We are IntechOpen, the world’s leading publisher of Open Access books
Built by scientists, for scientists

3,800
Open access books available

116,000
International authors and editors

120M
Downloads

154
Countries delivered to

TOP 1%
Our authors are among the most cited scientists

12.2%
Contributors from top 500 universities

WEB OF SCIENCE™
Selection of our books indexed in the Book Citation Index in Web of Science™ Core Collection (BKCI)

Interested in publishing with us?
Contact book.department@intechopen.com

Numbers displayed above are based on latest data collected.
For more information visit www.intechopen.com
Alternative Biotest on *Artemia franciscana*

Petr Dvorak¹, Katarina Benova² and Jiri Vitek³

¹University of Veterinary and Pharmaceutical Sciences
²University of Veterinary Medicine and Pharmacy
³Czech Republic

1. Introduction

Recent toxicology, in accordance with recommendations from the European Council, has demanded a decrease in the number of vertebrates used in toxicology testing and their partial replacement with invertebrate animals, plants or even organ, tissue, or cell cultures. During the last 50 years various invertebrate species have been tested for their sensitivity to many chemical or physical agents to prove their possible use for pre-screening tests. To the most valuable organisms available for ecotoxicity testing belong crustaceans of the *Artemia* genus, commonly known as brine shrimps.

2. Characterisation of *Artemia franciscana*

The taxonomic status of the *Artemia* genus is as follows (Martin & Davis, 2001):
Subphylum: Crustacea Brünnich, 1772
Class: Branchiopoda Latreille, 1817
Subclass: Sarsostraca Tasch, 1969
Order: Anostraca Sars, 1867
Family: Artemiidae Grochowski, 1896
Genus: Artemia Leach, 1819.

To this genus belong the following species:

*A. salina, monica, urmiana, franciscana, persimilis, sinica, tibetiana*, sp. Pilla & Beardmore 1994, and parthenogenetic population(s) of Europe, Africa, Asia, and Australia. In this report we use *A. franciscana* Kellogg 1906, distributed in America, Caribbean, and Pacific islands.

Populations of the *Artemia* genus are widely distributed in all continents except for Antarctica. Species of this genus inhabit more than 500 salt lakes, but not seas, of temperate, subtropical, and tropical zones. They are very well adapted to high salinity waters (up to 70 g.L⁻¹, but can survive even at 250 g.L⁻¹, Ruppert & Barnes, 1994) with fairly low diversity of living organisms and absence of predators or competitive species. In these environments the evolution of *Artemia* species is favoured by the abundance of microorganisms such as bacteria, protozoa and algae that are the basis of the *Artemia* diet (Amat, 1985).

Brine shrimps come in many colours - from white to pink, shadow, or green. The different colours probably result from specific diets and environmental conditions. Females can reach
the size of 30 mm (most often 12 - 18 mm), males are smaller. Their body is divided into three parts: head, thorax, and abdomen. On the head there is a pair of stalked, lateral, compound eyes and a single, median, unstalked naupliar eye, and two pairs of antennae. The latter are sexually dimorphic. Those of the adult males are modified to form a claspers organ to hold the female during copulation. The female antennae are smaller but much thicker and have a sensorial function. The remainder of the body consists of the segmented thorax, consisted of 11 segments, and posterior abdomen. Each thoracic segment bears a ventral pair of leaf-like appendages, known as phyllopods, used for swimming, respiration, and feeding. Finally, the abdomen consists of 6 segments and a telson – the posterior end of the body. The abdomen of females carries a bunch of spherical-like eggs (cca 200 μ in diameter). Their colour varies from creamy to almost blackish brown (Anderson, 1967; Benesch, 1969; Hertel, 1980).

The brine shrimp larva – nauplius is pink or rather auburn, its size about 0.4 mm. Its body consists of a head and short thorax (Fig. 1). On the head there is a dark, red or black, median naupliar eye and two pairs of antennae. The second pair is used for swimming and feeding, while the first pair is a sensory organ.

Artemia franciscana is resistant to high osmotic stress of the hypersaline environments. Its nauplii drink water and secret Na⁺ and Cl⁻ ions. The adults ingest water by mouth and anus. The capability to ingest water, containing bacteria and debris as well, is developed in the 2nd instar stage (Kikuchi, 1971). The larvae hatch usually after 20 - 48 hrs of embryonic development and reach their terminal size after 8 - 10 days, during which they undergo about 17 molts (Gilchrist, 1960).

The life span of A. franciscana varies from 2 to 4 months in dependence on salinity and temperature (Browne et al., 1991). Reproduction of the genus Artemia may proceed in two modalities: sexual or parthenogenetic. Both, sexual and parthenogenetic populations occur all over the world except for Americas where only sexual populations have been reported (Browne, 1992). Coexistence of both sexual and parthenogenetic strains has been recorded in various habitats; however, partial overlapping in space and/or time may occur. The parthenogenetic reproduction results in unisexual, female populations. While the bisexual specimens have stable number of chromosomes (2n = 42), the parthenogenetic populations may be diploid, triploid, tetraploid, or even pentaploid (Hentschel, 1968).
Females after insemination produce eggs that start to develop immediately after laying. But, under unfavourable conditions dormant stages, known as cysts, are produced. Their metabolism is inhibited and they can survive, even in extreme conditions, for dozens of years. In the cyst there is an embryo in the stage of gastrula, enwrapped with a hard outer membrane. In natural habitats the cysts gather on the water surface, and then drift ashore where they survive the unfavourable season. When the cysts occur in salt water their metabolic activity is again restored and in 20 hrs hatches the umbrella stage that quickly develops to a free swimming nauplius (Browne et al., 1991).

*Artemia* cysts are produced mainly in the USA, Australia, China, or Saudi Arabia but other countries are also involved. The recent annual yield of the cysts is about 2 000 t. They are produced for the aquaculture or aquarium practice as food for tropical fish. One of the most important sources of the cysts is the Great Salt Lake in Utah, USA. In the northern part (Gunnison Bay) *Artemia salina* cysts are produced in 16 oz. (454 g) cans, while in the southern part (Gilbert Bay) *Artemia franciscana* is produced and packed in 15 oz. (425 g) cans. The latter cysts are more delicate (more than 300 000 specimens per 1 g). The best quality products are sold as the Premium class, and this quality was used in our experiments (“Maxima brine shrimp eggs” produced by the Sanders firm in Utah).

3. Alternative methods

Alternative methods are as follows:

- biotests on plants (e.g. mustard roots, soya, or rice; Horowitz, 1970; Kratky & Warren, 1971) or algae (e.g. *Selenastrum capricornutum*, *Chlorella kessleri*, or *Chlamydomonas reinhardtii*; Fouradzieva et al., 1995);
- biotests on invertebrates (e.g. *Daphnia magna*, *Tetrahymena pyriformis*, *Asellus aquaticus*, genus *Tubifex*, or flatworms; Balls et al., 1992);
- biotests on vertebrates in early stages of development, before a nervous system is developed and the specimens do not feel any pain (Bagley et al., 1994);
- in vitro methods, i.e., cell, tissue, or organ cultures (Fentem & Balls, 1993), and
- mathematical and computer simulations (Pazourek, 1992).

The methods mentioned above have some disadvantages. Most of them are time consuming, require skilled workers, and often are expensive. These disadvantages may be overcome by the alternative microbiotests, in which the effect of dissolved agents on unicellular or small multicellular organisms is evaluated. For such tests are convenient various simple organisms, such as bacteria, fungi, algae, protozoa, or invertebrates (Blaise, 1991).

In the Multicenter Evaluation of In Vitro Toxicity (MEIC) program the special list of chemical agents have been proved in four standardized tests of the acute toxicity realized on the following species: *Artemia franciscana*, *Streptocephalus proboscideus*, *Brachionus calyciflorus*, and *Brachionus plicatilis*. The mortality and toxic effects expressed as LC$_{50/24}$ (i.e., the concentration of an agent at which 50 per cent of the tested animals are dead after 24 hrs) were chosen as criteria of the toxicity. Very good correlations of results among the different testing systems have been found. Furthermore, positive correlations between the tests on lower organisms and conventional tests on rats have also been achieved (Calleja & Persoone, 1992).
The “toxkit” tests are based on dormant stages – cysts or ephippia - that hatch up to 24 hrs before the start of a test. Their advantage is particularly the elimination of time and space consuming laboratory maintenance of the test organisms. Commercially available are ROTOXKIT F, ROTOXKIT M, THAMNOTOXKIT F, ARTOXKIT M, etc.

3.1 Alternative biotests on Artemia species

Suitability of the Artemia species for the toxicity studies was recognized 80 years ago (Boone, 1931). The first bioassay on Artemia salina was offered by Michael et al. (1956). Twenty four years later the standardized acute toxicity bioassay - Artoxkit M (Microbiotests, Inc., Mariakerke /Gent/ - Belgium) was developed (Vanhaecke et al., 1980). The reliability and validity of this commercially available test have been confirmed by a large international study (Vanhaecke & Persoone, 1981). Later on, Solis et al. (1993) developed the Artemia Microwell Cytotoxicity Assay.

Various modifications of the biotests on Artemia species have been used for the acute or rarely chronic toxicity testing of a large number of inorganic substances: such as cadmium (Hadjispyrou et al., 2001; Sarabia et al., 1998a, 2002, 2006; Brix et al., 2006), mercury (Sarabia et al., 1998b), chromium (Hadjispyrou et al., 2001), stannum (Hadjispyrou et al., 2001), zinc (Brix et al., 2006), copper (Browne, 1980, Brix et al., 2006), arsenic (Brix et al., 2003), potassium permanganate, potassium bichromate, and silver nitrate (see Boone, 1931), phenolic compounds (Guerra, 2001), and trace elements (Petrucci et al., 1995). From the organic substances have been tested: organic solvents (Barahona-Gomariz et al., 1994), acrylonitrile (Tong et al., 1996), antifouling agents (Okamura et al., 2000), oil dispersants (Zillioux, et al., 1973), phorbol esters (Kinghorn et al., 1977), phthalates (Van Wezel et al., 2000), carbamates (Barahona & Sánchez-Fortún, 1998), atropine (Barahona & Sánchez-Fortún, 1998), anesthetics (Robinson et al., 1965), anthelminths (Oliveira-Filho & Paumgarten, 2000), herbicides, insecticides, pesticides (Varó et al., 1997, 2002), mycotoxins (Schmidt, 1985; Panigrahi, 1993; Hlywka et al., 1997), pharmaceuticals (Touraki et al., 1999, Parra et al., 2001), pollutants (Knust & Sodergren, 1994), opiates (Richter & Goldstein, 1970), various plant extracts (Cáceres et al., 1998), or toxins (Granade et al. 1976; Vezie et al., 1996; Beattie et al., 2003). The tests on the genus Artemia have also been used to specify biological effects of some physical factors, such as ionizing radiation (Grosch & Erdman, 1955; Easter & Hutchinson, 1961), radionuclides (Boroughs et al., 1958), or UV (Dattilo et al., 2005). In the project Biostack carried out on Apollo 16 the effects of cosmic irradiation on cysts of Artemia franciscana were studied (Ruther et al., 1974; Graul et al., 1975; Heinrich, 1977).

3.2 Advantages and disadvantages of the Artemia species for toxicity test

The Artemia species have been found convenient for various short- or long-term toxicity testing. In spite of this fact, several criticisms against their suitability for such purpose have been published (Persoone & Wells, 1987; Nunes et al., 2006). The most important of them is the lower sensitivity of the Artemia species to several chemical or physical agents in comparison to the other invertebrate test organisms (Sorgeloos et al., 1978; Song and Brown, 1998, Okamura et al., 2000; Guerra, 2001; Nalecz-Jawecki et al., 2003; George-Ares, et al., 2003; Mayorga et al., 2010). The second disadvantage is a decreased solubility of some
chemical substances in saline or sea medium (e.g. Mayorga et al., 2010), however, this problem may be overcome by using convenient co-solvents (see bellow).

In general, the reliability of results of the *Artemia* tests may be affected by various conditions of a test, such as temperature, pH, chemical composition of the medium, oxygen, photoperiod, nutrients, some population effects, type of sexual reproduction, etc. (Soares, et al., 1992). For example, George-Ares et al. (2003) reported dependence of the *Artemia* test on the concentration and composition of medium, or on the age of nauplii. Some populations of *Artemia* species may consist of strains with different reproduction strategies (sexual versus parthenogenetic reproduction) and different sensitivity to tested agents. But, Triantaphyllidis et al. (1994) suggested a simple method to separate nauplii of bisexual and parthenogenetic strains by hatching at different temperatures, moreover specific strains may be distinguished by DNA-analysis (Abatzopoulos et al., 1997). Of course, the effects of almost all test factors mentioned above may be easily overcome by the strict standardization of test conditions. An important disadvantage of all alternative tests is their unsuitability to test the chemical agents that require metabolic activation in mammals.

On the other hand, the *Artemia* alternative tests offer many advantages to favour them as convenient for the standardized testing in ecotoxicology: high adaptability to variety of testing conditions, high fecundity, bisexual versus parthenogenetic reproduction strategies, small body size, varied nutrient resources, high hatchability, simple availability, and low cost of the tests. High sensitivity of the *Artemia* specimens to some chemical agents has also been reported. For example, Oliveira-Filho and Paumgarten (2000) found the higher sensitivity of *Artemia* to niclosamide in comparison with *Daphnia similis* or *Ceriodaphnia dubia*. Hlywka et al. (1997) have shown similar sensitivities to the mycotoxin fumonisin B1 in the *Artemia* test and the chicken embryo screening test. And Parra et al. (2001) compared the sensitivity of the acute toxicity *Artemia* test of several plant extracts with corresponding tests on mice and found significant correlations. Undoubtedly, the *Artemia* test is a suitable, sufficiently accurate, simple, and inexpensive alternative to pre-screening chemical toxicity with mammals. Of course, all results derived from the *Artemia* or any other invertebrate test have to be validated by bioassays on mammals.

Our test is based on the cysts produced for purposes of the aquaculture and aquarium practice. The test was established in 1992 (Dvorak, 1995), especially for the extensive dynamic studies including simultaneous treatments with two agents, or possibly of a radiation and a chemical agent. The total expenses of our tests are lower than those of the commercial toxkits.

### 4. Methods

#### 4.1 Hatching of cysts

Cysts are hatched in water of the following composition (Table 1). The total concentration of salts is 4.7%. In the following text this water is specified as the “sea water”.

In water of this quality the brine shrimps mature in 37 days and start their reproduction, providing they are fed with a convenient diet, and the sea water is changed every third day. The hatching proceeds at temperature of 25 °C and the first larvae occur after 24 hrs. During the hatching process the sea water is aerated by a membrane compressor.
4.2 Solubility of chemical agents

Solubility of various chemical agents in 4.7% sea water is often decreased. Consequently, in our pharmacological studies the concentration of sea water is reduced to 0.9% (i.e. the concentration of the blood salts). But, in this low concentration of salt water *A. franciscana* does not consume glucose; consequently, in such low concentration of medium this sugar cannot be used to prolong the test.

The solubility of some low soluble chemical agents may be improved by specific co-solvents. For example, increased solubility of some insoluble purine inhibitors of cyclin-dependent kinases may be achieved by the non-ionic tensides: polysorbate 80 and poloxamer Pluronic F68.

The co-solvent is a non-water solvent that can be mixed with water and the resulting mixture has increased capability to solve some chemical compounds. For example, dimethylsulfoxide (DMSO) is used as solvent and co-solvent for its ability to solve the most of organic or inorganic chemical compounds, as well as an enhancer of some medicines into skin. It is a colourless liquid, hygroscopic and miscible with water and many other organic solvents (Rowe et al., 2006). DMSO is included in some veterinary medicines. In medicaments used in human medicine it is used only rarely for some undesirable effects. The analgetic, antiflogistic, vasodilatative, myorelaxative, and antiviral effects of DMSO have been reported (Jacob & Herschler, 1986; Rowe et al., 2006). The systemic toxicity of this drug in vivo is rather low (Rowe et al., 2006).

The tweens are used in the pharmaceutical, cosmetics or food industries. Polysorbate 80 (Tween 80) is the tenside widely used in pharmaceuticals as helping agents for the preparation of oral or parenteral suspensions. In parenteral medicines the content of this agent must not exceed the concentration in which it causes a hemolysis of erythrocytes (Rowe et al., 2006). It is an oil-like, yellowish but clear liquid with a specific odour and rather bitter taste. This substance is miscible with water or ethylacetate, but insoluble in fatty oils or a paraffine liquid. The pH value of a 5% water solution varies from 6.0 to 8.0. Chemically it is a polyoxyethylenesorbitanmonooleate and it consists of the partial esters of sorbitol and its anhydrides, copolymerized with cca 20 moles of ethyleneoxide per mol of sorbitol and sorbitol anhydrides with various fatty acids, mainly the oleic acid. The toxicity of polysorbate 80 is rather low – the acute toxicity LD₅₀ in mice treated perorally is 25 g.kg⁻¹.

| salt (per analysis) | g.L⁻¹ |
|---------------------|------|
| NaCl                | 23.900 |
| MgCl₂.6H₂O          | 10.830 |
| CaCl₂.6H₂O          | 2.250  |
| KCl                 | 0.680  |
| Na₂SO₄.10H₂O        | 9.060  |
| NaHCO₃              | 0.200  |
| SrCl₂.6H₂O          | 0.040  |
| KBr                 | 0.099  |
| H₃BO₃               | 0.027  |

Table 1. Composition of hatching water.
(Rowe et al., 2006). We used polysorbate 80 produced by the Jan Kulich firm (Hradec Kralove, Czech Republic) applied in mixture with DMSO. Concentration of the stock solution of polysorbate 80 was 200 g L\(^{-1}\). In our experiments we combined the concentrations 10.0 g L\(^{-1}\) of polysorbate 80 and 5.0 g L\(^{-1}\) of DMSO.

Poloxamers are non-ionic agents used in pharmaceuticals as emulgators, surfactants, wetting agents, or solubilisers. In pharmaceuticals, they are widely used as helping agents for various medications. These agents are non-toxic, non-irritable and are not metabolised in the living organisms. For their inability to cause a hemolysis (Rowe et al., 2006) they are used in various parenteral medicaments. Poloxamers are commercially produced under the names of Lutrol® or Pluronic® (e.g. commercial name of the Poloxamer 188 used in this study is Pluronic F68). The acute toxicity of these agents applied in mice is 15 g kg\(^{-1}\), if applied perorally, or 1 g kg\(^{-1}\), when applied parenterally (Rowe et al., 2006). In our experiments we used a concentration of 10.0 g L\(^{-1}\) applied with DMSO.

4.3 Treatment with polychlorinated biphenyls and ionizing radiation

Delor 103 is one of the polychlorinated biphenyls (PCB’s). These compounds produce colloidal solutions. The initial delor 103 colloidal solution in salt water was obtained by three-day agitation. The excessive undissolved delor 103 was then removed. For the treatment we used the concentration of 4.5 ng L\(^{-1}\). This final concentration was detected by the capillary gas column chromatography analysis (41 peaks identified; from dichloride to pentachloride derivatives). The Kovats index was used as a method of evaluation.

As source of ionizing radiation we used the beta emitter strontium 89 in solution with the volume activity of 33 kBq L\(^{-1}\). The isotope was supplied by the Amersham Comp. as strontium chloride dissolved in water. The manufacturer guarantees specific strontium activity of 1.85 - 7.40 GBq g\(^{-1}\) and purity < 0.5% \(^{88}\)Sr < 0.1% \(^{90}\)Sr, with pH within the 5.0 - 9.0 range. The final activity used in experiments was obtained by re-calculation of the original activity to the reference date. Potassium dichromate K\(_2\)Cr\(_2\)O\(_7\) of analytical grade was used in the concentration of 5 mg L\(^{-1}\) and cadmium chloride CdCl\(_2\) 2.5H\(_2\)O in the final concentration of 20 mg L\(^{-1}\).

4.4 Subacute toxicity

Ten freshly hatched nauplii are placed into a polystyrene Petri dish (diameter 60 mm) by a thin plastic Pasteur pipette. The total volume of salt water is adjusted to 9 ml per dish. All the agents tested are dissolved in sea water, always in a concentration ten times higher than that finally required for a treatment. Consequently, to a dish with 9 ml of the standard sea water 1 ml of the tenfold concentrated stock solution is supplemented (in case of the control dishes 1 ml of the standard salt water). If the simultaneous treatment of two agents is tested, the original volume of sea water with specimens per dish is 8 ml and then each agent is supplemented in 1 ml of its stock solution.

The biotest proceeds in darkness, at temperature of 20 ± 0.5 °C, in covered dishes, and without aeration. The specimens are not fed, and consequently, under these conditions they start to die of starvation after 120 hrs. When the temperature is increased to 24 °C the lifespan of unfed nauplii is shortened to about 96 hrs.
For every experimental combination five Petri dishes (50 specimens in total) are used. Alive specimens are counted after 24, 48, 72, 96, or 120 hrs (in case of prolonged tests). The dead nauplii are not removed but their presence has not any deleterious effect on the final results of the biotest. If more than 10% of the experimental specimens died in the control group, the exposure was discarded. The evaluation of results of an experimental series should be always performed by one person.

As dead we consider the specimens that do not move even after the stimulation by movement of water caused by rotation of the dish. The evaluation is carried out under a microscope or magnifying glass. To express results of such a test, two terms are, generally used: mortality and lethality. The first means the percentage of dead specimens from the total number of originally healthy specimens. On the other hand, lethality means the percentage of dead specimens from all ill specimens. We use the latter term because, in fact, all specimens exposed to the agents tested at first become ill and only then some of them die.

4.5 Long term toxicity

The prolongation of life span of nauplii in biotests may be achieved by feeding. However, utilisation of various artificial diets, algae or yeast is always complicated with the contamination with some undesired chemical substances or metabolites. Hence, we prolong the Artemia test by the treatment with 3% solution of glucose that is used by the specimens as a source of energy. The other parameters of prolonged tests correspond to those of the standard test. The evaluation of results is carried out in steps of 24 hrs for 10 days (Dvorak et al., 2005).

4.6 Expression of concentrations

Concentrations of agents may be expressed in toxicology in mmol.L⁻¹ or mg.L⁻¹ (in some papers is used ppm unit, but it is not included in the International System of Units). The first method is used to compare different chemical compounds which toxicity depends on only part of the molecule, such as cations of metals, or molecules accompanied with bound molecules of water. In these cases the concentration is expressed only for the active part of the molecule (e.g. mmol Cd²⁺.L⁻¹). This expression accentuates rather more the chemical properties of the molecule than its biological effects. Actually, a biological effect often depends on physical properties of the agent, particularly on its solubility and absorption. Consequently, we prefer the expression of concentrations in units of weight, providing that the precise chemical composition of the substance is included. For this reason we express the drug concentrations in our experiments in mg.L⁻¹.

4.7 Evaluation of results

All 50 specimens (i.e., 5 Petri dishes with 10 specimens each) of one experimental combination are evaluated as a whole. Each specimen is judged as alive or dead (criterion see above). Significance of results is calculated according to Hayes (1991). The lethality is expressed in percentage of all specimens used. Results are displayed in the form of 3D charts – concentrations versus time of treatment.

4.8 Validity of results

The principle validity criterion of results is the mortality in control dishes lower than 10%. This criterion is generally used in the Artemia, as well as, in Daphnia toxicity tests. Because the
specimens in our experimental system are not fed and starve, it is sometimes difficult to keep this criterion at the exposures higher than 72 hrs. In this case, we terminate the experiments at this exposure, in spite of the fact that in the exposure of 96 hrs the nauplii are the most sensitive to some toxic agents and difference between the treated and control group is the highest. In the prolonged experiments (120 hrs or more) in medium supplemented with glucose (see above) we use the higher criterion of mortality (20%). The glucose is probably not sufficient to secure the optimal development of the experimental specimens. Consequently, we generally finish experiments when the mortality of the control group exceeds 20%.

5. Results and discussion

5.1 Simultaneous treatment with $^{89}$Sr and low concentrations of cadmium, chromium, and delor 103

For principal information about the treatment with polychlorinated biphenyls and ionizing radiation see 4.3. Results are summarized in Fig. 2. Neither the treatment with delor 103 or $^{89}$Sr per se, nor the simultaneous treatment with both agents did not affect survival of the experimental specimens (results of the separate treatments are not included in figure, simultaneous treatment see ‘PCB + Strontium 89’ in Fig. 2). On the contrary, potassium dichromate increased the lethality up to 27% (at 72 hrs). The simultaneous treatments with two agents (cadmium + potassium dichromate) or with all four agents altogether (cadmium + potassium dichromate + delor 130 + $^{89}$Sr) resulted in the expressive increase in lethality of the experimental specimens.

![Image](https://www.intechopen.com)

Fig. 2. Effect of simultaneous treatment with strontium 89 [33 kBq.L$^{-1}$], PCB - delor 103 [4.5 ng.L$^{-1}$], cadmium chloride (CdCl$_2 \cdot$2H$_2$O) [Cadm.chloride; 20 mg.L$^{-1}$], and potassium dichromate [Potass.dichromate; 5 mg.L$^{-1}$] on lethality of A. franciscana; CG – control group (no agents).
Isotope $^{89}$Sr is a beta emitter; consequently, it is impossible to satisfactorily convert its activity to a dose the experimental specimens actually receive during the exposure. For this reason, the strontium effects are given in the volume activity units of kBq.L$^{-1}$. The hexavalent chromium can damage DNA, decrease its synthesis and enhance the oxidative processes in cells. The analogous effects are induced by the ionizing radiation, as well.

Both agents - strontium 89 or delor 103 - attack the membrane integrity, the result of which may be the increased penetration of potassium dichromate or cadmium chloride into cells. Cadmium induces production of metallothioneins (e.g. Huska et al., 2008) that express, among others, the radioprotective effects (Benova et al., 2006). This study demonstrated the suitability of the Artemia test to evaluate effects of rather complex experiments with simultaneous treatments of several chemical and/or physical agents.

5.2 Changes in morphology of Artemia specimens during treatment with gamma rays or inhibitors of cyclin-dependent kinases

In our previous paper the lethality of A. franciscana after irradiation with the ionizing radiation was studied. The LD$_{50}$ value was estimated 96 hrs after the irradiation with 600 - 700 Gy (Dvorak & Benova, 2002). This result is in accord with the phylogenetic position of this species.

In the following experimental series the freshly hatched nauplii were irradiated by the gamma rays ($^{60}$Co) with 100, 250, 500, or 1000 Gy (dose rate of 2.7 kGy.h$^{-1}$). At the time of 72 or 96 hrs after the irradiation the specimens were killed and fixed with formaldehyde. The morphological changes were determined and documented by microphotography.

During the standard development of the control group we observed segmentation of the thoracic part and formation of appendages. The development of those structures in the specimens irradiated with 100 Gy (96 hrs after the irradiation) was comparable with control specimens. But after the irradiation with 250 Gy the segmentation was less apparent, and at the highest doses (500 or 1000 Gy) it was not observed at all.

Another morphological change observed after the irradiation was an atypical development of the intestine epithelium. In control specimens the ratio of the height of epithelium to the diameter of intestine lumen was 1 : 1.2. After irradiation the ratio changed: with 100 Gy to 1 : 1.5, with 250 Gy to 1 : 1.5, and even with 500 Gy to 1 : 1.8. When the doses increased to 1000 Gy the intestine epithelium was indistinguishable. The high sensitivity of intestine epithelium to irradiation corresponds with high sensitivity of this tissue in vertebrates caused by the high mitotic activity of intestine cells. Consequently, the effects of irradiation on the intestine epithelium in the Artemia species were consistent with the analogous response in vertebrates.

We also looked for some morphological changes caused by the inhibitors of the cyclin-dependent kinases – olomoucin or roscovitin to compare them with effects of the ionizing radiation. Both agents, in the concentration of 100 mg.L$^{-1}$, caused only slight changes in the intestine epithelium of nauplii stages of A. franciscana, but they did not affect the segmentation of thorax and further development of appendages. Also the length or morphology of the body were not affected by these drugs. In the concentration of 50 mg.L$^{-1}$ no effects on the intestine epithelium were apparent.
5.3 Toxicity of newly synthesized inhibitors of cyclin-dependent kinases

The synthetic inhibitors of cyclin-dependent kinases (CDKI) represent possible anticancer drugs. Their anticancer activities have been confirmed in clinical pilot studies. The first synthetic purine inhibitor of cyclin-dependent kinases was olomoucin (Vesely et al., 1994). Since that time a lot of other derivatives were synthesized (Hajduch et al., 1999; Sklenar, 2006). One of the most promising derivatives is the lipophilic roscovitin. In the *Artemia* experimental system we tested the toxicity of some newly synthesized CDKI, and the results compared with the toxicity of olomoucin.

The aim of this study was to test the acute toxicity of roscovitin and three other CDKI, namely TSP 1, TSP 2, and TSP 3 in sea water (salinity 0.9%). As co-solvents were used dimethylsulfoxide (DMSO), polysorbate 80 (TW), or Pluronic F68 (PL). Their toxicities were compared with the control groups (in charts labelled as CG).

The mixture of DMSO and PL occurred inconvenient because of the precipitation of roscovitin that started 24 hrs after dissolving. On the other hand, the mixture of polysorbate 80 and DMSO proved useful and the agents did not precipitate, at all.

![Fig. 3. Effect of olomoucin or roscovitin (100 mg.L⁻¹) dissolved in mixture of 10.0 g.L⁻¹ of polysorbate 80 (TW) plus 10.0 g.L⁻¹ of dimethylsulfoxide (DMSO) on lethality of *A. franciscana*. CG - control group without any TW or DMSO.](www.intechopen.com)
agents was 100 mg.L\textsuperscript{-1}. The toxicity of these agents was evaluated over 168 hrs. The values of lethality of both agents were significantly different from the toxicity of the control group (TW+DMSO) at the exposures higher than 96 hrs. Both agents, i.e., olomoucin and roscovitin gave the comparable results except for the exposure of 144 hrs, at which the lethality of roscovitin was significantly higher than that of olomoucin.

The simultaneous treatment with DMSO and polysorbate 80 in the concentrations used in our experiments did not cause any deleterious effects on the tested specimens (TW + DMSO). On the contrary, this treatment actually prolonged duration of the test as result of the polysorbate 80 used as energy source by the experimental specimens. Hence, results of the toxicity tests were compared with the control group treated with mixture of DMSO and polysorbate 80 (TW + DMSO).

In the following bar chart (Fig. 4) we compare toxicity of the newly synthesized CDKI, namely TSP 1 (=6-benzylamino-2-(2-aminoethylamino)-9-isopropyl-purine), TSP 2 (=6-benzylamino-2-[(E)-(4-aminocyclohexylamino)]-9-isopropylpurine), and TSP 3 (= 6-benzylamino-2-(3-aminopropylamino)-9-isopropyl purine) with the toxicity of olomoucin. All substances were dissolved in DMSO and used in the concentrations of 100 mg.L\textsuperscript{-1}. While the toxicities of TSP 1 or TSP 3 were comparable with the toxicity of olomoucin, TSP 2 occurred significantly more toxic than olomoucin. The toxicity of TSP 2 was even higher than that of cadmium; hence, this agent was discarded from the further pharmacotoxicological studies. In general, chromium, cadmium, or zinc are more toxic than olomoucin, roscovitin, TSP 1, or TSP 3 (Sklenar, 2006).

![Fig. 4. Comparison of effects of newly synthesised CDKI (TSP 1, TSP 2, and TSP 3) with effects of olomoucin (100 mg.L\textsuperscript{-1}) on lethality of A. franciscana. All substances were dissolved in dimethylsulfoxide (DMSO, 15 g.L\textsuperscript{-1}); CG - control group without DMSO.](#)
5.4 Anti-oxidative and pro-oxidative effects of ascorbic acid

The action of ascorbic acid on the oxidative effects of hydrogen peroxide (0.4 g.L\(^{-1}\)) was studied. Both, the pro-oxidative and anti-oxidative effects of ascorbic acid were evaluated. The first occurred at the concentration of ascorbic acid 0.3 g.L\(^{-1}\), while the latter at 0.1 g.L\(^{-1}\). The highest anti-oxidative effect was evaluated at the exposure of 96 hrs when the lethality decreased by 34% in comparison with that of hydrogen peroxide per se (Fig. 5).

The search for the antagonists of the oxidative effects of reactive forms of oxygen (free radicals) undoubtedly has been one of the priorities of recent pharmacology. Such antagonistic effects are generally dependant on the concentration of the drug used. In our experiments the ascorbic acid in a concentration of 0.1 g.L\(^{-1}\) partially decreased the oxidative effect of hydrogen peroxide. This finding is in accord with the well-known anti-oxidative efficiency of the ascorbic acid (Young et al., 1992). On the other hand, the higher concentration of ascorbic acid (0.3 g.L\(^{-1}\)) operated contrary and increased the final oxidative effect in the simultaneous treatment with 0.4 g.L\(^{-1}\) of hydrogen peroxide. These results are in agreement with the clinical studies showing that the treatments with ascorbic acid at concentrations higher than 1000 mg per day have the pro-oxidative effects, while the effects of the lower concentrations are contrary (Soska et al., 1994). Consequently, the alternative Artemia test proved competent for the extensive studies of simultaneous treatments with two agents.

![Fig. 5. Effects of simultaneous treatments with ascorbic acid (Asc.ac.; 0.1 or 0.3 g.L\(^{-1}\)) and hydrogen peroxide (Hydr.per.; 0.4 g.L\(^{-1}\)) on lethality of A. franciscana; CG - control group.](www.intechopen.com)
5.5 Anti-oxidative effect of *Orobanche flava* plant extract

To the *Orobanche* genus belong annual or perennial parasitic plants that lack chlorophyll, and consequently, fail to perform photosynthesis. They get the nutrients from their host dicotyledonous plants. In our experiments we used *Orobanche flava* (order Scrophulariales, family Orobanchaceae). The pharmacodynamic studies of this plant have shown the positive effects on fatigue, and supporting effects on the immunity system or male potency. From tissues of this plant various chemical substances have been extracted, such as phenylpropanoid glycosides, verbascoside, orobanchoside, tropones, tocochromanoles, fatty acids, phytosterols, carotenoids, or D-mannitol (Erickson, 1969). Some of these agents are supposed to produce anti-oxidative effects. Consequently, effects of the alcoholic extract of this plant on the treatment with hydrogen peroxide were evaluated by the *Artemia* alternative test.

The oxidative effects were induced by hydrogen peroxide in concentrations of 0.4 g.L\(^{-1}\) or 0.2 g.L\(^{-1}\). Use of such low concentrations of hydrogen peroxide is on principle in concordance with the real concentrations causing the oxidative effects in vivo. The co-solvent DMSO in concentration of 12.5 g.L\(^{-1}\) was used to improve solubility of the plant extract in sea water. In the other experiments, where the effect of the plant extract on the toxicity of DMSO was studied, the latter was used in the concentrations of 50 g.L\(^{-1}\) or 100 g.L\(^{-1}\). The alcoholic plant extract was dissolved in DMSO and applied in the concentrations of 0.05, 0.10, 0.25 or 0.50 g.L\(^{-1}\). All concentrations of the plant extract proved non-toxic, or they even decreased the lethality in comparison with the control dishes. At the concentration of 0.5 g.L\(^{-1}\) no dead specimens were found. This effect was probably caused by some carbohydrates present in the extract that served as sources of energy for the experimental specimens.

![Graph showing decrease in toxic effect of dimethylsulfoxide (DMSO) induced by the plant extract from *Orobanche flava* (Or); CG - control group (no agents); according to Hrbasova (2006).](www.intechopen.com)
In the first model experiment we studied the effect of the plant extract on toxicity of the co-solvent per se (Fig. 6). In two experimental combinations (100 g.L\(^{-1}\) DMSO + 0.50 g.L\(^{-1}\) of plant extract, or 50 g.L\(^{-1}\) DMSO + 0.25 g.L\(^{-1}\) of plant extract) the toxicity of DMSO was significantly reduced. The other experimental combinations proved rather ineffective.

In the second model experiment the anti-oxidative effects of the plant extract on the oxidative effects of hydrogen peroxide were evaluated. In all simultaneous treatments with the plant extract (0.05 g.L\(^{-1}\) or 0.10 g.L\(^{-1}\)) plus hydrogen peroxide (0.2 g.L\(^{-1}\) or 0.4 g.L\(^{-1}\)) the anti-oxidative effects of the plant extract were detected (Fig. 7). While in the combinations with 0.10 g.L\(^{-1}\) of the plant extract the anti-oxidative effects were significant, after 96 or 120 hrs, the effect of 0.05 g.L\(^{-1}\) occurred positive but insignificant. Thus, we came to the conclusion that the Artemia test had proved fully competent for the extensive studies of simultaneous treatments with combinations of drugs.

![Graph showing decrease in oxidative effect of hydrogen peroxide](image)

**Fig. 7.** Decrease in oxidative effect of hydrogen peroxide (Hp; 0.2 g.L\(^{-1}\) or 0.4 g.L\(^{-1}\)) induced by the plant extract from Orobanche flava (Or; 0.05 or 0.10 g.L\(^{-1}\)) dissolved in dimethylsulfoxide (DMSO); CG - control group treated with 3% glucose; according to Hrbasova (2006).

### 5.6 Prolonged treatment with cadmium

Cadmium, as well as zinc or copper, damage SH groups of proteins, resulting in effects analogous to ionizing radiation. These metals bind in tissues to metallothioneins so abundantly, that they may represent up to 11 per cent of the molecular weight of the metallothioneins. If the synthesis of metallothionein is insufficient the toxic effects of cadmium become apparent (Jaywickreme & Chatt, 1990). Lethal treatment of cells, such as
lymphocytes, with cadmium may result in the apoptosis (El Azouzi et al., 1994). On the other hand, production of the metallothioneins induced by cadmium may lead to the radioprotective effects (Benova et al., 2006). Genes that control production of the metallothioneins affect also the repair of DNA (Privezencev et al., 1996).

In this test survival of nauplii is prolonged by the treatment with 3% glucose. The aim was to study the effect of cadmium on Artemia in the prolonged test system (Fig. 8). The life span of nauplii was prolonged even up to 240 hrs. The mortality of nauplii in the control dishes treated with glucose decreased to only 4% after 216 hrs of exposure. Hence, 3% glucose proved useful for the prolongation of the Artemia test up to 10 days. As expected, the toxicity of cadmium increased with increasing concentration and exposure time.

The supplement of glucose to the Artemia test system even reduced the toxic effects of cadmium (Dvorak et al., 2005). This finding was significant for the exposure from 24 to 72 hrs and concentrations higher than 50 mg.L\(^{-1}\). The same relationship was valid also for the LC\(_{50}\) values expressed in mg.L\(^{-1}\) (value without glucose versus value with glucose): at 24 hrs - value 238 versus 482, at 48 hrs - value 250 versus 482, and at 72 hrs - value 195 versus 263. In the same paper we also compared LC\(_{50}\) values for three agents used to standardize the microbiotests, namely K\(_2\)Cr\(_2\)O\(_7\), CdCl\(_2\)•2H\(_2\)O, and ZnSO\(_4\)•7H\(_2\)O. Surprisingly, while the dependence of the toxicity of zinc sulphate on the exposure time was linear, that of the cadmium chloride was logarithmic, and that of potassium dichromate even quadratic.

Fig. 8. Effect of cadmium chloride on lethality of A. franciscana in test prolonged by glucose (3%), CG - control group supplemented with 3% glucose; according to Dvorak et al. (2005).
6. Conclusion

Eighty years of usage of the Artemia species in toxicology testing, and hundreds of papers published all over the world on this theme, have confirmed the exclusivity of this invertebrate genus not only in toxicology or pharmacology, but in biology and medicine as a whole. Our almost twenty years experience with A. franciscana have proved that the Artemia system is not only convenient for acute toxicity testing, but for extensive dynamic studies, as well, including model experiments with simultaneous treatments with two or more agents of chemical or physical character, as has been demonstrated in this chapter. The Artemia tests are fully competent to belong to the group of test systems used for the pre-screening of toxic agents.

7. Acknowledgment

The study was funded by the grant No. MSM6215712402 of the Ministry of Education, Youth and Sports of Czech Republic.

8. References

Abatzopoulos, T.J.; Triantaphyllidis, G.V.; Beardmore, J.A. & Sorgellos, P. (1997). Cyst Membrane Composition as a Discriminant Character in the Genus Artemia. (International Study on Artemia, LV). Journal of the Marine Biology Association of the United Kingdom, Vol.77, No.1, (February 1977), pp. 265-268, ISSN 0025-3154

Amat, F. (1985). Biología de Artemia. Informes Técnicos del Instituto de Investigaciones Pesqueras.

Anderson, D. T. (1967). Larval Development and Segment Formation in the Branchiopod Crustaceans Limnadia stanley King (Conchostraca) and Artemia salina (L) (Anostraca). Australian Journal of Zoology, Vol.15, pp. 47-91, ISSN 0004-959X

Bagley, D.M., Waters, D. & Kong, B.M. (1994) Development of a 10-day Chorioallantoic Membrane Vascular Assay as an Alternative to the Draize Rabbit Eye Irritation Test. Food and Chemical Toxicology, Vol.32, No.12, (December 1994), pp. 1155–60, ISSN 0278-6915

Balls, M.; Fentem, J. & Jooint, E. (1992). Issues: Animal Experiments. Hobson Publishing, Cambridge, pp. 2-15

Barahona, M.V. & Sánchez-Fortún, S. (1998). Toxicity of Carbamates to the Brine Shrimp Artemia salina and the Effect of Atropine, Iso-ompa and 2-PAM on Carbaryl Toxicity. Environmental Pollution, Vol.104, No.3, (1999), pp. 469-476, ISSN 0269-7491

Barahona-Gomariz, M.V.; Sanz-Barrera, F.; Sánchez-Fortún, S. (1994). Acute Toxicity of Organic Solvents on Artemia salina. Bulletin of Environmental Contamination and Toxicology, Vol.52, No.2, (May 1994), pp. 766-771, ISSN 0007-4861

Beattie, K.A.; Ressler, J.; Wiegand, C., Krause, E.; Codd, G.A.; Steinberg, C.E.W. & Pflugmacher, S. (2003). Comparative Effects and Metabolism of Two Microcysts and Nodularin in the Brine Shrimp Artemia salina. Aquatic Toxicology, Vol.62, (February 2003), No.3, pp. 219-226, ISSN 0166-445X
Benova, K.; Dvorak, P.; Falis, M. & Danova, D. (2006). Elimination of Negative Effects of cadmium in *Artemia franciscana* by Exposure to Ionizing Radiation. *Folia Veterinaria*, Vol.50, No.3, (Supplementum 2006), pp. 21-22, ISSN 03010724

Benesch, R. (1969). Zur Ontogenie und Morphologie von *Artemia salina* L. *Zoologische Jahrbucher. Abteilung für Anatomie und Ontogenie der Tiere*, Vol.86, pp. 307-458

Blaise, C. (1991) Microbiotests in Aquatic Toxicology: Characteristics, Utility and Prospects. *Environmental Toxicology and Water Quality*, Vol.6, No.2, (May 1991), pp. 145-155, ISSN 1053-4725

Boone, E. & Baas-Becking, L.G.M. (1931). Salt Effects on Eggs and Nauplii of *Artemia salina* L. The Journal of General Physiology. July 20, pp. 753-763. Available from http://jpg.rupress.org/content/14/6/753.full.pdf

Boroughs, H.; Townsley, S.J. & Ego, W. (1958) The Accumulation of Y90 from an Equilibrium Mixture of Sr90 – Y90 by *Artemia salina*. (L), Vol.3, No.4, pp. 413-317. Available from http://www.aslo.org/lo/lo/toc/vol_3/issue_4/0413.pdf

Brix, K.V.; Cardwell, R.D.; Adams, W.J. (2003). Chronic Toxicity of Arsenic to the Great Salt Lake Brine Shrimp, *Artemia franciscana*. *Ecotoxicology and Environmental Safety*, Vol. 54, No.2, (February 2003), pp. 169-175, ISSN 0147-6513

Brix, K.V.; Gerdes, R.M.; Adams, W.J. & Grosell, J. (2006). Effects of Copper, Cadmium, and Zinc on the hatching success of brine shrimp (*Artemia franciscana*). *Archives of Environmental Contamination and Toxicology*, Vol.51, No.4, (November 2006), pp. 580-583, ISSN 0090-4341

Browne, T. A. (1980). Acute Response versus Reproductive Performance in five Strains of Brine Shrimp exposed to Copper Sulphate. *Marine Environmental Research*. Vol.3, pp. 185-193, ISSN 0141-1136

Browne, R. A. (1992). Population Genetics and Ecology of *Artemia*: Insights into Parthenogenetic Reproduction. *Trends in Ecology and Evolution*, Vol.7, No.7, pp. 232-237, ISSN 0169-5347

Browne, R.A.; Li, M.; Wanigasekera, G.; Simonek, S.; Brownlee, D.; Eiband, G. & Cowan, J. (1991). Ecological, Physiological and Genetic Divergence of Sexual and Asexual (diploid and polyploid) Brine Shrimp (*Artemia*). In: Menon, J. (ed.): *Advances in Ecology*. Vol. 1. Trivandrum, India: Council of Research Integration. pp. 41-52

Cáceres, A.; López, B.; González, S.; Berger, I.; Tada, I. & Maki, J. (1998). Plants used in Guatemala for the Treatment of Protozoal Infections. I. Screening of Activity to Bacteria, Fungi, and American Trypanosomes of 13 Native Plants. *Journal of Ethnopharmacology*, Vol.62, No.3, (October 1998), pp. 195-202, ISSN 0378-8741

Calleja, M.C. & Persoone, G. (1992). Cyst-based Toxicity Tests: 4. The Potential of Ecotoxicological Tests for the Prediction of Acute Toxicity in Man as Evaluated on the First ten Chemicals of the MEIC Programme. *Alternatives to Laboratory Animals*, Vol.20, No.3, p. 396-405, ISSN 02611929

Dattilo, A.M.; Bracchini, L.; Carlini, L.; Loiselle, S. & Rossi, C. (2005). Estimate of the Effects of Ultraviolet Radiation on the Mortality of *Artemia franciscana* in Naupliar and Adult Stages. *International Journal of Biometeorology*, Vol.49, No.6, (July 2005), pp. 388-395, ISSN 0020-7128

Dvorak, P. (1995). Modified *A. salina* Test for Evaluation of Interactions of Heterogenous agents (In Czech). In: *Toxicita a biodegradabilita odpadu a latek vyznamnych ve vodnim*
prostredi. Sborník referátů z 7. konference, Milenovice. Výzkumný ústav rybárskeho a
hydrobiologického, Vodnany, pp. 25-29. ISBN 80-85887-02-9

Dvorak, P.; Benova, K. (2002). The Investigation of Interactions of Low Doses of Ionizing
Radiation and Risk Factors by Means of Artemia salina Biotest. Folia Veterinaria, Vol. 46, No. 4, pp. 195-197, ISSN 03010724

Dvorak, P.; Sucman, E. & Benova, K. (2005). The Development of a Ten-day Bio-test using
Artemia salina Nauplii. Biologia, Vol.60, No.5, (September 2005), pp. 593-597, ISSN
0006-3088

Easter, S.S. & Hutchinson, F. (1961). Effects of Radiations of Different LET on Artemia Eggs.
Radiation Research, Vol.15, No.3, pp. 333-340, ISSN 0033-7587

El Azzouzi, B.; Tsangaris, G.R.; Pollegrini, O.; Manuel, Y.; Benveniste, J. & Thomas, Y.
(1994). Cadmium Induces Apoptosis in a Human T Cell Line. Toxicology, Vol.88,
No.1-3, (March 1994), pp. 127-139, ISSN 0300-483X

Erickson, R. L. (1969). Hot Water–Soluble Glycosides: Location in the Tissue of Populus
grandidentata Bark. (Doctor’s Dissertation). The Institute of Paper Chemistry,
Appleton, Wisconsin. June 1969. pp. 66

Fentem, J. & Balls, M. (1993). Biology now! In: Developing Alternatives to Animal
Experimentation. Hobson Publishing, Cambridge

Fouradzieva, S.; Dittrat, F.; Lukavsky, J. (1995). Toxicity of Trichlorethylene on Green Algae
(In Czech). In: Toxicita a biodegradabilita odpadu a lak vyznamnych ve vodnim
prostredi. Sborník referátů ze 7. konference, Milenovice. Výzkumný ústav rybárskeho a
hydrobiologického, Vodnany, pp. 38-41, ISBN 80-85887-02-9

George-Ares, A.; Febbo,E.J.; Letinski, D.J.; Yarusinsky, J.; Safadi, R.S. & Aita, A.F. (2003) Use
of Brine Shrimp (Artemia) in Dispersant Toxicity Tests: Some Caveats. In: Proceedings of International Oil Spill Conference, 2003

Gilchrist, B.M. (1960). Growth and Form of the Brine Shrimp Artemia salina. Proceedings of the
Zoological Society of London, Vol.152, No.946, pp. 221-235, ISSN 0962-8452

Granade, H.R.; Cheng, P.C. & Doorenbos, N.J. (1976). Ciguatera, I. Brine Shrimp (Artemia
salina L.) Larval Assay for Ciguatera toxins. Journal of Pharmaceutical Sciences, Vol. 65,
No.9, pp. 1414-1415, ISSN 0022-3549

Graul, E.H.; Ruther, W.; Heinrich, W.; Alkofner, O.C.; Kaiser, R.; Pfohl, R.; Schopper, E.;
Henig, G.; Schott, J.U. & Bucker, H. (1975). Radiobiological Results of the Biostack
Experiment on Board Apollo 16 and 17. Life Sciences Research in Space, Vol.13,
pp. 153–159, ISSN 0006-3185

Grosch, D.S. & Erdman, H.E. (1955). X-ray Effects on Adult Artemia. Biological Bulletin,
Vol. 108, No.3, pp. 277-282. ISSN: 0006-3185

Guerra, R. (2001). Ecotoxicological and Chemical Evaluation of Phenolic Compounds in
Industrial Effluents. Chemosphere, Vol.44, No.8, (September 2001), pp. 1737-1747,
ISSN 0045-6535

Hadjispyrou, S.; Kungolos, A. & Anagnostopoulos, A. (2001). Toxicity, Bioaccumulation,
and Interactive Effects of Organotin, Cadmium and Chromium on Artemia franciscana. Ecotoxicology and Environmental Safety.
Vol.49, No.2, (June 2001), pp. 179-186, ISSN 0147-6513

Hajduch, M.; Havlicek, L.; Vesely, J.; Novotny, R.; Mihal, V. & Strnad M. (1999). Synthetic
Cyclin-dependent Kinase Inhibitors - New Generation of Potent Anti-cancer Drugs.

www.intechopen.com
Conference Information: 3rd International Symposium on Drug Resistance in Leukemia and Lymphoma, (March 1998), Amsterdam, Netherlands

Source: Drug resistance in leukemia and lymphoma III, Book Series: *Advances in Experimental Medicine and Biology*, Vol.457, pp. 341-353, ISSN 0065-2598, ISBN 0-306-46055-6

Hayes, W.J. (1991). Dosage and other Factors Influencing Toxicity. In: *Handbook of Pesticide Toxicology*. General Principles. Hayes, W.J., Laws, E.R. (Ed.), Academic Press. Vol. 1, pp. 39-97, ISBN 0-12-334161-2

Heinrich, W. (1977). Calculation of the Radiobiological Effects of Heavy Ions on Eggs of *Artemia salina* flown in the Biostack Experiments. *Life Sciences Research in Space*, Vol. 15, pp. 157–163, ISSN 0006-3185

Hentschel, E. (1968). Die postembryonalen Entwicklungsstadien von *Artemia salina* bei verschiedenen temperaturen (Anostraca, Crustacea). *Zoologischer Anzeiger*, Vol.180, pp. 73-384, ISSN 00445231

Hertel, H. (1980). The Compound Eye of *Artemia salina* (Crustacea). I. Fine Structure when Light and Dark Adapted. *Zoológische Jahrbücher für allgemeine Zoologie und Physiologie der Tiere*, Vol. 84, No.1, pp.1-14, ISSN 0044-5185

Hlywka, J.J.; Beck, M.M. & Bullerman, L.B. (1997). The Use of the Chicken Embryo Screening Test and Brine Shrimp (*Artemia salina*) Bioassays to Assess the Toxicity of Fumonisins B, Mycotoxin. *Food and Chemical Toxicology*, Vol. 35, No.10-11, (October-November 1997), pp. 991-999, ISSN 0278-6915

Horowitz, M. (1970). Notes on Bioassay Techniques for Several Soil-applied Substituted Ureas. *Israel Journal of agricultural Research*, Vol.21, No.2, pp. 281-284, IDS Number G2245

Hrabasova, L. (2006). Evaluation of Anti-oxidative Effects of Plant Extract of *Orobanche flava* by *Artemia salina* Biotest (In Czech). *Graduation thesis*. University of Veterinary and Pharmaceutical Science, Faculty of Pharmacy, Brno, p. 41

Huska, D.; Krizkova, S.; Beklova, M.; Havel, L.; Zahnalek, J.; Diopan, V.; Adam, V.; Zeman, L.; Babula, P. & Kizek, R. (2008). Influence of Cadmium(II) Ions and Brewery Sludge on Metallothinein Level in Earthworms (*Eisenia fetida*) – Biotransforming of Toxic Wastes. *Sensors*, Vol.8, No.2, (February 2008), pp. 1039-1047, ISSN 1424-8220

Kikuchi, S. (1971). The Fine Structure of the Alimentary Canal of the Brine Shrimp, *Artemia salina*: The Midgut. *Annual Report of Iwate Medical University, School of Liberal Arts and Sciences*, Vol.7, p. 15-47

Kinghorn, A. D.; Haarjes, K.K. & Doorenbos, N.J. (1977). Screening Procedure for Phorbol Esters using Brine Shrimp (*Artemia salina*) Larvae. *Journal of Pharmaceutical Sciences*, Vol.66, No.9, pp. 1362-1363, ISSN 0022-3549

Knulst, J. & Södergren, A. (1994). Occurrence and Toxicity of Persistent Pollutants in Surface Microlayers Near an Incineration Plant. *Chemosphere*, Vol.29, No.6, (September 1994), pp. 1339-1347, ISSN 0045-6355

Kratky, B.A. & Warren, G.F. (1971). The use of three simple rapid Bioassays on forty-two Herbicides. *Weed Research*, Vol.11, No.4, pp. 257-262, ISSN 0043-1737

Jacob, S.W. & Herschler, R. (1986). Pharmacology of DMSO. *Cryobiology*, Vol.23, No.1, (February 1986), pp.14-27, ISSN 0011-2240
Jayawickreme, C.K. & Chatt, A. (1990). Studies on Zinc and Cadmium - bound Proteins in Bovine Kidneys by Biochemical and Neutron Activation Techniques. *Biological Trace Element Research*, Vol.26-27, (July-December 1990), pp. 503-512, ISSN 0163-4984

Martin, J.W. & Davis, G.E. (2001). An Updated Classification of the Recent Crustacea. In: *Natural History Museum of Los Angeles County, Science Series 39*, Brown, K.V. (Ed.) (December 2001), pp. 1-124, ISSN 1-891276-27-1

Mayorga, P.; Pérez, K.R.; Cruz, S.M. & Cáceres, S. (2010). Comparison of Bioassays using the Anostacran Crustaceans *Artemia salina* and *Thamnocephalus platyurus* for Plant Extract Toxicity Screening. *Revista Brasileira de Farmacognosia-Brazilian Journal of Pharmacognosy*, Vol.20, No.6, (December 2010), pp. 897-903, ISSN 0102-695X

Michael, A.S.; Thompson, C.B. & Abramovitz, M. (1956). *Artemia salina* as a Test Organism for Bioassay. *Science*, Vol.123, No.3194, pp. 464, ISSN 0036-8075

Nalecz-Jawecki, G.; Grabinska-Sota, E.; & Narkiewicz, P. (2003). The Toxicity of Cationic Surfactants in four Bioassays. *Ecotoxicology and Environmental Safety*, Vol. 54, No.1, (January 2003), pp. 87-91, ISSN 0147-6513

Nunes, B. S.; Carvalho, F. D.; Guilhermino, L. M. & Van Stappen, G. (2006). Use of the Genus *Artemia* in Ecotoxicity Testing. *Environmental Pollution*, Vol.144, No.2, (November 2006), pp. 453-462, ISSN 0269-7491

Okamura, H., Aoyama, I., Liu, D., Maguire, R.J., Pacepavicius, G.J. & Lau, Y.L. (2000). Fate and Ecotoxicity of the New Antifouling Compound Irgarol 1051 in the Aquatic Environment. *Water Research*, Vol. 34, No.14, (October 2000), pp. 3523-3530, ISSN 0043-1354

Oliveira-Filha, E.C. & Paumgartten, F.J.R. (2000). Toxicity of *Euphorbia milii* Latex and Niclosamide to Snails and Nontarget Aquatic Species. *Ecotoxicology and Environmental Safety*, Vol.46, No.3, (July 2000), pp. 342-350, ISSN 0147-6513

Panigrahi, S. (1993). Biosassay of mycotoxins using terrestrial and aquatic, animal and plant species. *Food Chemistry and Toxicology*, Vol.31, No.10, (OCT 1993), pp. 767-790, ISSN 0278-6915

Parra, L.A.; Silva, Y.; Guerra, S.I.; Iglesias, B.L. (2001). Comparative Study of the Assay of *Artemia salina* L. and the Estimate of the Medium Lethal Dose (LD50) Value in Mice, to Determine Oral Acute Toxicity of Plant Extracts. *Phytotherapy*, Vol.8, No.5, pp. 395-400, ISSN 09447113

Pazourek, J. (1992). Simulation of biological systems (In Czech). Grada, ISBN 80-85623-13-7, Praha, Czech Republic, pp. 284

Persoone, G., Wells, P.G. (1987). *Artemia* in Aquatic Toxicology: A Review. In: *Artemia Research and its Applications. Morphology, Genetics, Strain Characterization, Toxicology*. Sorgellos P.; Bengtson, D.A.; Decleir, W. and Jaspers, E. (Eds.).Vol. I. Universa Press, Wetteren, Belgium

Petrucci, F.; Caimi, S.; Mura, G. & Caroli, S. (1995). *Artemia* as a Test Organism of Environmental Contamination by Trace Elements. *Microchemical Journal*, Vol.51, No.1-2, (February-April 1995), pp. 181-186, ISSN 0026-265X

Pilla, E. J. S. & Beardmore, J. A. (1994). Genetic and Morphometric Differentiation in Old World Bisexual Species of *Artemia* (the brine shrimp). *Heredity*, Vol.73, No.1, (July 1994), pp. 47-56, ISSN 0018-067X
Privezencev, K.V.; Sirota, N.P.; & Gaziev, A.I. (1996). Effect of Simultaneous Treatment of Cadmium and Gamma-radiation on Damage and Repair of DNA in Lymphoid Tissues of Mice (In Russian). Radiacionnaja biologija, radioekologija. Vol.36, No.2, pp. 234-239, ISSN 0869-8031

Richter, J.A. & Goldstein, A. (1970). The Effects of Morphine-like Compounds on the Light Responses of the Brine Shrimp Artemia salina. Psychopharmacologia, Vol.17, No.4, pp. 327-337, IDS Number G7382

Robinson, A.B.; Manly, K.F.; Anthony, M.P.; Catchpool, J.F. & Pauling, L. (1965). Anesthesia of Artemia Larvae: Method for Quantitative Study. Science, Vol.149, No.3689, pp. 1255-1258, ISSN 0036-8075

Rowe, R.C., Sheskey, P.J. & Owen, S.C. (2006). Handbook of Pharmaceutical Excipients. 5. Royal Pharmaceutical Society of Great Britain, 918 p., ISBN 0-85369-618-7, London, Great Britain

Ruppert, E.E. & Barnes, R.D. (1994). Invertebrate Zoology 6, Saunders College Publishing, ISBN 0-03-026668-8, New York, USA

Ruther, W.; Graul, E.H.; Heinrich, W.; Allkofer, O.C.; Kaiser, R. & Cuer, P. (1974). Preliminary Results on the Action of Cosmic Heavy Ions on the Development of Eggs of Artemia salina. Life Sciences Research in Space, Vol.12, pp. 69–74, ISSN 0006-3185

Sarabia, R.; Varó, I.; Torreblanca, A.; Del Ramo, J.J.; Pastor, A.; Amat, F. & Díaz-Mayans, J. (1998a). Accumulation of Cadmium in several Crains of Artemia. Cuadernos de Investigación Biológica. Vol.20, pp.435-438

Sarabia, R.; Torreblanca, A.; Del Ramo, J.J. & Díaz-Mayans, J. (1998b). Effects of low Mercury Concentration Exposure on Hatching, Growth and Survival in the Artemia Strain La Mata Parthenogenetic Diploid. Comparative Biochemistry and Physiology A-Molecular & integrative Physiology, Vol.120, No.1, (May 1998), pp. 93-97, ISSN 1095-6433

Sarabia, R.; Del Ramo, J.; Varó, I.; Diaz-Mayans & Torreblanca A. (2002). Comparing the Acute Exposure to Cadmium Toxicity of Nauplii from Different Populations of Artemia. Environmental Toxicology and Chemistry. Vol.21, No.2, (February 2002), pp. 437-444, ISSN 0730-7268

Sarabia, R.; Varó, I.; Amat, F. Pastor, A.; Del Ramo, J.J.; Díaz-Mayans, J. & Torreblanca, A. (2006). Comparative Toxicokinetics of Cadmium in Artemia. Archives of Environmental Contamination and Toxicology, Vol.50, No.1, (January 2006), pp. 111-120, ISSN 0090-4341

Schmidt, R. (1985). Optical Motility Test for the Detection of Trichothecenes using Brine Shrimps. Mycotoxin Research, Vol. 1, pp. 25-29

Sklenar, Z. (2006). Evaluation of Toxicity and Morphological Changes Induced by Purine Inhibitors of Cyclin-dependent Kinases (In Czech). Dissertation thesis. Faculty of Veterinary Hygiene and Ecology. University of Veterinary and Pharmaceutical Sciences, Brno. 138 p.

Soares, A.M.V.M.; Baird, D.J. & Calow, P. (1992). Interclonal Variation in the Performance of Daphnia magna Straus in Chronic Bioassays. Environmental Toxicology and Chemistry, Vol.11, No.10, (October 1992), pp. 1477-1483, ISSN 0730-7268
Solis, P.N.; Wright, C.W.; Anderson, M.M.; Gupta, M.P. & Phillipson, J.D. (1993). A Microwell Cytotoxicity Assay using Artemia salina (Brine Shrimp). Planta Medica, Vol. 59, No.3, (June 1993), pp. 250-252, ISSN 0032-0943

Song, M.Y. & Brown, J.J. (1998). Osmotic Effects as a Factor Modifying Insecticide Toxicity on Aedes and Artemia. Ecotoxicology and Environmental Safety, Vol.41, No.2, (October 1998), pp. 195-202, ISSN 0147-6513

Sorgeloos, P.; Remiche-van der Wielen, C. & Persoone G. (1978). The Use of Artemia Nauplii for Toxicity Tests A Critical Analysis. Ecotoxicology and Environmental Safety, Vol.2, pp. 249-255, ISSN 0147-6513

Soska, V.; Zechmeister, A.; Lojek, A. & Podrouzkova, B. (1994). Effect of L-ascorbic acid on Lipids and Free Radicals in Blood of Patients with Hyperlipoproteinemia (In Czech). Klinicka Biochemie a Metabolismus, Vol.23, No.2, pp. 86-88, ISSN 1210-7921

Tong, Z.; Hongjun, J. & Hualian, Z. (1996). Quality Criteria of Acrylonitrile for the Protection of Aquatic Life in China. Chemosphere, Vol.32, pp. 2083-2093, ISSN 0045-6535

Vanhaecke, P.; & Persoone, G. (1981). Report on an Intercalibration Exercise on a Short-term Standard Toxicity Test with Artemia Nauplii (ARC-test). Institut Snational de la Santé et de la Recherche Médicale (INSERM), Vol.106. pp. 359-376

Vanhaecke, P.; Persoone, G.; Claus, C. & Sorgeloos, P. (1980). Research on the Development of a Short-term Standard Toxicity Test with Artemia. The Brine Shrimp Artemia. In: Exology, Culturing. Use in Aquaculture. Vol.1, pp. 263-285

Van Wezel, A.P.; Van Vlaardingen, P.; Posthumus, R.; Crommentuijn, G.H.; Sijm, D.T.H.M. (2000). Environmental Risk Limits for Two Phthalates, with Special Emphasis on Endocrine Disruptive Properties. Ecotoxicology and Environmental Safety, Vol.46, No.3, (July 2000), pp. 305-321, ISSN 0147-6513

Varó, I.; Navarro, J.C.; Amat, F. & Guilhermino, L. (2002). Characterisation of Cholinesterases and Evaluation of the Inhibitory Potential of Chlorpyriphos and Dichlorvos to Artemia salina and Artemia parthenogenetica. Chemosphere, Vol.48, No.6, (August 2002), pp. 563-569, ISSN 0045-6535

Varó, T.; Taylor, A.C.; Ferrando, M.D. & Amat, F. (1997). Effect of Endosulfan Pesticide on the Oxygen Consumption Rates of Nauplii of Different Spanish Strains of Artemia. Journal of Environment Science and Health-Part B.: Pesticides, Food Contaminants and Agricultural Wastes, Vol.32, No.3, pp. 363-375, ISSN 0360-1234

Vesely, J.; Havlicek, L.; Strnad, M.; Blow, J.J.; Donella-Deana, A.; Pinna, L.; Letham, D.S.; Kato, J.; Detivaud, L.; Leclerc, S. & Meijer, L. (1994). Inhibition of Cyclin-dependent Kinases by Purine Analogues. European Journal of Biochemistry, Vol.224, No.2, (September 1994), pp. 771-786, ISSN 0014-2956
Vezie, C.; Sivonen, K.; Brient, L.; Bertru, G. & Lefeuvre, J.C. (1996). Development of Toxic Cyanobacteria in Western France-Detection of Toxicity with *Artemia salina* Tests. *Annales de Limnologie-International Journal of Limnology*, Vol. 32, No. 2, pp. 123-128, ISSN 0003-4088

Young, I.S.; Torney, J.J. & Trimble, E.R. (1992). The Effect of Ascorbate Supplementation on Oxidative Stress in the Streptozotocin Diabetic Rat. *Free Radical Biology and Medicine*, Vol. 13, No. 1, (July 1992), pp. 41-46, ISSN 0891-5849

Zillioux, E.J; Foulk, H. R.; Prager, J.C. & Cardin, J.A. (1973). Using *Artemia* to Assay Oil Dispersant Toxicities. *Journal Water Pollution Control Federation*, Vol. 45, No. 11, pp. 2389-2396, ISSN 0043-1303
This is a good book on upcoming areas of Ecotoxicology. The first chapter describes genotoxicity of heavy metals in plants. The second chapter offers views on chromatographic methodologies for the estimation of mycotoxin. Chapter three is on effects of xenobiotics on benthic assemblages in different habitats of Australia. Laboratory findings of genotoxins on small mammals are presented in chapter four. The fifth chapter describes bioindicators of soil quality and assessment of pesticides used in chemical seed treatments. European regulation REACH in marine ecotoxicology is described in chapter six. X-ray spectroscopic analysis for trace metal in invertebrates is presented in chapter seven. The last chapter is on alternative animal model for toxicity testing. In conclusion, this book is an excellent and well-organized collection of updated information on Ecotoxicology. The data presented in it might be a good starting point to develop research in the field of ECOTOXICOLOGY.

How to reference
In order to correctly reference this scholarly work, feel free to copy and paste the following:

Petr Dvorak, Katarina Benova and Jiri Vitek (2012). Alternative Biotest on Artemia franciscana, Ecotoxicology, Dr. Ghousia Begum (Ed.), ISBN: 978-953-51-0027-0, InTech, Available from: http://www.intechopen.com/books/ecotoxicology/alternative-biotest-on-artemia-franciscana
© 2012 The Author(s). Licensee IntechOpen. This is an open access article
distributed under the terms of the Creative Commons Attribution 3.0
License, which permits unrestricted use, distribution, and reproduction in
any medium, provided the original work is properly cited.