ABSTRACT

The meaning of alternative biotests is described and discussed. The paper also deals with the possible application of the development studies of the sea Artemia franciscana nauplius. Five-day biotests including the validation criteria are described. The possibilities of the biotests are very wide. Additionally to the standard applications in ecotoxicology, there is a possibility of modelling pharmacological experiments or monitoring the effects of ionizing radiation and the interaction with other chemicals.

KEY WORDS: Artemia salina; Artemia franciscana; microbiotests; animal protection

Introduction

In biology the phenomena are so complicated and the mutual interactions so frequent that the theoretical principles are only temporary and hypothetical. Even very precise human deductions are uncertain and they require the experimental verification. Two apparently incompatible tendencies are known from the middle of the last century in biomedical research. On one hand, this is an attempt to understand etiopathogenesis of many diseases and successively the development of enormous quantity of new substances with the need of their testing, and on the other hand there is pressure of the society which calls at least for mitigation of test animal suffering (Russell and Burch, 1959). This demand has led to the creation of the 3 R concept (i.e. Reduction, Refinement and Replacement). This strategy also involves substituting the test animals by cell or tissue cultures and lower organisms, for example, invertebrates or microorganisms (defined according to the Council of Europe 1976). The important difference which distinguishes the biological tests from other tests is in the complexity of live systems that cannot be simplified. If we use tissue or cells in a research, it is only a study of partial systems not the study of a simplified system (Pazourek, 1992).

The environment including water ecosystems is very often contaminated by low concentrations of various chemical compounds of foreign origin (Beňová et al., 2007). Although the resistance of test organisms to various substances is relatively well known, it is impossible to determine toxicity of mixtures of various components. Similar situation is also in medicine where information about the long-term effects of combined pharmaceuticals or the effects of their residuals on water organisms is relatively very poor (Sklenář et al., 2006). The foreign substances in low concentrations can interact with some physical factors. The effects can occur in some time, and their subacute or chronic effects can be expected (Dvořák and Beňová, 2002; Beňová et al., 2006).

Biotests

The tests to monitor the interactions should meet the following requirements: sufficient sensitivity to foreign substances; subacute to long-term nature in the time; high homogeneity of the individuals who will be relatively less sensitive to the external conditions of the experiment; high reproducibility of the experiments; as high as possible closeness of the system; simplicity of production and verification; price availability; and simple evaluation and simple statistical processing.

The standard biotests performed on fish or invertebrates have some disadvantages. From the methodological point of view, the biotests are highly demanding for both the time and the personnel qualification. Hence such experiments are too expensive. The generation II tests solve the problem mentioned. These are the alternative microbiotests in which unicellular or small multicellular organisms are exposed to liquid nitrogen when the specific effect is measured. Various microorganisms, e.g. bacteria, mushrooms, algae, protozoa
and invertebrates, are used (Blaise, 1991). The principle of so-called “toxkits” can lead to incubation of the test animals from the resting eggs (marked as cysts) within 24 hours before starting the test. This method can avoid the time-consuming and spatially demanded cultivation of the live organisms. ROTOXKIT F and M, THAMNOTOXKIT F, ARTOXKIT M and others are manufactured in large series. However, the greater production represents the research which is financially demanded. The affordable tests on Artemia franciscana (previously A. salina) discussed in this contribution, have been performed since 1992, that is, they started before Czech Republic approached to the import of the toxkit.

Biotests using brine shrimps of genus Artemia

One invertebrate routinely used in the different biotests for a long time is genus Artemia (order Anostraca order, class Branchiopoda) that forms an independent group of nauplii stages. The population of Artemia can be found in about 500 salt lakes and salt works in the temperate, sub-tropical, and tropical climate zones. Because they are adapted to high salinity, their biotops are characterized by the minimum diversity of genera and the absence of predators and food salinity, their biotops are characterized by the minimum tropical climate zones. Because they are adapted to high salinity, their biotops are characterized by the minimum diversity of genera and the absence of predators and food

...
must be evaluated separately after completing each test and compared with the control group. The range of individual attempts is limited due to the time demands during readout. The time span between the start and end of readout should not be too long. The results read by more than one person can lead to an undesired variability.

Validation for the subacute test should be secured by the mortality of the control group lower than 10%. However, this criterion is designed for the daphnia tests with an exposure of up to 48 hours without any feeding, or for the tests when the monitored objects are optimally fed. Because Artemia are hungry, they exhaust their energy reserves and the strict criterion within 120 hours cannot be always met. On the other hand, Artemia are very sensitive within this period for the presence of toxic agents and the maximum differences between the mortality of the control and tested groups are achieved.

To monitor a long-term effect of the substances on the organisms it is necessary to extend as much as possible the viability of tested organisms under the standard conditions. Because Artemia start to perish after 96 hours for food shortage, it is necessary to supply the standard energy sources. This can provide the addition of 3% glucose that prolongs the test up to ten days (Dvořák et al., 2005). In this test of prolonged toxicity the more moderate validation criteria were designed for the exposure longer than 120 hours, i.e. 20% mortality in the control groups because glucose does not represent full nutritive value. The test should be completed as soon as mortality would exceed 20%. For the monitoring of unstable substances the validation criterion is to determine the concentration of substances in the solution at the beginning and at the end of the experiment.

The widest use of the above mentioned test is in ecotoxicology. Because it is possible to monitor high numbers of specimens at the same time (up to 1,000), we can study the mutual interactions of chemical substances and their physical factors (Dvořák, 1999; Dvořák and Beňová, 2002; Beňová et al., 2006; Beňová et al., 2007).

The test in practice

The example of the generation II biotest application at Artemia franciscana in pharmacology represents the utilization in the primary toxicity screening of the new synthesized purine inhibitors of cyclin-dependent kinases. Toxicity was compared with toxicity of olomoucin and also with toxicity of risk elements – chromium, cadmium, zinc and boron. The experiment was designed as the toxicity test in the environment with 0.9% salinity (Sklenář et al., 2006).

The cosmic radiation effect on Artemia franciscana cysts was studied during the unique Biostack project performed on Apollo 16 board. Hatching occurred only at 10 percent at cysts exposed to cosmic radiation (Rutherford 1974).

Study on lethality of Artemia franciscana depending on the dose of gamma ionizing radiation determined LD₅₀ of 96 hours when exposed to 600–700 Gy (Dvořák and Beňová, 2002) which even corresponded to phylogenetic genus classification. The study which monitored morphologic changes at Artemia franciscana after gamma radiation exposure was presented at the 2nd Radiobiological Conference at Košice. Intestinal epithel is the most sensitive, and the changes of epitelial cells were already observed with the nauplii exposed to a dose of 100 Gy. In individuals exposed to a dose of 1,000 Gy intestinal epithel was completely destroyed. Loss of segmentation in thoracal area and cease of appendical formation were the next significant morphological changes (Dvořák et al., 2004).

The alternative biotests cannot replace the conventional tests in a full range at experimental mammals, but they can reduce remarkably their numbers.

Acknowledgement

This research was carried out in the frameworks of the Research Plan of the Ministry of Education, Youth and Physical Training of the Czech Republic MSM6215712402.

REFERENCES

Beňová K, Dvořák P, Falis M and Daňová D. (2006). Elimination of negative effects of cadmium in Artemia franciscana by exposure to ionizing radiation. Folia Vet 50, (3) Suplementum: 21–22.

Beňová K, Dvořák P, Falis M and Sklenář Z. (2007). Interaction of low doses of ionizing radiation, potassium dichromate and cadmium chloride in Artemia franciscana biotest. Acta Vet Brno 76: 35–40.

Blaise C. (1991). Microbiotests in aquatic toxicology-characteristics, utility and prospects. Environ Toxicol Qual 6: 145–155.

Conde F R, Hootman S R and Harris P J. (1972). Neck organ of Artemia salina nauplii. A larval salt gland. J Comp Physiol 80: 239–246.

Council of Europe. (1976). European convention for the protection of animals kept for farming purposes. European Treaty Series Nr. 87, Strasbourg, European Council 1976.

Dvořák P. (1999). Sledování interakcí nízkých expozic ionizujícího záření a cizorodých látek. In: Trans. 2. Radiobiologické konference s medzinárodnou účasťou venované 55. výročiu založenia UVL. UVL v Košiciach. 28–33.

Dvořák P. (1995). Eliminácia kyselín a ionizujúcích častíc. In: Zborník 2. Radiobiologické konference s medzinárodnou účasťou venované 55. výročiu založenia UVL. UVL v Košiciach. 28–33.

Dvořák P, Šuľman E and Beňová K. (2005). The development of a ten-day biotest using Artemia salina nauplii. Biologia Bratislave 60 (5): 593–597. 41 A P. (1995). Metod analízičkej pre le acou. Quad. litt. Ric. Acque 100: 342–344.

Michael A S, Thompson C G and Abramovitz M. (1956). Artemia salina as a test organism for bioassay. Science 123: 464.

Pazourek J. (1992). Simulace biologických systémů. Grada a.s., Praha.

Rother W, Grail E H, Heinrich W, Alloko E O, Kaiser Rand Cuer P. (1974). Preliminary results on the action of cosmic heavy ions on the development of eggs of Artemia salina. Life Sci 12: 69–74.

Russell W M S and Burch R L. (1959). The Principles of Humane Experimental Technique. Methuen, London.

Sklenář Z, Dvořák P and Beňová K. (2006). Možnosti využití biotestu s Artemia salina při studování toxikologických účinku inhiboru cizorododentních kináz. Klin Farmakol Farm 20: 62–65.

Copyright © 2009 Slovak Toxicology Society SETOX