecific sensitivity of organisms to chemical substances and other influences is the most difficult to adjust and it requires careful study of the issue.

Most individuals on the planet have unique properties. Even the microorganisms that multiply by division are capable of acquiring individual genetic differences through mutagenesis. As

the organization of life becomes more complex, individual differences multiply in the genotype, phenotype, and qualities acquired in the process of ontogenesis. This individual uniqueness is the reason for the different sensitivity of individuals of the same species to the toxicants acting on them. When using the bioassay methods, intraspecific differences in individuals lead to discrepancies in responses within the experimental sample, even if the so-called “clean lines” are used. For a reliable assessment of chemical risk, it is critical to study the contribution of uncertainty factors to interspecies and individual differences in the toxic process [Kasteel and Westerink 2021]. In this paper, the goal was to review and analyze the factors that form differences in the intraspecific sensitivity of organisms to chemical stress.

The Figure 1 shows main groups of factors to which this review is devoted.

GENETIC FACTORS

Mutations

The main reason for both interspecific and intraspecific differences in the reactions of organisms to toxic substances is genetic characteristics. Under natural conditions, biological species are represented by many populations. The degree of their isolation contributes to the formation of individual genotypes that do not go beyond the species genome, but distinguish representatives of populations in a number of properties. One

of these properties may be a different reaction of representatives of a species to chemical substances. Sensitivity to toxicants also fluctuates within a single population due to a certain number of mutations that occur in organisms throughout life.

Many chromosomal aberrations and mutations in individual major genes are well studied. They can be lethal or non-lethal, often manifesting in a phenotype, for example, the color of the eyes in the *Drosophila* fly or the color of daphnia. When conducting bioassays, such inhomogeneities of model populations are tried to be eliminated. If mutations are hidden, then the reproducibility of bioassay results begins to decline.

Some studies of mutations, which are more difficult to detect than the chromosomal changes, but significantly affect the sensitivity to toxicants, have appeared recently. Thus, the mitochondrial mutations can be both the cause of different responses of organisms and the consequence of a toxic process. In the experiments with human mitochondrial DNA (mtDNA), it was proven that the population is divided into separate haplogroups according to the susceptibility to chemical substances [Ball et al. 2021]. Such heterogeneity of the population, of course, will be characteristic of most test-cultures from bacteria and protozoa to mammals, since in most organisms, protein synthesis depends on the sequence of genes in mtDNA. For example, when bioassaying Paraquat – non-selective contact herbicide (methyl viologen) on the *Caenorhabditis elegans* nematode, it was shown that the mechanism of acute and chronic toxicity in several generations

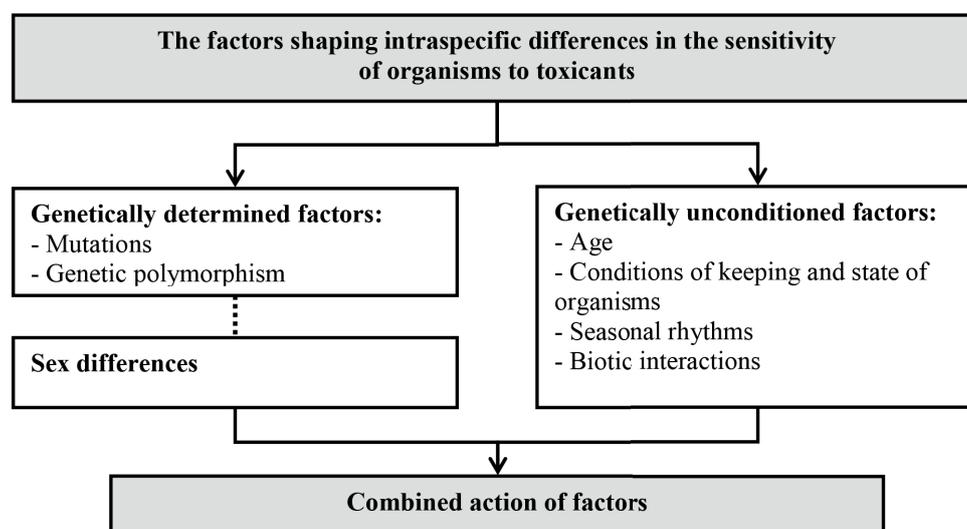


Figure 1. Review design

consisted of the changes in the mitochondrial physiology and mitochondrial mutations of mtDNA [Bora et al. 2021].

Moreover, the genomic drift in cells can differentiate the organisms of the same species according to the degree of their responses to chemical exposure. This new factor contributing to the reproducibility of bioassay results was shown in cell lines used in bioassay in vitro [Gutbier et al. 2018].

Genetic polymorphism

Genetic polymorphism, that is, the existence of two or more sharply differing alleles of the same gene in one population, is a mechanism and the root cause of many interindividual differences in organisms of the same species. For example, the human cytochrome P450 (CYP2D6) gene is known to have high polymorphism, which leads to wide ethnic and interindividual differences in the metabolism of toxicants and drugs [Bernard et al., 2006]. In [Dumont et al., 2020], the authors showed that seven genotypes of the aquatic plant *Myriophyllum spicatum* differed in the sensitivity to copper by a factor of 8.

Moreover, an example of bioassay can be white laboratory mice, as a rule, hybrids of the *Mus musculus domesticus* and *Mus musculus musculus* subspecies. Their populations are among the most commonly used test-systems for detecting and studying various effects, including the differences in the toxicity of substances at the intraspecific level. In the work of Rachel Church et al., using a genetically heterogeneous population of mice, it was shown that modern food additives can pose a potential hazard, the detection of which is complicated by the intraspecific sensitivity to a certain substance of only a part of the individuals in the population. The scientists, studying the effect of green tea extract on the body, showed that most mice tolerated a dose of 50 mg/kg equally well (daily for three days), but a small part of animals (16%) showed pronounced hepatotoxicity with liver necrosis at the level of 10 86.8% [Church et al., 2015].

The discussed facts about different intraspecific sensitivity to toxicants are explained by the fact that expressed stressor genes occur only in a part of the population [Oberholster et al. 2016], and then this leads to different metabolism of toxicants in the genetic lines of organisms [Tewes et al. 2018]. On the one hand, such mechanisms

can be called interfering factors for obtaining representative and reproducible results of bioassay, and on the other hand, the bioassay methods based on the search for genetic biomarkers of chemical stress were proposed [Oberholster et al. 2016]. Moreover, testing new substances and materials using genetic lines of test-organisms with different resistance to chemical attack will help to determine the risks to populations in real ecosystems more accurately.

Gender differences

A special case of genetically determined individual sensitivity to substances involves the gender-related differences. They can be formed due to different hormonal levels, different ratios of fat and water fractions in males and females, specific enzymatic differences inherent in a biological species. The team of authors [Artal et al. 2020] emphasizes the importance of gender accounting in ecotoxicology and bioassay. The scientists conducted a gene-specific analysis, studied the whole genome transcriptional profile of male and female organisms of *Parhyale hawaiiensis* amphipods after the exposure to the AgCl and Ag nanoparticles and showed that males changed the expression of genes related to peptidase and catalytic activity twice more often than females [Artal et al. 2020]. In the study with other amphipods, *Gammarus roeselmi*, the increased resistance of females to toxic stress (Cd) is explained by more efficient detoxification processes in females compared to males [Gismondi, Cossu-Leguille and Beisel 2013].

There are also contrary reports on the greater sensitivity of females compared to males. On the example of rats, it was shown that females are more sensitive than males, their skin is approximately twice more permeable to urea, benzoic acid, and cortisone than the skin of males [Kutsenko 2004]. The females of the *Culex pipiens* mosquito are more sensitive to chlorpyrifos than males [Delnat et al. 2019].

In homomorphic animals, which are often used in bioassay (crustaceans, amphibians, fish), the gender differences in sensitivity to toxicants can be missed due to an endocrine change of gender during the toxic exposure. The fact is that in the animals with homomorphic gender chromosomes, gender is formed by endocrine during the development of the organism. Among the newest xenobiotics, there are compounds that destroy the

endocrine system, which leads to a change in the differentiation of gonads in both males and females of affected animals. It was shown that the animals that are phenotypically identified as one gender, and when genetically determined they belong to the opposite gender appear as a result of such toxic effects [Burke and Henry 1999; Olmstead, Lindberg-Livingston and Degitz 2010]. This property of test-organisms, on the one hand, is used in bioassay of endocrine disruptors [Olmstead, Lindberg-Livingston and Degitz 2010]. On the other hand, there is a likelihood of creating a gender-heterogeneous sample during bioindication or the absence of taking into account such complex endocrine effects during bioassay.

Nevertheless, bioassay using heterosexual organisms of the same species can be promising for predicting the population effects occurring in natural populations.

FACTORS NOT ASSOCIATED WITH GENETIC FEATURES OF INDIVIDUALS

The next group of reasons for the individual sensitivity of organisms is not associated with genetic characteristics. These include age, body weight, the effect of pregnancy (for mammals) or disease, annual and circadian cycles, region of habitat (if test-organisms are removed from the environment immediately before research), nutrition, and etc.

Age

According to the laws of general toxicology, young individuals are more sensitive than adults to many types of exposure, including chemical stress [Bailey, Li and Potter 2016; In Vitro Environmental Toxicology ... 2017]. In the field of bioassay, the sensitivity of juveniles and adults is often compared. For example, juveniles of *D. magna* (less than 24 hours old) are 50% more sensitive than adults (9 days) to the effects of polystyrene microplastic particles [Eltemsah and Bohn 2019].

Immature (unformed) toxicological barriers in juveniles may be the reason for the opposite results when tested on adults and juveniles. Thus, in the studies of the action of aflatoxin B-1 (the fungal toxin aflatoxin B-1) at the dose of 6 mg/kg, the formation of liver tumors of newborn transgenic Big Blue mice (Neonatal Big Blue transgenic

mice) was observed, and in the experiment on adult mice, this dose did not induce tumors [Chen et al. 2010].

The comparison of the sensitivity of young and old individuals is reported less frequently. The reason is that from an ecological point of view, the viability of primarily young and adult individuals is necessary to preserve the population. However, in the field of pharmacological toxicology, such information is certainly important. This information is provided by the bioassays on isolated cell cultures. For example, the specimen doxorubicin and 1-epidoxorubicin, after 3 hours of exposure, showed the greatest cytotoxic effect in bone marrow cells from the donors over 40 years old [Sundman-Engberg, Tidefelt, Paul, 1998]. It is discussed that a decrease in metabolic activity in very young or very old people can increase the chemical toxicity of substances, and age-related diseases of people affect the metabolism of xenobiotics in the liver and their renal excretion, which delays the inactivation of toxicants [Dybing, Soderlund, 1999].

The variability of the bioassay results can be caused by even a slight difference in the age of the test-organisms. For example, in the experiments using small arthropod collembolans [ISO 11267 1998], the difference in the age of individuals in 1 day influenced the final result of bioassay, while the difference in the temperature of the experiment within 1°C did not have a noticeable effect [Crouau and Cazes 2003].

However, not all chemical substances appear to be age- and gender-related. In the original study [Moser and Padilla 2015], 20 commercial human liver samples (ages 11–83) were used as test-systems to study the metabolism and detoxification of organophosphorus and N-methylcarbamate pesticides. The authors found the differences in the action of different substances on liver cells, but, for the most part, these differences did not correlate with age or gender.

In any case, during bioassay, it is necessary to create model groups of test-organisms that are as close as possible in age. This is one of the factors influencing intraspecific sensitivity, which lends itself well to regulation.

The origin of test-cultures and conditions for their further cultivation

The potential for resistance to toxicants among individuals of the same species is often

determined by the habitat region in which the living organisms were initially selected for laboratory maintenance and cultivation. This is apparently due to the different amounts of metabolizing enzymes in individuals of the same species under different living conditions. Such a mechanism for formation of intraspecific differentiation of sensitivity to the hepatotoxicant aflatoxin B1 is shown in [Dohnal, Wu and Kuca 2014] using the example of humans and animals. Similar conclusions were made in the work on the accumulation of dioxins in the human body. The results of a model experiment suggested that the differences in body weight, gastrointestinal absorption, and feeding behavior may partially explain the variations in dioxin concentrations in human tissues and the possible interindividual tendency to accumulate these xenobiotics [Maruyama et al., 2002].

It is likely that the habitat with the entire set of environmental factors leaves an imprint on the genetic characteristics of populations of one species. In this regard, the work [Shrestha et al. 2011], explaining the relationship between evolutionary development, dietary habits and the degree of susceptibility to toxicants, is quite interesting. The authors [Shrestha et al. 2011] prove the hypothesis that the high sensitivity of the domestic cat (*Felis catus*) to phenolic drugs was formed evolutionarily under the long-term exposure of low doses of plant toxicants, which were consumed by cats when eating. This led to the inactivation of the genes responsible for detoxification of hazardous substances.

Laboratory cultivation of organisms increases their sensitivity to toxicants. For example, the sensitivity of *Rhepoxynius abronius* and *Eohaustorius estuaries* amphipods, which spent several weeks in the laboratory, increased 2–3 times compared to the individuals recently collected in their natural habitat [Meador 1993]. The author of the study explains this both by seasonal biorhythms of organisms and by a decrease in lipid content in organisms under artificial conditions. In addition, the sensitivity of natural and laboratory populations of organisms of the same species may differ due to the increased stress level of the latter, caused by a high density compared to the natural conditions and periodic manipulations with the culture. At the same time, the mechanism for reducing the resistance of laboratory cultures consists in a stressful change in the synthesis of melatonin, which in turn leads to desynchronization of

the daily and annual rhythms of organisms [Lopez-Patino et al. 2014].

Despite the fact that for many test-organisms, the conditions of their laboratory keeping are standardized, in practice it turns out that different laboratories use cultures of the same biological species, but they differ in key characteristics and sensitivity to toxicants. This was shown in the previous papers of the author when studying *D. magna* test-cultures in different laboratories. The differences in the average and maximum life expectancy, specific fertility, sensitivity to a model toxicant were shown [Olkova 2021a; Olkova 2021b]. The most likely reasons for such interlaboratory differences in individuals of the same species are differences in the chemical composition of cultivation waters, nutrition of organisms, cultivation protocols (density of model populations, temperature of keeping and temperature of the experiment, total volume of the cultivation medium, frequency of manipulations, etc.), on which detailed studies are available [Olkova et al. 2018; Terekhova et al. 2018].

Different sensitivity of individuals of the same species under cultivation conditions or experiments that differ from each other can become the basis for specialized bioassay methods that assess not only toxicity, but also other ecologically important processes. For example, differentiation of the degree of toxic effect under conditions of temperature variability during bioassay can be used to predict the consequences of global warming. At the same time, interdependent processes occur, which are reflected in the concepts of TICS (“toxicant-induced climate change sensitivity”) and CITS (“climate-induced toxicant sensitivity”) [Delnat et al. 2019; Meng, Delnat and Stoks 2020].

Thus, the origin of organisms and conditions for their further maintenance significantly affect their sensitivity to chemical stress. However, the differences in responses associated with these factors are most often observed not in one model population, but between cultures of different laboratories. These intraspecific differences can persist for a long time even when different model populations are placed in absolutely identical conditions. Confirmation was found by the author in several scientific bioassay laboratories, which separately contained the test-cultures of amphipods and cladocera taken from other laboratories. Probably, there are already mutations that have become entrenched in the population.

Annual and circadian rhythms of organism activity

Most of the characteristics of the test-organism used as test-functions in bioassay have natural variations which are not caused by chemical exposure, but associated with circadian (daily) and circannual (seasonal) rhythms. Sometimes, these fluctuations are very significant, so they need to be studied and taken into account in order to obtain reliable research conclusions. It was shown that the highest acetylcholinesterase activity of freshwater fish *Cnesterodon decemmaculatus* is in summer and it decreases by 40% in winter [Menendez-Helman et al. 2015]. A similar winter decrease in resistance to the toxicant $K_2Cr_2O_7$ in *D. magna* was observed, the maximum differences between the LD_{50} ($K_2Cr_2O_7$) indices were in winter and spring ($p=0.02$), and the difference in responses in other seasons was not significant [Olkova et al. 2018]. The animals of the continental climate are characterized by a winter period of dormancy, which explains such seasonal phenomena. The work [Bernal-Rey et al. 2020] also highlights the existence of seasonal variations in the dose-response relationship, which may be associated with variations in the metabolism of pollutants.

There are studies, the authors of which claim that the test-organisms they offer, for example, the *Myriophyllum aquaticum* macrophyte, does not have significant seasonal variations in sensitivity [Turgut 2006]. However, the maximum difference between the data obtained in different months of the year in this study was 23%, which cannot be ignored when interpreting the results.

There are very few studies on the influence of the circadian rhythms of organisms on the results of bioassay, although it is known that circadian variations in the activity of animals are also reflected in the metabolism of substances, including toxicants coming from outside [Svarc-Gajic 2009; Mammalian Toxicology 2015]. The study by Kang et al. provides the evidence that the circadian cycles of *Nilaparvata lugens* affect the effectiveness of imidacloprid. The scientists explain this by two peaks in the expression of cytochrome P450 genes during the day [Kang et al. 2017]. When studying the effect of deltamethrin on *Anopheles gambiae* mosquitoes, it was shown that among the metabolic detoxification enzymes there are those that depend on the time of day (glutathione-S-transferases) and have constant

activity (oxidases and esterases) [Balmert et al. 2014]. Thus, endogenous physiological and biochemical processes with a circadian rhythm can affect the results of bioassay even within the framework of experiments on one biological species.

In part, the problem of the influence of the rhythm of the vital activity of organisms on their responses is solved by the transition from the absolute values of the assessed indicators to the relative ones (in comparison with the control). However, when solving such important environmental issues as developing maximum permissible concentrations of substances, determining the “weak link” of the ecological system, forecasting environmental risks, it is necessary to take into account the seasonal sensitivity of organisms.

Biotic factors

Biotic factors are a set of relationships between living organisms, including intraspecific and interspecific interactions.

The cultures of many aquatic organisms, such as fish, crustaceans, and molluscs, are not sterile; therefore, both synergistic and antagonistic interactions between the representatives of this model microcosm can be expected [Sulcius, Slavuckyte and Paskauskas 2017]. The contribution of gut microbes to the metabolism and individual toxicity of substances for various animals and humans is increasingly recognized [Li, He and Jia 2016]. For cultures of zooplankton organisms (daphnia, rotifers) the appearance of cyanobacteria in cultivation water is dangerous, since they are antagonists in relation to the organisms with a filtering type of nutrition, and they also release microcystin toxins [Liang et al. 2020; Asselman et al. 2013]. There is often a need to introduce a different biologic species into the culture as feed, and factors such as abundance and frequency of feeding can create differences in sensitivity to toxicants among members of the same species [Elendt and Bias 1990].

In the earlier work [Olkova et al. 2018], it was shown that the density of model cultures of test-organisms affects the lifespan and fertility of *D. magna* – these are the parameters the variations of which are assessed when determining the toxicity of the test medium. Confirmation of this hypothesis can be seen in the work devoted to the effect of the pesticide on the dragonfly larvae *Ischnura elegans* under the conditions of

competition between individuals and in their individual, isolated, keeping. Intraspecific competition appeared to increase the toxicity of chlorpyrifos [Op de Beeck, Verheyen and Stoks, Robby 2018]. This means that when developing laboratory bioassay methods, it is necessary to create model groups of organisms with the density corresponding to natural populations.

In addition to the density of the model population, the intraspecific sensitivity of individuals to toxicants is influenced by the phase of laboratory population cycles. It was shown that the populations during the growth phase are the most resistant to chemical stress, and model groups in the peak phase of population growth turned out to be the most sensitive [Woo, East and Salice, 2020].

In bioassay interactions of individuals of one biological species with representatives of another species are rarely taken into account, since monocultures are most often used as test-cultures. However, there are studies aimed at researching toxic effects on the community of organisms. In this case, the so-called microcosms are created, i.e. artificial communities. Monteiro et al. conducted an experiment on the effect of water-soluble oil fractions on the on nematode assemblages and suggested that the interspecies interactions change the sensitivity of individual species [Monteiro et al. 2019]. This means that the sensitivity of a particular species in a community and in a monoculture may differ.

Thus, biotic interactions cannot be excluded from the spectrum of factors that create interindividual sensitivity to toxicants, but they need to be regulated, maintained within certain limits, as it is usual, for example, with temperature or lighting.

COMBINED ACTION OF FACTORS

Of course, there may be a situation when intraspecific differentiation of sensitivity to pollutants is created by several factors at once, that is, their combined effect is observed. The published report [Dybing and Soderlund 1999] raises the questions about complex effects of age, disease and genetic polymorphic changes in the body on individual differences in susceptibility to toxicants. The work [Delnat et al. 2019] presents the evidence that the TICS concept, which connects the thermal sensitivity of organisms and their resistance to toxicants, may also depend on the gender of the experimental animal. Op de Beeck et

al showed that the effect of chlorpyrifos depends on both the ambient temperature and the level of competition between individuals of the same species [Op de Beeck, Verheyen and Stoks 2018].

Under natural conditions, physical, chemical and biological stressors will change with the season and climate, which leads to the changes in the bioavailability of substances, their interactions with each other and, in some cases, to an increase in the toxicity of chemical substances [Noyes et al. 2009]. The advantage of bioassay is that the combined acting factors can either be regulated or taken into account when discussing experimental results.

The authors [Wong and Carmona 2021] showed that intraspecific variability of traits makes a significant contribution to the functional diversity of the population. From the ecological point of view, the heterogeneity of a biological species is a response to the combined action of environmental factors. Using probabilistic methods of mathematical statistics, it is possible to record intraspecific variability of traits in the individuals of the same species [Wong and Carmona 2021]. The authors also propose taking into account the factors of individual sensitivity using chemical-specific adjustment factors [Kasteel and Westerink 2021]. Probably, such approaches can be used to standardize the cultures of organisms in bioassays and improve the accuracy of bioassays.

CONCLUSIONS

Thus, the factors that determine the intraspecific diversity of the sensitivity of organisms to toxicants are divided into the parameters determined by the genetic characteristics of the species and the individual organism, and not depending on them. This review shows how these factors, in all their diversity, create heterogeneity in responses of organisms of one biological species. In natural ecosystems, this is a process necessary for the survival of a species under the conditions of periodic chemical stress, and in bioassay this is a reason for the scatter of data of toxicological experiments.

Genetically, unconditioned factors such as age, conditions of cultivation of organisms and experiments, biotic factors are easier to standardize and control than the genetic characteristics of organisms. Various mutations, genetic drift and polymorphism contribute to so-called “misses” of

experiments, which are often inexplicable. Only recent studies have shown how cultures of test-organisms or cell cultures of the same species can be divided into intraspecific genetic lines with different resistance to toxicants.

The degree of differentiation of individual sensitivity to chemicals will be lesser in the stenobiont biological species compared to the eurybiont species. This principle is explained by more significant adaptive capabilities of eurybionts formed during evolution. Despite this, eurybionts are more often used in bioassay, because they are easier to cultivate, and they do not create significant restrictions on the parameters of the tested media, like stenobionts.

REFERENCES

1. Asano, S. 2006. Standard bioassay method using insects for quality control of *Bacillus thuringiensis* products – The history and problems. *Japanese journal of applied entomology and zoology*, 50(2), 101–114. DOI: 10.1303/jjaez.2006.101
2. Asselman, J., Meys, J., Waegeman, W., De Baets, B., De Schampelaere, K.A.C. 2013. Combined exposure to cyanobacteria and carbaryl results in antagonistic effects on the reproduction of *Daphnia pulex*. *Environmental Toxicology and Chemistry*, 32(9), 2153–2158. DOI: 10.1002/etc.2296
3. Bailey, S., Li, D., Potter, D.M. 2016. General Toxicology, Safety Pharmacology, Reproductive Toxicology, and Juvenile Toxicology Studies. In: *Tonclinical statistics for pharmaceutical and biotechnology industries*. Book series: *Statistics for Biology and Health*, 231–261 p.
4. Ball, A.L., Bloch, K.M., Rainbow, L., Liu, X., Kenny, J., Lyon, J.J., Gregory, R., Alfirevic, A., Chadwick, A.E. 2021. Assessment of the impact of mitochondrial genotype upon drug-induced mitochondrial dysfunction in platelets derived from healthy volunteers. *Archives of toxicology*. DOI: 10.1007/s00204-021-02988-3
5. Balmert, N.J., Rund, S.S.C., Ghazi, J.P., Zhou, P., Duffield, G.E. 2014. Time-of-day specific changes in metabolic detoxification and insecticide resistance in the malaria mosquito *Anopheles gambiae*. *Journal of insect physiology*, 64, 30–39. DOI: 10.1016/j.jinsphys.2014.02.013
6. Bernal-Rey, D.L., Cantera, C.G., Afonso, M.D., Menendez-Helman, R.J. 2020. Seasonal variations in the dose-response relationship of acetylcholinesterase activity in freshwater fish exposed to chlorpyrifos and glyphosate. *Ecotoxicology and Environmental Safety*, 187, 109673. DOI: 10.1016/j.ecoenv.2019.109673
7. Bernard, S., Neville, K.A., Nguyen, A.T., Flockhart, D.A. 2006. Interethnic differences in genetic polymorphisms of CYP2D6 in the US population: Clinical implications. *Oncologist*, 11(2), 126–135.
8. Bora, S., Vardhan, G.S.H., Deka, N., Khataniar, L., Gogoi, D., Baruah, A. 2021. Paraquat exposure over generation affects lifespan and reproduction through mitochondrial disruption in *C. elegans*. *Toxicology*, 447, 152632. DOI: 10.1016/j.tox.2020.152632
9. Burke, W.H., Henry, M.H.G. 1999. Gonadal development and growth of chickens and turkeys hatched from eggs injected with an aromatase inhibitor. *Poultry science*, 78(7), 1019–1033. DOI: 10.1093/ps/78.7.1019
10. Chen, T., Heflich, R.H., Moore, M.M., Mei, N. 2010. Differential Mutagenicity of Aflatoxin B-1 in the Liver of Neonatal and Adult Mice. *Environmental and Molecular Mutagenesis*, 51(2), 156–163. DOI: 10.1002/em.20518
11. Church, R.J., Gatti, D.M., Urban, T.J., Long, N., Yang, X., Shi, Q., Eaddy, J.S., Mosedale, M., Ballard, S., Churchill, G.A., Navarro, V., Watkins, P.B., Threadgill, D.W., Harrill, A.H. 2015. Sensitivity to hepatotoxicity due to epigallocatechin gallate is affected by genetic background in diversity outbred mice. *Food and Chemical Toxicology*, 76, 19–26.
12. Crouau, Y., Cazes, L. 2003. What causes variability in the *Folsomia candida* reproduction test? *Applied Soil Ecology*, 22(2), PII S0929–1393(02)00128–2, 175–180.
13. Crouau, Y., Cazes, L. 2003. What causes variability in the *Folsomia candida* reproduction test? *Applied Soil Ecology*, 22(2), 175–180, PII S0929–1393(02)00128–2.
14. Delnat, V., Tran, T.T., Verheyen, J., Dinh, K.V., Janssens, L., Stoks, R. 2019. Temperature variation magnifies chlorpyrifos toxicity differently between larval and adult mosquitoes. *Science of the Total Environment*, 690, 1237–1244. DOI: 10.1016/j.scitotenv.2019.07.030
15. Delnat, V., Tran, T.T., Verheyen, J., Dinh, K.V., Janssens, L., Stoks, R. 2019. Temperature variation magnifies chlorpyrifos toxicity differently between larval and adult mosquitoes. *Science of the Total Environment*, 690, 1237–1244. DOI: 10.1016/j.scitotenv.2019.07.030
16. Dette, H., O'Brien, T.E. 2004. Efficient experimental design for the Behrens-Fisher problem with application to bioassay. *American statistician*, 58(2), 138–143. DOI: 10.1198/0003130043259
17. Dohnal, V., Wu, Q., Kuca, K. 2014. Metabolism of aflatoxins: key enzymes and interindividual as well as interspecies differences. *Archives of Toxicology*, 88(9), 1635–1644.
18. Dumont, E.R., Larue, C., Michel, H.C., Gryta, H., Line, C., Baque, D., Gross, E.M., Elger, A. 2020. Genotypes of the aquatic plant *Myriophyllum*

- spicatum with different growth strategies show contrasting sensitivities to copper contamination. *Chemosphere*, 245, 125552. DOI: 10.1016/j.chemosphere.2019.125552
19. Dybing, E., Soderlund, E.J. 1999. Situations with enhanced chemical risks due to toxicokinetic and toxicodynamic factors. The conference: ILSI Europe Workshop on the Significance of Excursions of Intake above the Acceptable Daily Intake (ADI), Milan, Italy. *Regulatory Toxicology and Pharmacology*, 30(2), S, S27–S30, part 2.
 20. Elendt, B.-P., Bias, W.-R. 1990. Trace nutrient deficiency in *Daphnia magna* cultured in standard medium for toxicity testing. Effects of the optimization of culture conditions on life history parameters of *D. magna*. *Water Research*, 24(9), 1157–1167. DOI: 10.1016/0043-1354(90)90180-E
 21. Eltemsah, Y.S., Bohn, T. 2019. Acute and chronic effects of polystyrene microplastics on juvenile and adult *Daphnia magna*. *Environmental Pollution*, 254, 112919 (A). DOI: 10.1016/j.envpol.2019.07.087
 22. Gismondi, E., Cossu-Leguille, C., Beisel, J.-N. 2013. Do male and female gammarids defend themselves differently during chemical stress? *Aquatic toxicology*, 140, 432–438. DOI: 10.1016/j.aquatox.2013.07.006
 23. Gutbier, S., May, P., Berthelot, S., Krishna, A., Trefzer, T., Behbehani, M., Efremova, L., Delp, J., Gstraunthaler, G., Waldmann, T., Leist, M. 2018. Major changes of cell function and toxicant sensitivity in cultured cells undergoing mild, quasi-natural genetic drift. *Archives of toxicology*, 92(12), 3487–3503. DOI: 10.1007/s00204-018-2326-5
 24. Hansen, P.H., Blasco, J., Delvalis, T.A., Poulsen, V., van den Heuvel-Greeve, M. 2007. Biological analysis (Bioassays, Biomarkers, Biosensors). In: *Sustainable Management of Sediment Resources: Sediment Quality and Impact Assessment of Pollutants*. Book series: Sustainable Management of Sediment Resources, Volume 1, 131–161, DOI: 10.1016/S1872-1990(07)80076-6
 25. *In Vitro Environmental Toxicology – Concepts, Application and Assessment*. 2017. Ed. by G. Reifferscheid, S. Buchinger. In: *Advances in Biochemical Engineering-Biotechnology*. Cham: Springer, 157, 324 p. ISBN 978-3-319-45906-6.
 26. ISO 11267. 1998. Soil quality-inhibition of reproduction of *Collembola* (*Folsomia candida*) by soil pollutants. Inter. Stand. Org. Ed. Geneve. 16 p.
 27. Josko, I. Oleszczuk, P. 2014. Phytotoxicity of nanoparticles-problems with bioassay choosing and sample preparation. *Environmental science and pollution research*, 21(17), 10215–10224. DOI: 10.1007/s11356-014-2865-0
 28. Kang, K., Yang, P., Pang, R., Yue, L., Zhang, W. 2017. Cycle affects imidacloprid efficiency by mediating cytochrome P450 expression in the brown planthopper *Nilaparvata lugens*. *Insect molecular biology*, 26(5), 522–529. DOI: 10.1111/imb.12313
 29. Kasteel, E.E.J., Westerink, R.H.S. 2021. Refining in vitro and in silico neurotoxicity approaches by accounting for interspecies and interindividual differences in toxicodynamics. *Expert opinion on drug metabolism & Toxicology*. DOI: 10.1080/17425255.2021.1885647
 30. Kokkali, V., Van Delft, W. 2014. Overview of commercially available bioassays for assessing chemical toxicity in aqueous samples. *Trends in Analytical Chemistry*, 61, 133–135. DOI: 10.1016/j.trac.2014.08.001
 31. Kutsenko, S.A. 2004. *Fundamentals of toxicology*. St. Petersburg, Russia: Foliant, 715 p. ISBN 5-93929-092-2.
 32. Li, H., He, J., Jia, W. 2016. The influence of gut microbiota on drug metabolism and toxicity. *Expert opinion on drug metabolism & Toxicology*, 12(1), 31–40. DOI: 10.1517/17425255.2016.1121234
 33. Liang, Y., Shao, L., Jiang, Q.C., Yang, J.X. 2020. Changes in the life-cycle parameters and glutathione-related antioxidant defense system of rotifer *Brachionus calyciflorus* under the combined stress of microcystin-LR and ammonia. *Aquatic ecology*, 54(1), 243–256. DOI: 10.1007/s10452-019-09739-8
 34. Lopez-Patino, M.A., Gesto, M., Conde-Sieira, M., Soengas, J.L., Miguez, J.M. 2014. Stress inhibition of melatonin synthesis in the pineal organ of rainbow trout (*Oncorhynchus mykiss*) is mediated by cortisol. *Journal of experimental biology*, 217(8), 1407–1416. DOI: 10.1242/jeb.087916
 35. *Mammalian Toxicology*. Ed. by Abou-Donia M. B. New York: Wiley, 2015. 720 p. – ISBN 978-1119940418.
 36. Maruyama, W. Yoshida, K. Tanaka, T. Nakanishi, J. 2002. Possible range of dioxin concentration in human tissues: Simulation with a physiologically based model. *Journal of Toxicology and Environmental Health, Part A*, 65(24), 2053–2073.
 37. Meador, J.P. 1993. The effect of laboratory holding on the toxicity response of marine infaunal amphipods to cadmium and tributyltin. *Journal of experimental marine biology and ecology*, 174(2), 227–242.
 38. Menendez-Helman, R.J., Ferreyroa, G.V., Afonso, M.D., Salibian, A. 2015. Circannual rhythms of acetylcholinesterase (AChE) activity in the freshwater fish *Cnesterodon decemmaculatus*. *Ecotoxicology and Environmental Safety*, 111, 236–241 DOI: 10.1016/j.ecoenv.2014.10.017
 39. Meng, S.D., Delnat, V., Stoks, R. 2020. Mosquito larvae that survive a heat spike are less sensitive to subsequent exposure to the pesticide chlorpyrifos. *Environmental Pollution*, 265(PT A), A, 114824. DOI: 10.1016/j.envpol.2020.114824

40. Monteiro, L., Moens, T., Lynen, F., Traunspurger, W. 2019. Effects of the water-soluble fraction of a crude oil on freshwater meiofauna and nematode assemblages. *Ecotoxicology and environmental safety*, 176, 186–195. DOI: 10.1016/j.ecoenv.2019.03.083
41. Moser, V.C., Padilla, S. 2015. Esterase detoxication of acetylcholinesterase inhibitors using human liver samples in vitro. *Toxicology*, 353, 11–20.
42. Noyes, P.D., McElwee, M.K., Miller, H.D., Clark, B.W., Van Tiem, L.A., Walcott, K.C., Erwin, K.N., Levin, E.D. 2009. The toxicology of climate change: Environmental contaminants in a warming world. *Environment international*, 35(6), 971–986. DOI: 10.1016/j.envint.2009.02.006
43. Oberholster, P.J., Hill, L., Jappie, S., Truter, J.C., Botha, A.M. 2016. Applying genotoxicology tools to identify environmental stressors in support of river management. *Chemosphere*, 144, 319–329. DOI: 10.1016/j.chemosphere.2015.08.024
44. Olkova, A. 2021a. Control of Suitability of the Culture *Daphnia magna* Straus for Bioassays of Aquatic Environments, Taking into Account Demographic Indicators of Model Populations. *Water*, 13(1), 47. DOI: 10.3390/w13010047
45. Olkova, A.S. 2021b. Develop a strategy bioassay aquatic environments given multifactorial responses of test organisms. Dissertation of Doctor of Biological Sciences, Vladimir, Russia: Vladimir State University named after Alexander Grigorievich and Nikolai Grigorievich Stoletovs, 359 p. http://diss.vlsu.ru/uploads/media/Dissertacija_Olkova_itog.pdf
46. Olkova, A.S., Berezin, G.I. 2019. Study on the sensitivity of certified bioassays to water pollution with modern herbicides: model experiments. *Water and ecology: problems and solutions*, 2(78), 111–119. DOI: 10.23968/2305–3488.2019.24.2.111–119
47. Olkova, A.S., Kantor, G.Y., Kutyavina, T.I., Ashikhmina, T.Y. 2018. The importance of maintenance conditions of *Daphnia magna* Straus as a test organism for ecotoxicological analysis. *Environmental Toxicology and Chemistry*, 37(2), 376–384. doi: 10.1002/etc.3956
48. Olmstead, A.W., Lindberg-Livingston, A., Degitz, S.J. 2010. Genotyping sex in the amphibian, *Xenopus (Silurana) tropicalis*, for endocrine disruptor bioassays. *Aquatic toxicology*, 98(1), 60–66. DOI: 10.1016/j.aquatox.2010.01.012
49. Op de Beeck, L., Verheyen, J., Stoks, R. 2018. Competition magnifies the impact of a pesticide in a warming world by reducing heat tolerance and increasing autotomy. *Environmental Pollution*, 233, 226–234. DOI: 10.1016/j.envpol.2017.10.071
50. Shrestha, B., Reed, J.M., Starks, P.T., Kaufman, G.E., Goldstone, J.V., Roelke, M.E., O'Brien, S.J., Koepfli, K.P., Frank, L.G., Court, M.H. 2011. Evolution of a Major Drug Metabolizing Enzyme Defect in the Domestic Cat and Other Felidae: Phylogenetic Timing and the Role of Hypercarnivory. *PLOS ONE*, 6(3), e18046 DOI: 10.1371/journal.pone.0018046
51. Slabbert, J.L., Venter, E.A. 1999. Biological assays for aquatic toxicity testing. *Water Science and Technology*, 39(10–11), 367–373. doi: 10.1016/S0273–1223(99)00300–5
52. Sulcius, S., Slavuckyte, K., Paskauskas, R. 2017. The predation paradox: Synergistic and antagonistic interactions between grazing by crustacean predator and infection by cyanophages promotes bloom formation in filamentous cyanobacteria. *Limnology and Oceanography*, 62(5), 2189–2199. DOI: 10.1002/lno.10559
53. Sundman-Engberg, B., Tidefelt, U., Paul, C. 1998. Toxicity of cytostatic drugs to normal bone marrow cells in vitro. *Cancer Chemotherapy and Pharmacology*, 42(1), 17–23.
54. Svarc-Gajic, J. 2009. *General Toxicology*. NY, USA: Nova Science Publishers, 287 p.
55. Terekhova, V.A. 2011. Soil Bioassay: Problems and Approaches. *Eurasian soil science*, 44(2), 173–179. DOI: 10.1134/S1064229311020141
56. Terekhova, V.A., Wadhia, K., Fedoseeva, E.V., Uchanov, P.V. 2018. Bioassay standardization issues in freshwater ecosystem assessment: test cultures and test conditions. *Knowledge and management of aquatic ecosystems*, 419, 14. DOI: 10.1051/kmae/2018015
57. Tewes, L.J., Michling, F., Koch, M.A., Muller, C. 2018. Intracontinental plant invader shows matching genetic and chemical profiles and might benefit from high defence variation within populations. *Journal of ecology*, 106(2), 714–726. DOI: 10.1111/1365–2745.12869
58. Turgut, C. 2006. The growth stability and sensitivity of parrotfeather to a reference toxicant (3,5-dichlorophenol) throughout a 1-year period. *Fresenius environmental bulletin*, 15(5), 462–464.
59. Wong, M.K.L., Carmona, C.P. 2021. Including intraspecific trait variability to avoid distortion of functional diversity and ecological inference: Lessons from natural assemblages. *Methods in Ecology and Evolution*. DOI: 10.1111/2041–210X.13568
60. Woo, T.J., East, A., Salice, C.J. 2020. Intraspecific interactions affect outcomes of pulse toxicity at different *Daphnia magna* population phases. *Environmental pollution*, 267, 115398. DOI: 10.1016/j.envpol.2020.115398
61. Zovko, M., Vidaković-Cifrek, Ž., Cvetković, Ž. Bošnjir, J., Šikić, S. 2015. Assessment of acrylamide toxicity using a battery of standardised bioassays. *Archives of Industrial Hygiene and Toxicology*, 66(4), 315–321. doi: 10.1515/aiht-2015–66–2715