Ecotoxicology and environmental protection

Dr. Éva Milinki

Introduction

As a result of intensive industrial and agricultural development, and the intensification of consumerism in developed countries, the amount of unnatural substances released into the environment has increased dramatically in the past decades. The above phenomenon has led to the occurrence of many environmental disasters, that is we ourselves have caused the impoverishment of the biodiversity of wildlife in environmental elements, and the appearance of numerous diseases related to pollution. Mankind has ignored the fact that the same effect that is harmful to other living organisms and can cause their destruction, also poses a human health risk and can lead to serious problems. The intertwining between ecotoxicological and environmental research, the publication of the findings has greatly contributed to the emergence of a more environmentally-conscious generation in which commitment to a healthy environment is a kind of an „attitude”.

Ecotoxicology, as an interdisciplinary science, plays an important role in shaping this attitude, promotes the ability to integrate knowledge, and an approach to natural scientific knowledge as a whole. Today the boundaries of competence between the different disciplines can no longer be drawn clearly, instead of fragmentation a complex approach to the problems is indispensable. An integrated approach is required to understand the ecotoxicological processes, to put the relationships in an appropriate professional context, therefore the chapter provides an adequate foundation for deepening the multidisciplinary approach of students participating in higher level VET and BSc courses on the subject.

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1. Introduction to Ecotoxicology

1.1 A Brief History of Toxicology

The name toxicology is derived from the Greek word „toxikon" - which means an arrow. The use of poisons seems to be as old as mankind. Ancient Indian tribes already used in their fights the small poison dart frogs living in South-America. The skin secretions of these frog species (Dendrobates, Phyllobates species) contain alkaloids that are among the most potent natural poisons in the world (Photo 1). The Indians used this poisonous skin secretion to coat the tips of their blow darts. Besides the toxic effect of substances found in nature, man soon discovered that they had healing properties as well, when applied in an appropriate amount and form.

Photo 1: Dendrobates truncatus (Zsolt Benkó)

Ancient Egyptian Culture: In the 4th-3rd millennium B.C. medicinal herbs were already cultivated in Egypt, as they were regularly used for healing. The Ebers Papyrus – found with a mummy – already recorded several plants effectively used for the treatment of diseases (e.g. white wormwood, saffron, hemlock). And henna in paste form was used as a fungicide (Photo 2).

Photo 2: The Ebers Papyrus

Ancient Greek and Roman culture:

Hippocrates (460 B.C. - 377 B.C.)

An ancient Greek physician, known as the founder of medicine and medical ethics. He emphasized the importance of observation and the healing power of nature. He advocated the use of medicinal herbs in the treatment of diseases. He prepared potions and ointments for his patients from gentian, cinnamon, and hemlock. Hippocrates is therefore regarded as the founding father of both modern medicine and natural medicine.

He recognized the hazard of overdose, and proclaimed that even substances found in nature should be applied only in an appropriate amount: **„The majority of maladies may be cured by the same things that cause them.”**

Cornelius Celsus (50 B.C. - 25 A.D.)

A Roman encyclopaedist, his book on medicine entitled „De Medicina Libri” described 250 medicinal herbs, underlining the positive effects of mallow and fennel (Animation 1).

Animation 1: Medicinal herbs used in antiquity

Curled mallow, a member of the Malvaceae, and fennel stimulate the digestive system, their metabolic effects can help to control body weight. The knowledge of both the therapeutic and the toxic effect of hemlock dates back to antiquity. It has a beneficial effect in the treatment of asthma, however at higher concentrations the juice of spotted hemlock is so toxic that it was used as a method of execution, those sentenced to death were forced to drink hemlock. In larger amounts it causes respiratory paralysis.

Middle Ages:

Paracelsus (1493 – 1541)

A physician and naturalist, the father of toxicology. He prepared his medicines mainly from medicinal herbs and metals (he made potions and ointments). He used as medicine otherwise toxic substances, e.g. mercury, sulphur and iron compounds. Paracelsus proved with experiments that the amount of the substance entering the body plays a primary role in the toxic effect.

He proclaimed that the dose makes the poison. **"All things are poison and nothing is without poison: the dose alone makes a thing not poison."**

Paracelsus was the founder of medical chemistry. His work led to the rapid development of pharmacology. A series of originally herb-based medicines were prepared semi-synthetically or synthetically. These synthetic preparations do not occur naturally, thus they are foreign substances to the human organism, and therefore they have numerous side- and after-effects.

1.2 The Place and Role of Ecotoxicology within the Field of Toxicology

Originally toxicology was a field of medicine and pharmacology, but developed into an independent discipline. The most famous practitioners of toxicology in the last century, e.g. Claude Bernard, were pharmacists. They studied poisoning due to drug overdose, and its symptoms. The intensive development of industrial and agricultural production played a primary role in the fact that toxicology became an independent discipline. In particular, the large-scale development of the chemical industry should be noted: numerous new chemical substances appeared (modern plastics, fertilizers, pesticides) and within a few decades they caused a crisis as pollutants in environmental systems.

As a consequence of these changes, now there are several new subdisciplines within the field of toxicology (KISS 1997):

- Human toxicology
- Environmental toxicology
- Ecotoxicology
- Industrial and agricultural toxicology
- Food toxicology
- Occupational toxicology
- Chemical-analytical toxicology

In addition to the fields listed above, in the past decade significant development has been made in the field of genotoxicological research, and today we are witnessing the emergence of nanotoxicology.

Ecotoxicology is a multidisciplinary science that combines the findings of several disciplines at a higher quality level providing a new approach, and integrates the results achieved so far (Animation 2). Its most important foundation is ecology, as a science, as based on that the interactions between species in the ecosystem, and the role of toxic substances in the changes occurring in the structure and function of the ecosystem can be determined.

Animation 2**:** The interdisciplinarity of ecotoxicology (based on D. Connell et al.)

Toxicology (science of poisons): is a science that studies toxic substances, the physical and chemical properties of poisons, their detection, production and effects on living organisms.

The field of study of **ecology** was already defined by Ernst Haeckel in 1866: „the interactions between living organisms and their environment at the level of individuals, populations (interbreeding groups of individuals of the same species living together in the same biotope at the same time), communities and the biosphere (the part of the Earth where life exists)".

The term **ecotoxicology** was coined by Truhaut, who defined ecotoxicology as „the branch of toxicology concerned with the study of toxic effects, caused by natural or synthetic pollutants, to the constituents of ecosystems (animal, including human, vegetable and microbial) in an integral context."

Callow (1993) defined ecotoxicology as a field of science that "studies known and new pollutants and their ecological effects on the environment". Besides the findings of toxicology and ecology, ecotoxicology integrates and utilizes the results of physiology, chemistry, mathematics, geology, genetics and microbiology as well. Chemical tests are indispensable for the understanding of abiotic and biotic interactions. And mathematical, computer modelling is necessary for predicting future changes, and for extrapolation.

1.3 An Overview of the International and National Situation of Ecotoxicological Research

Changes of the past decades (the intensive development of industrial and agricultural production, changes in lifestyle) have made necessary the reinterpretation and understanding of the processes occurring in the ecosystems. As a result of human activity, new material and energy flows have been created and are created even today, threatening the integrity of our environment. As a consequence of anthropogenic impacts, the previous interactions between abiotic and biotic systems have loosened up, and new interactions have developed instead of them. As a negative result of these interactions, not only the environment, but the existence and future of the whole mankind is also threatened. The biosphere providing the conditions of life is no longer able to adapt to the changes caused by these accelerated processes. Today the effects exerted on the environment by the technosphere, created as a result of human activity, are more decisive than the effects caused by the forces of nature. The diverse effects of the technosphere on the biosphere, and the chain of – usually negative – responses given to them by the biosphere have led to an environmental crisis.

The wide range of foreign pollutants released into environmental systems has resulted in a more effective extension of both ecological and toxicological research. However, initially these tests were conducted in parallel rather than complementing each other.

Therefore ecologists for a long time failed to recognize the relationships between the new chemical substances released into the environment and the changes observed in the ecosystems. They focused their attention primarily on the effects of climate change, invasive species, and research into biodiversity. Toxicologists, on the other hand, concentrated on determining the properties of new chemicals, developing methods for setting thresholds, and failed to analyze the actual effects on the ecosystems.

The breakthrough was the publication of the work of Rachel Carson entitled „Silent Spring” (CARSON 1962). Her book drew attention to the adverse environmental consequences of chemical substances and their metabolites, and she already recognized

that the toxic effect of a given substance can be significantly influenced by interactions between substances. Carson was the first one to point out the dangers of DDT, she revealed evidence of its carcinogenic effect, with this she launched the first environmental movements, and it is also thanks to her that state-level environmental institutions were established. As a result of her persistent work, the World Wide Fund for Nature (WWF) called for a global ban on the use of DDT.
Research in the past decade has supported the assumption that both ecological and ecotoxicological tests are required for the realistic assessment of environmental risks, and for taking the necessary measures (DE ZWART et al. 2005). The importance of ecotoxicological tests has been highlighted by some industrial accidents with serious consequences, e.g. the Minamata Bay Disaster, or the Seveso Chemical Disaster near Milan in Italy.

In Japan wastewater containing mercury was released into Minamata Bay from a company producing acetaldehyde (1938). Until 1970 300 people died due to the accident as a result of the pollution, and this drew the attention of the world to the hazards of heavy metal pollution. The methylmercury released from the chemical plant accumulated in fish and shellfish, and passed through the food chain to people living in the area. Mercury, due to its harmful effect on the nervous system, caused coordination and other serious movement disorders, blindness and dementia. Due to its harmful effect on fetal development, many children were born with birth defects in the villages around Minamata Bay.
In Italy an environmental disaster occurred in 1976 due to an explosion at a chemical plant in Seveso near Milan. A cloud of toxic gas containing dioxin was released, and the soil in the surrounding areas was polluted to such an extent that it was safe for residents to move back only 3 years later. As a result of the poisoning, first skin disease, then liver, kidney, immune system damage, and cancerous degeneration were recorded in the exposed persons in the years after the disaster.

In the field of ecotoxicological research, seven markedly distinct periods can be identified in the past 60 years (BLAISE 1998):

- 1950s (dark ages – environmental aspects were not considered at all, the effects of pollutants were not studied)
- 1960s (pollution mapping started, toxicological testing, mainly fish-testing)
- 1970s (pollutant emission regulations, setting thresholds)
- 1980s (a shift towards an ecotoxicological approach)
- 1990s (the widespread use of microbiological testing)
- 2000s (the appearance of ecotoxicogenetic methods)
- 2010s (the emergence of nanoecotoxicology)

Nanotechnology works with very small sizes, it studies changes at the atomic level, with the objective to effect the directed assembly of atoms of the desired substance. This technology allows the creation of nanometre-sized objects (in the field of information technology and medicine, today almost unimaginable results can be expected in nanotechnology, starting a chain reaction of innovation). However, the emergence of this technology involves potential environmental hazards as well (COLVIN 2003). Nanoscale metal oxides are present in every ecosystem, and wildlife has adapted to them through evolution. Nevertheless, artificially created nanoparticles can pose a potential hazard (HANDY et al. 2008), but at present such statements can only be regarded as mere speculation (NOWACK 2009).

The national fields of ecotoxicological research follow the international trend. The serious pollution of environmental systems, the occurrence of disasters is not an unknown phenomenon in Hungary either. The effects of the cyanide and heavy metal pollution of the Rivers Szamos and Tisza in 2000 could be felt for years. After the passing of the pollution wave 150 tons of dead fish were collected. In the year following the cyanide pollution, there were specific expectations about whether the Hungaricum Tisza mayfly (*Palingenia longicauda*) survived the natural disaster, and the spectacular swarming would take place in the summer. Fortunately, the expert reports clearly found that no species became extinct as a result of the pollution, only their population declined, and mayfly swarming could be seen on certain sections of the river as usual (Photo 3).

Photo 3: Mayfly swarming area

The effects of the cyanide and heavy metal pollution could be felt for years, and the heavy metal load in sediments due to deposition can become mobilized and threaten the surrounding wildlife through the food chain at any time (FLEIT 2001, CSENGERI et al. 2001, LAKATOS et al.2003, REGŐS et al. 2005).

The red sludge disaster of 2010 near Ajka in Hungary should also be mentioned among the environmental disasters caused by human negligence. The dam of a red sludge reservoir located between the village of Kolontár and the town of Ajka, and owned by the Hungarian Aluminium Production and Trade Company (MAL) collapsed, and hundreds of thousands of cubic metres of highly alkaline, caustic sludge was spilled over an area of about 40 square km (Animation 3).

Animation 3: The red sludge disaster in the region between Kolontár and Ajka (source: MTI/Sándor H Szabó)

The red sludge pollution covered almost everything on an area of 40 square km, devastated the wildlife of the Creek Torna, and 10 people lost their lives. The Hungarian tragedy triggered international cooperation and support. Red sludge had not caused a disaster of this magnitude before in the world. The disaster recovery work was started jointly with experts from the European Union.

In addition to the harmful effects of industry, agriculture is another major emitter of pollutants. Pesticides drift away from the area of application (off-target effect), get washed into natural waters (run-off effect), and exert their effects in non-target organisms (DARVAS & POLGÁR 1998). The group of chlorinated hydrocarbons was the first group of pesticides in the case of which adverse properties, such as accumulation, persistence, biomagnification, and carcinogenicity, drew attention to the hazards of chemical plant protection. Agricultural ecotoxicology, in addition to assessing the toxic effect of the applied pesticides, is also concerned with the applicability of a given agent, the practical aspects of approval (DARVAS & SZÉKÁCS 2006, VÁRNAGY 1995, 2005).

Today, in accordance with the importance of the field, ecotoxicological research is a completely independent field of research under the direction of the Hungarian Academy of Sciences, and in higher education ecotoxicology, as a subject, is offered as an independent course.

1.4 The Relationship between Ecotoxicology and Environmental Protection

On the basis of ecotoxicological tests it can be predicted how chemicals released into the environment modify the structure and function of a given ecosystem, what degree of risk they present to living organisms. Naturally, based on our present knowledge the effects on the whole ecosystem and their consequences cannot be explored, however the results of toxicological and ecotoxicological tests can be extrapolated to real biological communities. Ecotoxicology starts from the environmental concentration of a given chemical, and on the basis of the available data tries to assess the environmental risk of the tested substance (GRUIZ et al. 2001).

Toxicological tests form an important part of environmental impact assessment, the objective of which is to assess the changes occurring in environmental conditions as a result of human activity. In impact assessment, on the basis of a preliminary impact study and a detailed impact assessment, the environmental risk of a given chemical can be quantified (FÖLDI & HALÁSZ 2009). This quantifiability helps in making the appropriate environmental protection, environmental management and environmental policy decisions (Figure 1).

Figure 1: The role of ecotoxicology in environmental protection (based on K. GRUIZ)

The impact of chemicals released into environmental systems can be assessed on the basis of the dose-response and concentration-response relationships. And on the basis of the obtained results the non-harmful concentrations and the proposed thresholds can be determined.

Test Questions:

Circle the correct answer!

1) Who made the following statement? „The dose makes the poison. All things are poison and nothing is without poison: the dose alone makes a thing not poison."
   a) Hippocrates
   b) Paracelsus
   c) Cornelius Celsus
   d) Callow
2) What is the field of study of ecotoxicology?
   a) the interactions between living organisms and their environment
   b) the ecological effects of new pollutants on the environment
   c) the understanding of normal physiological phenomena
   d) ethological observations

Match the letters to the correct numbers!

a) Minamata disease b) dioxin c) Silent Spring d) red sludge e) works with very small sizes

3) Rachel Carson
4) methylmercury
5) the environmental disaster in Seveso
6) nanotechnology
7) Kolontár

Decide which of the following statements are true, and which are false (mark with T or F)!

8) Heavy metals in the sediments of waters can become mobilized at any time.
9) The toxic effect of a substance is not dose-dependent.
10) The results of ecotoxicological tests are a great help in making environmental protection, environmental management and environmental policy decisions.

2. The Basic Concepts of Toxicology and Ecotoxicology

2.1 Factors Influencing Toxicity

Toxicity is the special physical, chemical and biochemical activity of substances that poses a potential hazard to living organisms. Toxicity cannot be expressed with a single parameter, it is the function of several variables (KISS 1997).

The toxicity of a given substance is determined primarily by the following factors:

- dose
- duration of action
- method of exposure
- species used for testing
- bioavailability

2.1.1 Dose

The amount of a substance administered to, absorbed by a living organism (mg/kg of body weight). The toxicity of the same dose can vary as a function of body weight (Animation 4).

Animation 4: Body weight-dose relationship

The toxicity of every substance can be characterized by a dose-effect function. This function shows how the degree of the harmful effect increases as the dose of the given substance is increased (Figure 2).

Figure 2: Dose-effect curve (J. Szőnyi)

The biological response suitable for detecting, indicating a harmful effect at a tested dose is called a symptom. The Anglo-Saxon literature uses the term „endpoint" for this. The developing symptoms can have varying degrees of intensity (ranging from mild to severe), or can be described in the form of „have/have not" (0 or 1) (Figure 3).

Figure 3: Dose-effect function (parametric levels for changes in the carbon monoxide concentration in blood and the developing symptoms) (based on I. KISS)

The effect of a given substance is determined not on the basis of the response of a single individual, but a population of multiple individuals. Members of the population have different sensitivity to the tested substance, therefore the incidence of toxic symptoms in the population shows some degree of deviation. If all individuals in the population had the same sensitivity to the effect, then none of the individuals would be destroyed up to a certain threshold, and all of them would be destroyed above the threshold. However, by increasing the amount of the effect, a gradual increase can be observed in the number of destroyed test organisms, as individual members of the population have different sensitivity to the tested substance or effect (NÉMETH 1998).

The relationship between the probability of an effect and exposure gives an S-shaped curve. The dose-effect curve is also a sigmoid curve, where individuals more sensitive than the average (hypersensitive) are shown in the left-hand part of the curve, and more tolerant individuals (hyposensitive) in the right-hand part. The slope of the dose-response function can be different for different substances. The steeper the obtained curve, the higher its reliability, as then the individual differences are smaller. In toxicology usually mortality is used to assess the symptoms, as it can be measured clearly and expressed quantitatively. When expressing the symptoms in the form of „have/have not", the toxicity value changes between 0 and 1. 1 is the lethal value, which is regarded as the most severe symptom in toxicology. The incidence of mortality as a function of dose shows a normal, Gaussian distribution (Figure 4).

Figure 4: Gaussian normal distribution curve

The 50% response rate is at the median of the Gaussian curve. Moving away from this in the ± direction, the incidence decreases as a function of the standard deviation (SD). 68.3% of the obtained data are within the ± SD range, 95.5% within the ± 2SD range, and 99.7% within the ± 3SD range. Conventionally the confidence interval corresponding to the confidence level of 95% obtained within the 2SD standard deviation range is accepted in practice. The abscissa of the dose-response function shows the dose, or a natural logarithm there of, while the ordinate shows the percentage incidence of the symptom (mortality) produced by the given dose.

LD_{50} value: median lethal dose is used as a measure of toxicity, it is the dose of the tested substance required to kill 50% of the test organisms after a single treatment (given in mg/kg of body weight). The literature usually gives the acute oral LD_{50} value measured in rats (Figure 5, Table 1).

Figure 5: LD_{50} value (mg/kg of body weight) for aldrin measured in rats (J. SZŐNYI)

Substance	**LD_{50} (mg/kg of body weight)**
Ethyl alcohol	10 000
Common salt	4000
Morphine	900
Sodium phenobarbital	150
Strychnine	2
Nicotine	1
d-Tubocurarine	0.5
Tetrodotoxin	0.1
Dioxin	0.001
Botulin toxin	0.00001

Table 1: LD_{50} values in mg/kg of body weight for various substances in rats (I. KISS)

LC_{50} value: the concentration required to kill 50% of the test organisms. Ecotoxicologists use the environmental concentration value instead of the dose, as in the case of organisms forming an ecosystem it is uncontrollable how much of a substance present in the environment enters the tested individuals. Here the method used in human toxicology is not feasible, as a human toxicologist studies the biological responses of the test animals by administering (by feeding, injection) a known dose.

Probit Analysis: both LD_{50} and LC_{50} give a probability value, and the values obtained are not necessarily the same when the tests are repeated under the same conditions and with the same doses, but in different populations. Impact assessment often uses probit units instead of probability, then the S-shaped curve can be replaced by a straight line. The use of probit analysis makes easier the performance of toxicological tests and the evaluation of the obtained results. As a sigmoid dose-effect curve is obtained in toxicological testing only if the tests are performed on a large number of individuals and at a wide range of concentrations of the given substance. That would be very time-consuming and expensive, and would involve the destruction of many test organisms. In probit analysis the percentage probability value (P) is transformed into a probit value (Pr) (Table 2).

%	0	1	2	3	4	5	6	7	8	9
0	-	2.67	2.95	3.12	3.25	3.36	3.45	3.52	3.59	3.66
10	3.72	3.77	3.82	3.87	3.92	3.96	4.01	4.05	4.08	4.12
20	4.16	4.19	4.23	4.26	4.29	4.33	4.36	4.39	4.42	4.45
30	4.48	4.50	4.53	4.56	4.59	4.61	4.64	4.67	4.69	4.72
40	4.75	4.77	4.80	4.82	4.85	4.87	4.90	4.92	4.95	4.97
50	5.00	5.03	5.05	5.08	5.10	5.13	5.15	5.18	5.20	5.23
60	5.25	5.28	5.31	5.33	5.36	5.39	5.41	5.44	5.47	5.50
70	5.52	5.55	5.58	5.61	5.64	5.67	5.71	5.74	5.77	5.81
80	5.84	5.88	5.92	5.95	5.99	6.04	6.08	6.13	6.18	6.23
90	6.28	6.34	6.41	6.48	6.55	6.64	6.75	6.88	7.05	7.33
-	0.0	0.1	0.2	0.3	0.4	0.5	0.6	0.7	0.8	0.9
99	7.33	7.37	7.41	7.46	7.51	7.58	7.65	7.75	7.88	8.09

Table 2: The transformation of P% into a probit value (FINNEY)

It can be seen from the table that the 50% value corresponds to a probit value of 5. By transforming the P% values obtained in toxicological testing into probit values, and by connecting the obtained dots with a straight line, the probit value of 5 is projected onto the X-axis and the LD_{50} is obtained (Figure 6).

Figure 6: Mortality probit – function (based on I. KISS)

2.1.2 Duration of Action

One of the main factors in the classification of toxicological tests is the duration of testing:

- **Acute toxicity tests** (short-term): Usually 24 - 96 hour tests, to determine the response to a single dose of a potentially hazardous substance. In these tests the mortality % is determined, the monitoring of reproduction is not possible. Readily absorbed poisons often have an acute effect, acute tests can be used well to assess direct toxicity (DICKSON et al. 1992). In acute toxicity measurement, because of the short duration of testing, a possible error is that the effect occurs only after the end of the test. The LD_{50} or LC_{50} value determined from the dose-response curve is given as an indicator of acute effects. In addition to the LC_{50} value, the EC_{50} value is also used. EC_{50} is the concentration that causes some adverse effect in 50% of the test organisms.
- **Chronic toxicity tests** (long-term observations): They usually last for 20-30 days, occasionally even for 200 days. During the test the physiological, morphological, reproductive, or nutrition biological effects are studied at lower concentrations of a potentially hazardous substance administered in multiple, repeated doses. From the point of view of the strength and nature of the effect, the time interval between the repeated doses of the given substance (frequency of exposure) is also important. In chronic long-term testing habituation, tolerance can develop to the potentially hazardous substance, or a risk of accumulation may exist.

  The following values are determined as endpoints for chronic toxicity:

- **NOEC** (No Observed Effects Concentration) – the highest concentration at which no effects are observed

- **NOEL** (No Observed Effects Level) – the highest dose at which no effects are observed
- **NOAEC** (No Observed Adverse Effects Concentration) – the highest concentration at which no adverse effects are observed
- **NOAEL** (No Observed Adverse Effects Level) – the highest dose at which no adverse effects are observed
- **LOEC** (Lowest Observed Effects Concentration) – the lowest concentration at which effects are observed
- **LOEL** (Lowest Observed Effects Level) – the lowest dose at which effects are observed
- **MATC** (Maximum Allowable Toxicant Concentration) – the maximum allowable concentration of a pollutant

The NOEC value and the LOEL value can be calculated from each other: NOEC = LOEC/2. The MATC value can be given as the average of the LOEL value and the NOEC value.
On the basis of the indicators obtained in the acute and chronic tests, the Acute-Chronic Ratio (ACR) can be calculated, and for some groups of compounds the ACR value has been determined (Giesy et al. 1989):

$$\mathbf{ACR = LC_{50} / NOAEL}$$

where LC_{50} – is the LC_{50} value obtained in a 96-hour acute test
NOAEL – is the highest dose at which no adverse effects are observed in a chronic test

The role of the Acute-Chronic Ratio: for chemical substances belonging to the same group of compounds, on the basis of the results obtained in the acute test the NOAEL value for chronic toxicity can also be given.

2.1.3 Method of Exposure, Routes of Exposure

In toxicology exposure is defined as the contact of a potentially toxic substance with a living organism at a given dose. For a known dose it is important to determine the way in which the given substance enters the organism, and its bioavailability.

The most common routes of exposure:

- oral – entry by mouth, can cause anatomical and functional changes in the gastrointestinal system
- inhalation – the tested substance enters the organism by breathing in, it is absorbed in the lungs
- dermal – exposure by skin contact
- other parenteral routes, e.g. intravenous, intramuscular (into a muscle), subcutaneous (under the skin)

The strength of the response to the toxic effect varies with the different routes of exposure. Naturally, direct entry into the blood (intravenous route) has the strongest effect. In practice this can occur when drugs are administered. Potentially toxic substances from our environment enter primarily through the skin, the respiratory system and the alimentary tract.

Dermal exposure:

The best defence system is an intact skin surface. In vertebrate organisms the stratified keratinized epithelium can provide adequate protection against various chemical substances. The horny layer (stratum corneum) forming the surface of the skin, as the first line of defence, is very resistant to mechanical and chemical influences. An intact skin surface mostly prevents the entry of toxic substances, a damaged skin, however, can be a source of hazard. Studies have shown that damage to the horny layer significantly reduces the protection against xenobiotics (man-made substances foreign to the environment). Detergents also damage the skin considerably, facilitating the entry of hazardous substances.

Inhalation exposure:

Poisons entering by inhalation can be readily absorbed through the thin epithelium of the lung, and the toxic substance, by passing through the walls of the capillaries, spreads all over the organism via the bloodstream. The rate of delivery is determined by the relative solubility in blood of the steams or gases entering the organism by inhalation. If the toxic substance is dust, or enters the organism bound to smoke particles, it can also get into the bloodstream by macrophage phagocytosis.

Oral exposure:

Substances entering and absorbed from the alimentary tract can get into the intestinal epithelial cells by passive or active transport. Passive transport occurs by diffusion, while active transport is accomplished by means of carrier molecules. Active transport can also occur against a concentration gradient by using ATP energy.

Liver plays an important role in the removal of toxic substances, that is in the process of detoxification. Liver cells convert the toxic substances with the help of enzymes. They can either leave the organism with the bile, or become water soluble and excreted in the urine through the kidneys. The detoxification capacity of the organism is affected by many factors, e.g. the amount of the toxic substance entering the organism, its water solubility, and the sensitivity of the individual to the given toxic substance.

2.1.4 Species Dependence of Toxicity

The toxic effect of the same toxic substance can be very different even for taxonomically closely related species. It is exactly this selective toxicity that plant protection tries to exploit. The differences between species result from differences in anatomy, metabolic characteristics (formation of metabolites with different effects, differences in accumulation and excretion), and differences in genetic factors (ANDERSON et al. 2008). There is no close relationship between the environmental concentration of a given substance and the dose absorbed by living organisms. In addition to the species differences listed above, the ratio of the absorbed dose to the environmental concentration is also influenced by the shape of the living organism, the specific surface area of its body. The amount of substance absorbed from the environment is species dependent, and significant differences can be observed in this field.

2.1.5 Bioavailability

The negative biological effect of a chemical is significantly influenced by its absorbability and bioavailability. Bioavailability is therefore an important factor in the assessment of the environmental hazard, risk of a pollutant. The concentration and bioavailability of a given pollutant can be different (Figure 7). The value shown by chemical analysis is not necessarily higher than the biologically available amount, therefore the ratios shown in the figure can change significantly in both space and time.

Figure 7: The relationship between biological, chemical and real concentration (K. GRUIZ)

The pollutant concentration found in the environment can be considerably lower for substances that have a high tendency for bioaccumulation and thus accumulate in living organisms, but the opposite can also occur. Then only a fraction of the concentration found in the environment can be detected in living organisms. One of the main objectives of ecotoxicological testing is to estimate bioavailability. The entry of a tested pollutant into a given biological system is influenced by many factors: the physical-chemical properties of the substance (molecular weight, octanol-water partition coefficient, water solubility, vapour pressure, boiling point), environmental factors (pH, redox potential, enzyme reactions), and other interactions occurring in the medium. Interactions between chemical substances are not detectable by chemical analysis, although they can result in summed, decreased or enhanced toxicity (additive, antagonistic, synergistic effects).

In analytical measurements the toxic substance is extracted by solvents, then its environmental concentration is inferred using direct proportionality, that is the signal-concentration relationship is linear. The curves of toxicological tests are sigmoid curves, just as we have seen with the dose-response curves, the saturation curve shows the saturation of a hypothetical receptor with molecules of the toxic substance (Figure 8).

Figure 8: The concentration-signal relationship in analytical and ecotoxicological measurements

The situation outlined above is further complicated by the different routes of exposure, the presence of more hypothetical receptors, and the wide range of the methods of entry into cells and availability. The behaviour of different chemical substances changes upon entering a biological system. The microflora of the soil, the digestive enzymes in the human organism convert the entering substances, modifying their bioavailability. Biotransformation processes can produce metabolites that are even more toxic than the initial substances. Biotransformation usually occurs in two steps in an organism:

- first a primary product is produced by oxidation, reduction or hydrolysis
- then the primary product is bound to water soluble compounds (e.g. glutathione, glycine, cysteine, sulphates) and joins different endogenous metabolic pathways, or is excreted

Modelling of bioavailability: digestion experiments are used to model bioavailability. The pollutant part separated from the matrix by digestive enzymes can be regarded as biologically available. This separated part can be absorbed and can pass through the epithelium of the digestive system, thus getting into the blood and lymph circulation. In the organism it can be converted into other compounds through biotransformation mechanisms, or excreted with the bile. The bioavailability of a given substance is greatly determined by the route of exposure by which it enters the organism (the bioavailability of substances administered orally is lower), and the contact time and the type of the transport process can also be a modifying factor.

2.2 Criteria for Toxicological and Ecotoxicological Testing

Ecotoxicological tests directly detect the actual toxicity of the environment, or environmental samples, the bioavailability of the tested substance, or substances.

With environmental samples the combined impact of pollutants can be measured, the synergistic and antagonistic factors can be separated, effect modifications can be monitored. The results of ecotoxicological tests can be used for developing the thresholds that are accepted in practice. The tests are usually conducted under laboratory conditions, as then constant environmental conditions can be ensured, and that allows the standardization of the tests. They are relatively simple to perform, easy to reproduce, and give reliable results. In Hungary there are many standards for the purposes of toxicological testing, but the European OECD guidelines provide an even wider choice.
In laboratory testing given components can be selected individually, and their biological effect can be observed separately. However, some disadvantages also stem from the advantages listed above. The artificial laboratory conditions differ greatly from the natural environment of living organisms. The physical, chemical, biochemical and biological processes, transformations taking place there can significantly modify the toxicity of a given element, or compound.
In laboratory tests conducted **on pure samples** the concentrations measured usually with chemical analytical methods are proportional to the toxic effect, in environmental samples, however, differences can be observed. Biologically non-available pollutants present in high concentrations can have a negligible ecotoxic effect (e.g. certain chromium compounds, highly apolar hydrocarbons). Toxicity is influenced by the degree of oxidation of the pollutant as well. In aquatic ecosystems the biofilm formed on the surface of the sediment can also modify toxicity, as it shows a level of activity different from both the solid phase and the pore water. An ecotoxic effect can occur even in cases where it is not supported by the results of chemical tests (e.g. the chemical substance in question is still unknown, the pollutant is in a form undetectable by analytical methods, or there is an additive or synergistic effect). Highly toxic intermediate, side or end products can be generated during biodegradation as well. The problem is further complicated by the selection of organisms used for testing.
The list of test organisms recommended for certain pollutants, or types of pollution, is still undeveloped. While in human toxicology there are already well-established methods and a wide database, in the field of ecotoxicology there is no uniform methodology. One

of the main expectations with respect to the test organisms is that they should have a wide geographic spread, occur in large numbers in their natural habitat, play an important structural and functional role in the ecosystem, and acclimate easily to artificial laboratory conditions. Organisms meeting these criteria usually have a high degree of adaptability, are stress tolerant in their natural environment, and cope well with the pollution of the environment. In most cases, however, they are not sufficiently representative of the other species of the ecosystem. Adequate results are obtained only if the tests are performed on different living organisms, or within a given group of living organisms on species with different sensitivities. For classification purposes the value obtained from the most sensitive test organism is relevant. Some of the tests are so-called single-species tests. These tests give reliable results for the assessment of an environmental impact posing a hazard to the given species, but it is difficult to extrapolate the obtained data to real ecosystems. A better solution is to perform the tests on multiple species. The literature often recommends the use of combined tests (BREITHOLTZ et al. 2006). The reliability of testing increases when organisms representing different trophic levels are used, or the tests are performed with species utilizing different feeding strategies. Research has shown that for many chemicals the sensitivity of e.g. the aquatic flora to a given compound is lower than that of the aquatic fauna. Naturally, in many cases the opposite is true. As no organism has equal sensitivity to all pollutants (DÉVAI et al. 1992). The practice of using only specimens of the same age, and performing the tests on only healthy individuals for the sake of standardization, can also prevent a realistic assessment. Organisms of different ages have different sensitivities, the larval and early juvenile stages of development are the most vulnerable. It can also be stated that not only healthy individuals or populations can be found in nature, and they can also have very different sensitivities (ADELMAN et al. 1976). In the case of using stock cultures for the tests, reduced genetic variability and the fact that some organisms can become habituated, acclimated to various pollutants should be taken into account. Tests performed on trout have shown that the LC_{50} value for acclimated specimens was almost two times higher than for non-acclimated individuals.

Thus the requirements for test organisms are diverse, the most important expectations are summarized below:

- they should have a wide geographic spread
- they should play an important structural and functional role in the ecosystem
- they should be sufficiently representative of the given biological community
- they should be readily available or collectable (from their natural environment or a stock collection)
- they should be easy to keep, and breed under laboratory conditions (cultures with known history, genetics)
- they should be sensitive to several types of chemicals
- they should not be pathogenic species

The organisms used for toxicological testing can be selected from the most diverse groups of living organisms, thus there are e.g. bacteriological tests, algal tests, seedling tests. With respect to animals, invertebrate organisms most commonly used for testing are the crustaceans (Daphnia, Ceriodaphnia, Cyclops species), shellfish and nematodes. Vertebrates used for testing include primarily fish and small mammals.

Test Questions

Circle the correct answer!

1) What does the term hypersensitive individual mean?

a) it does not give a response to environmental changes

b) it is tolerant to environmental changes

c) it is characterized by a more sensitive response than the average

d) it gives a hyperactive response

2) What does the value 1 mean in toxicology?

a) all individuals have survived

b) a change occurred in the behaviour of the tested individuals

c) the lethal value

d) the test was performed on one individual

Match the letters to the correct numbers!

a) median lethal dose b) digestion experiments c) short-term d) shows a normal distribution e) the lowest concentration at which effects are observed

3) Gaussian curve

4) LOEC

5) acute toxicity test

6) bioavailability

7) LD_{50}

Decide which of the following statements are true, and which are false (mark with T or F)!

8) Plant protection is based on the exploitation of selective toxicity.

9) The environmental risk of a given chemical is not influenced by its bioavailability.

10) Acute toxicity tests can determine the bioaccumulation of toxic substances.

3. Ecotoxic Factors in Environmental Systems

The chemical load on environmental systems as a result of the changes of the past decades (the intensive development of industrial and agricultural production, changes in lifestyle) is an increasing problem both locally and globally. Chemicals are expected to be in widespread use in the future as well, instead of their elimination stricter control should be exercised over their use, as overchemicalization has led to structural and functional changes in the ecosystems.

3.1 The Relationship between Ecosystems and Ecotoxicology, the Complexity of Ecosystems, the Ecological Risk of Chemicals

Ecosystems can be regarded as self-regulatory systems, their proper functioning is ensured by their dynamic equilibrium state. This dynamic biological equilibrium developed over a long time, and in the past decades human activity has dramatically intervened in this self-regulatory system. In an ecological sense biological self-regulation is over, instead of it human transformation activity plays a decisive role. Nevertheless, the self-regulatory ability of ecosystems is not unlimited, they can be loaded only up to a certain tolerance limit, beyond that limit the regulatory mechanisms and regeneration processes are unable to cope (VÁRNAGY 1995).

Ecotoxicological tests provide the data required for assessing the environmental risks of chemicals. On the basis of its physical-chemical properties, chemical structure, the expected biological effect of a given chemical can be predicted within certain limits. Naturally, the fact that in environmental systems the actual toxicity of a chemical can be modified by many effects (e.g. UV radiation, temperature, pH, interactions with other substances) should not be left out of consideration. In the case of xenobiotics the assessment of biodegradability, convertibility is particularly difficult. These man-made unnatural substances often cannot be broken down by living organisms, their biochemical transformation is unsolved.

The understanding of ecosystem-level toxicity requires a complex attitude and approach. A given chemical interacts with a concrete individual, but the consequences of the effect affect the whole ecosystem (CAMPBEL 1993). For many chemicals the recognition of their ecotoxic and human health hazard took decades. In the vicinity of certain chemical plants more and more cases with similar symptoms were recorded over the years, and after chemical disasters serious damage to the health of the population living there could be detected. The disease appearing from the 1950s among those living in the vicinity of Minamata Bay in Japan as a result of mercury pollution, and the ITAI-ITAI disease appearing also in Japan as a result of cadmium-contaminated rice, called attention to the hazards of toxic heavy metals classified as micropollutants. From the chemical disasters the Seveso disaster (1976) in Italy, causing serious dioxin pollution, and the industrial accident in Basel (1986) should be mentioned (Photo 4).

Photo 4: Chemical disasters in Seveso and Basel

As a result of the disaster at the Basel chemical plant of the Sandoz factory, the water of the River Rhine turned red. Large amounts of dioxin and pesticides were released into the environment, causing the significant destruction of wildlife in the River Rhine. With this pollution the river received a higher load in a few hours than in the previous years in total.

The serious ecological and human health consequences of these accidents led to the compilation of a so called „black list" of the most hazardous chemicals, the ATSDR list of the top 20 hazardous substances includes toxic heavy metals, volatile organic compounds (VOCs), polychlorinated biphenyls (PCBs), polycyclic aromatic hydrocarbons (PAHs) and pesticides (Figure 9).

Figure 9: The ATSDR list (based on Incze-Lakatos)

The ATSDR, the American Agency for Toxic Substances and Disease Registry assesses the risks of chemicals posing a threat to health, and determines the scope of necessary measures.

3.2 Micropollutants, as Environmental Stress Factors (the Environmental and Human Health Effects of Heavy Metal and Pesticide Pollution)

From the pollutants recently micropollutants have come to the focus of attention. They can be detected only in small amounts, but they can have a harmful effect even at low concentrations (e.g. taste- and odour-affecting substances, carcinogens, mutagens, teratogens). Their negative effect can be observed in our waters even at a concentration of μg/l, manifested primarily in their toxicity and accumulation potential. Because of their ecotoxicity, special attention should be given to heavy metals classified as inorganic micropollutants, and pesticides classified as organic micropollutants.

Both groups are characterized by non- or low bioavailability, therefore they can accumulate in living organisms. In biochemical reactions they can be converted into compounds that are even more toxic than the initial substances. Because of their bioaccumulation and transport through the food chain, they are an increasingly serious problem not only from the point of view of environmental protection, but for human health as well (KIPPLER et al. 2007, SOHÁR & VARGA 2003).

Micropollutants have become the environmental stress factors of our age, their toxic effect can even multiply as a result of their interactions with each other, or substances in their surroundings. In addition to the harmful effects of industry, in our century agriculture has appeared as another major emitter of pollutants. All chemicals released into the natural environment can become harmful substances outside a certain concentration range. This becomes particularly apparent, if their effects are summed or enhanced in an additive or synergistic manner (McGEER et al. 2007).

Substances produced naturally are degraded biochemically, then get back into the biogeochemical cycle. On the other hand, biologically active, but unnatural substances have considerable persistence and become enriched in ecological systems. Persistence means the time for which a chemical compound stays in a well-defined region of the natural environment (KISS 1997, MILINKI & MURÁNYI 1997, SÁNDOR et al. 2000). As a result of these processes the concentration of a given pollutant can be orders of magnitude higher in living organisms than in the environment, and this effect can even multiply through the food chain (HODSON 1988).

3.2.1 The Environmental Impacts of Heavy Metals, the Consequences of Heavy Metal Pollution

In an everyday sense the term heavy metal often refers to a group of toxic metals, although heavy metals include essential metals as well, e.g. copper or zinc. Essential metals are indispensable for physiological processes, readily available biologically, but can be toxic above a given concentration. Heavy metals are defined on the basis of density. Metals with a density higher than 5 g/cm^3 can be regarded as heavy metals. The amount of heavy metals released into the environment and mobilized as a result of human activity (e.g. extraction of raw materials, energy production, metal processing industry, agriculture) can exceed by orders of magnitude the metal content released naturally from the geochemical cycle. They become enriched primarily through the food chain (FÖRSTNER & WITTMANN 1979, SÁNDOR et al. 2006).

The heavy metal content of a given environmental system is a sensitive indicator of anthropogenic pollution, as it cannot be removed biologically. The anthropogenic enrichment factor can reach a value of $10^2 - 10^5$ compared to the natural level. In an equilibrium state the mobilization of heavy metals accumulated in the sediment and soil, and their accumulation in living organisms would not occur. However, due to changes in the environmental factors, the previously non-available heavy metals pose a potential hazard to living organisms (CSENGERI et al. 2001). Biologically non-available metals in the soil and sediment can be present in environmental systems as chemical time bombs (RONCAK et al. 1997, GRUIZ et al. 1998). Previously non-available toxic substances in environmental elements with a high binding capacity can become mobilized at any time.

Therefore the measurement of the heavy metal load of the soil or sediment is more suitable for detecting the potential threat to the given system (GRUIZ et al. 2001).

No separate ecological and environmental health threshold systems have been developed. For surface waters few data are available for processing on the heavy metal content of the sediment. Data sets looking back over a longer period can be found only for the Danube, Tisza and Balaton. For the Tisza detailed sediment tests have been performed on the upper, middle and lower sections of the river following the cyanide and heavy metal pollution in 2000 (FLEIT & LAKATOS 2002). These tests clearly support the opinion that changes detectable in the sediment are better indicators of catchment-level disturbance effects than the instantaneous concentration values measured in the water body. In international monitoring practice emphasis is shifting from the water-phase to the sediment-, or soil-phase. The comparability, equivalence of data is a serious problem both internationally and nationally. The opportunity for comparison with historical data is often lacking, or the available data sets cover only a short period of time. In the absence of a reference area - for the determination of the natural background concentrations of heavy metals – the method of taking soil and sediment core samples can be used. The amounts of micropollutants detectable in successive layers reflect the timeline of pollution.

The absorption of heavy metals is influenced by several abiotic and biotic factors. From the abiotic factors the temperature, the pH, the oxygen content dissolved in the water, and the hardness of the water should be noted. From the biotic factors the differences between species, the age, the size, and the differences in adaptability are relevant. Experiments have demonstrated that in aquatic environments an increase in the water temperature increases the intensity of the absorption of heavy metals. It can be observed in many invertebrate organisms that in the range of 10-15 C° the acute toxicity of heavy metals is undetectable, but in the range of 25 -30 C° their toxicity is significantly enhanced (WANG 1987). The toxicity of heavy metals is also influenced by the hardness, salinity, and pH value of the water. With a decrease in the pH value the toxicity increases, at higher pH values the absorbability of heavy metals decreases.

Heavy metals, due to their unpredictable conversion processes, in many cases can be regarded as more hazardous than other pollutants. In the past decades the concentrations of heavy metals in environmental systems have increased by orders of magnitude, and they have significantly accumulated in the tissues of living organisms through the food chain. The ITAI-ITAI disease caused by cadmium and the MINAMATA disease caused by methylmercury compounds in Japan called attention to the hazards of this tissue accumulation of heavy metals.

In the case of the **MINAMATA disease**, the number of cases of neurological damage increased in the population over many years. Doctors reported trembling, deformed and stiff joints assuming abnormal positions („breathing wooden dolls"), in hundreds of patients the disease had a fatal outcome. The chemical factory in Minamata Bay originally released less toxic inorganic mercuric sulphate into the water of the Bay, where microorganisms converted this compound into highly toxic organic methylmercury. The produced methylmercury can easily cross the cell membrane of living organisms, the blood-brain barrier, and can exert its health damaging effect.

The **ITAI-ITAI disease** calls attention to the consequences of chronic cadmium exposure. The consumption of cadmium-contaminated rice in Japan caused skeletal abnormalities, osteoporosis, and in the case of long-term exposure renal failure (Photo 5).

Photo 5: The MINAMATA and the ITAI-ITAI disease

The symptoms mentioned above appear in people exposed to cadmium in Hungary as well. Osteoporosis in men living in the vicinity of Gyöngyösoroszi is 300% above the national average.

In the soil members of the microflora are sensitive to an increase in the cadmium concentration, therefore, as a result of adaptation processes, they have developed resistance-mechanisms by means of which they neutralize cadmium by binding it to proteins, making it biologically non-available. The threshold for cadmium in soil is 0.5 mg/kg.

Heavy metals can appear in different forms in the natural environment, their toxicity is determined by their speciation. They can form complexes with other molecules in the environment, and as a result of complex formation the concentration of free hydrated metal ions decreases, the rate of transport processes, their physiological role can change. A critical condition occurs when a given concentration value reaches the boundary between an ecologically insignificant effect and a long-term change in the ecosystem. It is difficult to predict the bioavailability of toxic metals occurring naturally or semi-naturally. The toxicity mechanisms of metals are implemented through very complex processes. Upon entering a living organism, the given heavy metal interacts with functional groups of enzymes or other proteins. By inhibiting functional groups of proteins, they disrupt physiological processes. The toxicity of a given metal can also increase significantly in the case of competition with another metal or metals (GALVEZ et al. 2007). Competition can develop between certain metal ions for the active centre of a given enzyme (e.g. zinc-cadmium, calcium-cadmium). The more toxic cadmium can take the place of zinc and calcium, and this competitive inhibition can lead to phosphate metabolism disorders, as well as severe osteoporosis, bone fragility. Oxidative metal ions (e.g. chromate ion) that are carcinogenic over a longer period of exposure can also cause poisoning.

The toxicity of heavy metals is further complicated by the fact that through interactions occurring between them, they can significantly modify the biological absorbability, physiological role of each other (PELGROM et al. 1994, NORWOOD et al. 2003, POHL et al. 2003). The nature of the occurring interactions is determined by the type, concentration of the given heavy metal, the ratio of the metals to each other, and the physical-chemical and biological parameters of the environment (GLOVER et al. 2004). At lower concentrations of cadmium and zinc, upon combined exposure an antagonistic effect can be observed (BRZÓSKA & MONIUSZKO-JAKONIUK 2001). At low concentrations zinc protects cells against apoptosis and oxidative stress upon cadmium exposure. In the presence of zinc the binding of cadmium to metallothionein (MT) increases, the resistance of cells to cadmium strengthens. MT is a multifunctional protein that can bind metal cations, and plays an antioxidant role as well. Upon heavy metal exposure MT protein synthesis is increased in living organisms (USENA et al. 2007). Heavy metals exert their adverse biological effect through inhibiting the neutralization of reactive free radicals in the organism, increasing oxidative stress and apoptosis (LEONARD et al. 2004, PULIDO & PARRISH 2003). From heavy metals, exposure to cadmium shows the highest free radical formation in cells.

3.2.2 The Effects of Pesticide Pollution in our Environment

The significant increase in the use of pesticides in the past decades can be regarded as a consequence of intensive agricultural production. Pesticides are preparations of natural or chemical origin, containing a substance or a mixture of substances suitable for destroying or controlling pests damaging plants, plant parts, or stored crops. They can destroy wildlife in the soil, cause soil degradation, and through run-off pollute the groundwater and surface waters (LENGYEL & FÖLDÉNYI 2003). Because of their widespread use, today 53% of our surface waters and piped drinking water contain pesticide residues. The provisions relevant to surface waters are specified in Hungarian Standard No. MSZ 12749/1993 (Table 3).

designation of active substance	**I excellent μ/l**	**II good μ/l**	**III tolerable μ/l**	**IV polluted μ/l**	**V heavily polluted μ/l**
chlorinated hydrocarbon lindane	0.1	0.2	0.5	2.0	> 2.0
organic phosphoric acid ester	0.1	0.2	0.5	2.0	> 2.0

malathion					
triazine derivatives	0.5	1.0	2.0	5.0	> 5.0

Table 3: Thresholds for pesticides in each water quality class

In order to protect drinking water and surface waters, the EU developed a priority list of pesticides (EUROPEAN PARLIAMENT AND COUNCIL 76/464/EEC, EC 2003). This determined the maximum allowable thresholds for pesticides in water for human consumption. Although today a downward trend can be observed in the use of pesticides, the available data do not always reflect the reality. With an increase in the number of small family farms, and due to illegally imported pesticides, it is difficult to determine the actual pesticide use (BALOGH 2004, OCSKÓ 2005, TOMPA 2005). Pesticides are grouped according to the pest organisms they control (Figure 10).

Figure 10: Groups of pesticides (based on Darvas-Vörös)

In developing countries more insecticides, while in developed regions more herbicides are used (VÁRNAGY 1995). With respect to the use and applicability in practice of a pesticide, in recent years primarily persistence, toxicity and bioaccumulation have been taken into consideration (VÁRNAGY 2005). The behaviour of pesticides can be very different in different environmental systems. Their toxicity is determined primarily by their mobility in the soil, water solubility and accumulation potential. The less soluble a compound is in water, the more resistant it is to biochemical degradation, and the more it accumulates in the tissues of living organisms. The toxicity and persistence of a pesticide can be different. This is well demonstrated by the differences between early pesticides and pesticides used today.

DDT, used from the 1950s for its insecticide effect, was less toxic, but as it was a very persistent compound, it greatly accumulated in environmental systems, and through the food chain in the fatty tissues of living organisms (RUIGIANG et al. 2007). Although parathion and its derivatives replacing DDT are characterized by rapid degradation, that is they are less persistent, experiments have shown that they are much more toxic to mammals (PÁLFI 2001). The degradation of pesticides occurs through biological, chemical and photochemical processes. The degree of photodegradation is one of the decisive factors in the environmental impact of a given pesticide. Metabolites formed in photochemical processes can reduce or enhance the toxicity of the given agent. An increase in light-induced toxicity has been shown in benthic macroinvertebrate species in waters (HATCH et al. 1999).

Test Questions

Circle the correct answer!

1) What are xenobiotics?

   a) food additives

   b) the collective name for substances found in nature

   c) cosmetics

   d) unnatural substances

2) What causes the ITAI-ITAI disease?

   a) copper poisoning

   b) spoiled food

   c) cadmium poisoning

   d) virus infection

Match the letters to the correct numbers!

a) pesticides b) enhancing effect c) chemical time bomb d) pesticide used against insects e) MT f) essential metal g) abiotic factor h) metals with a density higher than 5 g/cm^3

3) cadmium-binding ability

4) heavy metal content of the soil, sediment

5) insecticide

6) synergism

7) heavy metals

8) copper

9) photodegradation

10) temperature

4. Agricultural and Industrial Pollutants Posing the Highest Risk, and their Environmental Impacts

Because of the intensive chemicalization of agriculture and the multiplication of industrial production, chemical substances with unknown effects have entered the environmental systems and through that the human organism in significant concentrations. Almost 10 million chemicals of different structure are registered in the world, from this 100 000 are commercially available, approved substances. Compounds generated by industry, as well as pesticides and fertilizers are ever-present potential sources of hazard in our environment. And their effects can even be enhanced through interactions with other chemicals. With respect to human health, combinations of stimulants, preservatives and drugs can be particularly hazardous.

From the pesticides chlorinated hydrocarbons, organic phosphoric acid esters and triazine derivatives should be noted. In addition to the pollution originating from agriculture, numerous toxic substances enter the environmental systems through industrial activity and transportation, and from households as well. Shocking cases of environmental pollution with polychlorinated biphenyls (PCBs), dioxins and polycyclic aromatic hydrocarbons (PAHs) have been reported in the world press. Representatives of the compounds listed above occur everywhere in our environment, and through their accumulation we will feel their health damaging effects for years, or even decades to come.

4.1 Chlorinated Hydrocarbons

Chlorinated hydrocarbons, in widespread use in the 1950s, remain a serious environmental and health problem to this day. One of them, DDT (dichloro-diphenyl-trichloromethylmethane) was widely used all over the world, it proved to be an excellent insecticide, and in tropical areas it was used effectively to curb malaria. Within a few years after the introduction of DDT pests resistant to the agent appeared, and it became increasingly evident that, as it was a highly persistent compound, it accumulated in environmental systems, and through the food chain in the fatty tissues of living organisms. Due to its lipophilic properties, it causes damage to fat-rich organs, primarily the central nervous system is affected, but liver and kidney functions are also disrupted. It increases myocardial sensitivity to adrenaline, and thus it can cause ventricular fibrillation.

Because of its health damaging effect, the use of DDT was banned from 1968, nevertheless it can be found in foodstuffs of animal origin to this day. Human population studies have shown that today the amount of DDT in fatty tissues is still 15-20 mg/kg (Figure 11).

Figure 11: DDT residues in the fatty tissues of residents of Budapest (based on Sohár – Matyasovszky, NIFNS – National Institute for Food and Nutrition Science)

The effects of aldrin, dieldrin and lindane compounds are similar to those of DDT. These polychlorinated organic compounds are POPs (Persistent Organic Pollutants), and are present everywhere in the environment (ubiquitous pollutants). They persist for a long time, become enriched in the food chain, accumulate in fatty tissues, cross the placental barrier, and are also excreted in breast milk (levels detected in breast milk reach 340 mg/l). In the 1970s Hungary was one of the most heavily polluted countries with respect to the total DDT content of breast milk (Figure 12). 90% of polychlorinated compounds enter the human organism through food, they get into the food chain from residues persisting in the environment. They are particularly hazardous, as their biological effect is manifested in toxicity occurring later (teratogenicity, carcinogenicity, immunotoxicity).

Figure 12: Comparison of the average total DDT contents of breast milk in different countries between 1970 and 1980 (based on Sohár – Matyasovszky, NIFNS)

4.2 Organic Phosphoric Acid Esters

Organic phosphoric acid ester derivatives are less persistent pesticides. Pesticides belonging to this group exert their effect by blocking the activity of enzymes. They inhibit acetylcholinesterase activity, and thus prevent the breakdown of acetylcholine at cholinergic synapses into choline and acetic acid. The consequences of poisoning include cardiac and respiratory paralysis.

4.3 Triazines and their Derivatives

Triazines are primarily weed killers, herbicides. Until the 1990s active substances of this type were applied in large quantities to arable soils. Therefore these compounds, or their residues can be detected in groundwater everywhere (e.g. atrazine, terbutryn, prometryn). The high concentration of triazine in soil poses a serious threat to future drinking water resources.

The environmental hazards of pesticide pollution, their detection: the pesticide pollution of soil poses serious hazards, as it gets washed into our waters, where it becomes almost impossible to remove. The water treatment technologies available today are in most cases not suitable for the removal of pesticide derivatives. Data on diffuse pollution of agricultural origin are received by the Environmental Inspectorates and the Services for Plant Protection and Soil Conservation, then they are processed by the National Environmental Information System (NEIS). Pesticides are listed in the Water Framework Directive (WFD) as so called „priority substances". The list of hazardous organic „priority substances" includes the following pesticides: endosulfan, diuron, simazine. According to the WFD the monitoring of these substances is not solved in Hungary and the surrounding countries. Three automatic monitoring stations have been installed on the Hungarian section of the River Tisza, but they are less suitable for the detection of the negative ecological effects of pesticides. The development of systems for that kind of measurements is currently underway, but the target date for completion is 2015. The installation of comprehensive soil and water monitoring systems is indispensable, as in the case of hazardous exposure only they will allow the exploration of cause-and-effect relationships, the determination of the short- and long-term effects of pesticides on the stability of a given ecosystem.

4.4 Polychlorinated Biphenyls (PCBs)

PCB (polychlorinated biphenyl) compounds enter the organism through polluted air, polluted food and contact with old electrical equipment. The toxicity of PCB compounds depends on the number and location of chlorine atoms. Their degree of persistence increases with the degree of chlorination. They are poorly soluble in water, but highly soluble in organic solvents and the fatty tissues of living organisms, they have bioaccumulation and biomagnification potential. Organic compounds with a high chlorine content are resistant to biodegradation.

In 2001 the Stockholm Convention banned the production of PCBs. Industry used them primarily as a dielectric fluid for transformers and capacitors, or as a softening agent in the production of adhesives, paints and plastics. Today they can be detected all over the world from air, soil and sediment samples. PCB pollution is particularly significant in the Baltic Sea.

Routes of exposure: they enter the organism primarily through polluted food, their concentration can increase by orders of magnitude through the food chain, and food packaging materials can also cause contamination.

Adverse effects: They accumulate in fat-rich tissues (nervous system, breasts, testes, ovaries). They are carcinogenic (cause cancer), reduce reproduction, weaken the immune system, they can cause liver damage, memory disorders, motor disorders, headaches, and numbness.

NIFNS studies have shown that in the Hungarian population the PCB content of human fatty tissue significantly increases with age (Figure 13), and the decrease in tissue PCB levels over the years has been slight.

Figure 13: Total PCB content of human fatty tissue samples as a function of age (NIFNS)

In 2001 Hungary also signed the Stockholm Convention, and accordingly, took regulatory measures to prevent the release of PCB compounds into the environment, but as the half-life of PCBs ranges from 1 to 70 years, their elimination is a very slow process.

4.5 Polychlorinated p-Dibenzodioxins and Dibenzofurans (Dioxins)

Dioxins have a varying number of chlorine atoms and aromatic rings. Similarly to PCBs, their toxicity depends on the number of chlorine atoms. The most toxic is 2,3,7,8-tetrachloro-p-dibenzodioxin (TCDD). Dioxin-type compounds can be found everywhere in the environment, they are very stable and persistent. They have bioaccumulation and biomagnification potential. They do not occur naturally, but are produced as the by-products of various chemical processes. Major dioxin emitters are waste incinerators, coal-fired power plants, metallurgy, and the chemical industry.

As a result of industrial accidents and human negligence, several dioxin scandals have been reported in recent years. Dioxin was released into the environment in chemical disasters in Seveso, Italy, and at the Sandoz factory in Basel. From incidents involving the dioxin contamination of food, the dioxin contamination of guar gum imported from India in 2007 and the dioxin contamination of eggs originating from Germany in 2011 should be noted.

Routes of exposure: they enter the organism primarily through polluted food, dioxins and PCBs typically occur together in food.

Adverse effects: similarly to polychlorinated biphenyls, they accumulate in fatty tissues, they are the most hazardous compounds, they are carcinogenic, they can cause neurological disorders, chronic rashes, chloracne, liver damage, diabetes, and thyroid dysfunction. They damage the genetic material, that is they have been proven to be genotoxic.

Different dioxin compounds have different biological effects, their toxicity is expressed by the so called toxic equivalent (TEQ), compared to the most toxic TCDD. Dioxin levels are higher in high-fat food, and lower in fruits and vegetables.

EU Directive 2002/69/EC sets the maximum levels for dioxins in food and determines the tolerable daily intake (TDI).

4.6. Polycyclic Aromatic Hydrocarbons (PAHs)

PAH compounds consist of aromatic rings, they are less water-soluble, their most important representative, benzo(a)pyrene is highly carcinogenic. PAHs can occur naturally as well, e.g. from forest fires, or can be synthesized by algae and certain higher plants.
However, the amount of polycyclic aromatic compounds produced naturally is minimal compared to PAH compounds released into the environment as a result of human activity. Such organic substances are formed during the combustion of fossil fuels, the exhaust gases of motor vehicles contain more than 30 kinds of PAHs, from which 10 have been proven to be carcinogenic. PAH pollution in the soil is very high in the vicinity of petrol stations and oil refineries. The concentration of PAHs in plants grown in these areas is also very high. In towns PAH exposure as a result of transportation poses a serious hazard. The organism is also exposed through food: smoked meat, overcooked food, repeatedly used cooking oil are all potential sources of hazard.
Adverse effects: they are tumorigenic, damage the immune system, PAH compounds can seriously harm aquatic organisms. EU Directive 2005/69/EC specifies the provisions applicable to PAH compounds.
In summary it can be stated that the substances described above, which are particularly hazardous to living organisms, have a number of common characteristics: each is characterized by a high tendency for bioaccumulation, high persistence, a tendency for biomagnification, they accumulate mainly in the fatty tissues of living organisms, where they can become mobilized at any time. They are common all over the world, they can be found in high concentrations in different environmental systems, and they induce structural and functional disorders through their ecotoxic and human-toxic effect. They have low biodegradability, the efficiency of the defence and adaptation of wildlife to these compounds is not sufficient enough.
Global warming affecting the whole planet raises new problems with respect to highly hazardous chemicals. As a result of warming, chemicals banned decades ago get washed into environmental systems. These substances were carried by air currents over long distances, and now, as a consequence of warming, they get mobilized as „frozen stocks”.

Test Questions

Match the letters to the correct numbers!

a) TCDD b) ubiquitous pollutant c) PAH compounds d) PCB compounds e) acetylcholinesterase inhibitors f) pesticides

1) can occur naturally, e.g. from forest fires

2) organic phosphoric acid esters

3) are listed in the WFD as priority substances

4) present everywhere

5) the most toxic dioxin

6) can also get into food from food packaging materials

Decide which of the following statements are true, and which are false (mark with T or F)!

7) The toxicity of PCB compounds is not influenced by the number of chlorine atoms.

8) Dioxin compounds do not occur naturally, they are produced only as the by-products of chemical processes.

9) Persistent compounds are rapidly eliminated from the organism, as they have a low tendency for bioaccumulation.

10) The concentration of a given pollutant is always higher in environmental elements than in the tissues of living organism.

5. Types of Toxicological Tests

In toxicological testing it is always the test objective, the aspects of the test that determine which test is relevant to the solution of the given problem.

Toxicological tests can be classified:

a) on the basis of the duration of testing (acute and chronic tests)
b) on the basis of the used test-organisms (single-species and multi-species tests)
c) on the basis of the observed physiological processes (e.g. reproduction tests, growth tests)
d) on the basis of the tested level of organization (tests at molecular, individual, population, community and ecosystem level)
e) as laboratory or field toxicity tests
f) as tests in a static or a dynamic system
g) as tests on „pure compounds” and/or environmental samples
h) as tests on terrestrial or aquatic organisms

Acute tests: short-term tests (24-96 hour tests), usually single-species tests, and are suitable for the assessment of direct toxicity. Acute toxicity tests allow the determination of minimum and maximum mortality rates, the assessment of the hazard of toxicity, and in certain cases with appropriate care they can be reasonable substitutes for ecosystem-level field tests (DICKSON et al. 1992). Short-term express methods are suitable mostly for the determination of acute toxicity. The results provide information on the presence of a toxic substance and the degree of its harmless effect (harmless concentration, median tolerance limit).

Chronic tests: long-term tests are also suitable for the observation of anatomical, physiological, nutritional, endocrinological, reproductive, attitudinal and behavioural changes. Tests lasting longer than 96 hours are classified as subchronic tests. Chronic toxicity tests can last for 20-30 days, or in rare cases even for 200 days. These tests, in addition to the survival time, are also suitable for the determination of the allowable, or harmless concentration of a given toxic substance. Thus the results of long-term tests give a more realistic picture of the actual toxicity of a given pollutant, in fast-reproducing species the adverse biological effect of a chemical (e.g. mutagenic, carcinogenic, cytotoxic changes) can be monitored over several generations.

5.1 The Use of Single-Species and Multi-Species Tests

5.1.1 The Role of Single-Species Tests in the Detection of Toxicity

Acute toxicity tests are usually single-species tests, and the test objective determines which test-organism is the most appropriate. Naturally, different aspects should be considered when we want to determine the toxic effect of a given substance or substances in aquatic or terrestrial ecosystems. There are differences in the number and nature of the routes of exposure, in the case of aquatic organisms e.g. the total body surface gets into contact with the pollutant, thus the adverse effects through the outer epithelium, the digestive system and the respiratory system can add up. The method of exposure is significantly influenced by whether the tested potentially toxic substance is in a liquid or a solid phase (RUFLI et al. 1998). The routes of exposure in a given system vary depending on the stability, hydrophilic or hydrophobic nature of the tested chemical substance (BREITHOLTZ & WOLLENBERGER 2003). In the natural environment it is more complicated to explore the routes of exposure than under laboratory conditions. The toxicity and absorbability of a substance is determined not only by its water solubility, but also by how the given process takes place as a result of the combined interaction of abiotic and biotic factors (CONRAD et al. 2002, GATERMANN et al. 2002). The method of exposure is greatly influenced by the habitat conditions, the form of feeding, and the type of metabolism. In addition to the primary toxicity affecting one or more given species, today the assessment of the effect and risk of secondary toxicity through the food chain also plays an increasingly important role (ESCHER & HERMENS 2002).

In recent years particular emphasis has been placed on the determination of the possible routes of exposure of substances of hydrophobic nature, and on the development of standardized methods suitable for the assessment of their environmental risk.
The exposure of organisms living in polluted environmental systems can occur in complex and complicated ways. For soil- and sediment-dwelling species special routes of exposure are possible.

In ecotoxicological testing one of the most difficult tasks is to select the most relevant organism for the test:

- If our primary objective is the protection of a species, then that species and the organisms that provide the food base for it should be tested.
- If we want to find out the toxic effect of a given pollutant, the testing should be repeated with species playing an important structural or functional role in the given biological community (in general, three species representing different trophic levels should be selected).

In summary it can be stated that single-species tests are very useful for predicting toxicity, but do not allow extrapolation to the whole ecosystem, as the oversimplification of ecological interactions can lead to false conclusions (SCHMITT – JANSEN et al. 2008).

Statistical methods for the evaluation of single-species tests:

a) **Graphical interpolation:** toxicity endpoints are determined on the basis of dose-response and concentration-effect curves (LC_{50}, LD_{50}, EC_{50}, ED_{50}). The disadvantage of the method is that a confidence interval cannot be calculated.
b) **Probit method:** the most commonly used procedure, the data sets obtained with this method are transformed into probability units (probit units). A confidence interval can be determined easily.
c) **Logit method:** it is also based on the transformation of data. After the transformation of data it finds the best-fitting curve.

Programs suitable and available for the processing of data are e.g. TOXSTAT, SAS-PROBIT, SPSS-PROBIT (GRUIZ et al. 2001).

5.1.2 The Characteristics of Multi-Species Tests and the Criteria for their Use

Multi-species tests allow the testing of interactions between species (e.g. prey-predator relationships, competition), and the exploration, modelling of the system of relationships within a biological community.

Micro- and mesocosm models, and in a wider sense field experiments can be regarded as multi-species tests.

Microcosm models: laboratory experiments, where the size, volume of the microcosm model is not standardized. Experiments performed in flasks of a few hundred millilitres and in aquariums of several hundred litres are both classified as microcosm tests (Animation 5). In practice, around 22 methods have been developed for laboratory microcosm models (GEARING 1989).

Animation 5: Laboratory microcosm experiments (EKF-Eszterházy Károly College, Institute of Biology)

Mesocosm models: in general, they are implemented outdoors, often in the form of artificial ponds, swamps, reservoirs, gardens, or artificial forests. Mesocosm experiments are suitable for the simulation of actual ecosystem-level processes. Mesocoms tests constitute a transition between microcosm and field experiments.

Field tests: extrapolation to the whole ecosystem is the safest in these experiments, however, their implementation is very expensive. The obtained results are suitable for resolving „lab-to-field” problems, that is they give an acceptable answer to the question of how much and to what extent do laboratory tests reflect the processes occurring in real ecosystems.

Statistical evaluation of the results of multi-species tests: the processing, interpretation of data is more difficult than in single-species tests. The repeatability and standardizability of these tests is highly problematic. For the analysis of the data multivariable methods are used, by means of which the relationships, regularities found in ecological systems can be explored. Primarily two methods are suitable for such evaluation: PCA (principal component analysis) and NCAA (nonmetric clustering and association analysis).

5.2 Ecotoxicological Tests, Measurement Endpoints

The subjects and measurement endpoints of ecotoxicological tests can cover all levels of organization in biological systems. Methods used in other branches of toxicology are often integrated and utilized in the tests. For assessing the actual toxicity of an environment, or an environmental sample, monitoring can range from the level of biochemical, genetic, cytological tests, through individual behavioural manifestations, to changes occurring at population and ecosystem level (Figure 14).

Figure 14: The subjects of ecotoxicological tests at different levels of organization

5.2.1 The Use of Geno- and Cytotoxicity Tests in Ecotoxicology

In ecotoxicological tests the assessment of the genetic risk of toxic substances released into the environment is often indispensable. Today many chemicals are released into the environment that have been shown to damage the genetic material, and through that they can cause birth defects, cancerous diseases. Just think of the constantly recurring dioxin scandals shocking the public.

Dioxin: is carcinogenic (causing cancer) due to the inhibition of nucleic acid synthesis, and in dioxin contaminated regions more infants are born with birth defects. In the southern part of Italy, in the so called „death triangle” (near Naples), in recent years around 1200 illegal dump sites have been created, and as a consequence, the number of cases of cancerous diseases has increased dramatically among the people living there, and an increasing number of children have been born with birth defects. Skin abnormalities have included chloracne, typically characteristic of dioxin exposure (Photo 6).

Photo 6: The consequences of dioxin poisoning

Symptoms of dioxin poisoning can appear anywhere and at any time, as this group of compounds is present everywhere in the environment (ubiquitous). Its ecosystem-level effects and hazards have been described in details in a previous chapter.

Upon entering a living organism, many industrial and agricultural poisons are capable of binding to the DNA or the chromosomes, and induce structural changes in them. These substances causing mutations are called mutagens, and the term mutation means a sudden change in the genetic material.

Chemicals and various impacts (e.g. ionizing radiation) causing genetic damage can exert their mutagenic effect at three levels:

- genome mutations (changes in the number of chromosomes)
- gene mutations (genetic changes causing the appearance of a new allele; usually it is the result of a point mutation changing the DNA base sequence)
- chromosome mutations (changes in the structure of the chromosomes, and thus in the functioning of the genes on them)

A mutation can affect the gametes, then the damage occurring as a consequence of the mutation is inherited. When a mutation affects the somatic (body) cells, a pathological process starts at the level of the individual, which can lead to e.g. cancerous diseases. Somatic mutations are not inherited.

Mutagenic substances:

- destructive compounds – inducing the formation of reactive free radicals (e.g.

H_2O_2, nitrates, nitrites, certain heavy metals)

- alkylating compounds – transfer their alkyl groups to nucleotides in the DNA (e.g. mustard gas derivatives, epoxides, diazo compounds, nitroso compounds)
- substituted compounds – nucleotide base analogues, that is their chemical structure is similar to the DNA bases, therefore in DNA synthesis they are incorporated into the nucleotides instead of the DNA bases, thus a defective DNA structure is formed.

Naturally, in most cases the mutations occurring in an organism are not manifested, as cellular so called repair mechanisms eliminate these changes. However, with increasing environmental pollution, the number of mutations can increase so dramatically that the repair mechanisms can no longer repair these changes effectively, or the repair mechanisms themselves can also be damaged by the mutagenic substances.

Genetic changes and their consequences are known and studied primarily with respect to human health, such tests have not been conducted yet at ecosystem level. In ecotoxicological tests the mutation inducing effect of certain chemicals is considered primarily in the case of approval procedures for pesticides, and the classification of hazardous wastes.

Mutagenicity tests are suitable for the detection of genetic changes: these are indirect tests and the experiments can be performed in vivo or in vitro.

In vivo experiment (the meaning of the term is – in life): testing in a living organism, where the test organism is exposed during the experiment (e.g. feeding the test animal with the potentially toxic chemical, or applying various doses of radiation). The responses of the test organism are monitored (e.g. nutritional, respiratory, movement, attitudinal and behavioural changes).

In vitro experiment (the meaning of the term is – in glass, in a test tube): testing not in a living organism, but on cells, cell cultures taken from a living organism, then maintained under laboratory conditions.

In mutagenicity tests damage to the genetic material can be indicated by e.g. a change in enzyme activity, cell division dysfunction, chromosome abnormalities.

Genotoxicity is measured most commonly by using standardized bacterial tests, e.g. the AMES-test detects changes in enzyme activity as a result of a mutagen.
Micronucleus tests are suitable for observing disorganized cell division as a result of a mutagen.

AMES – reverse mutation test (OECD 471)
A standardized test using *Salmonella typhimurium* and *E. coli* strains. From the amino acids, the *Salmonella* strain cannot synthesize histidine, while *E.coli WP2* cannot synthesize tryptophan. Therefore, for their growth histidine and tryptophan needs to be added to the medium. As a result of mutagenic substances, some of the bacterial cells regain their ability to synthesize histidine and tryptophan, respectively, and thus they are able to grow in a histidine- or tryptophan-free medium. The more bacterial colonies form, the stronger the mutagenic effect of the tested substance (Animation 6).

Animation 6: Mutagenicity tests: AMES-reverse mutation test, micronucleus test (based on M. Molnár, BME – Budapest University of Technology and Economics)

Micronucleus test
Micronuclei are cytoplasmic bodies smaller than the nucleus, with a nuclear membrane, forming in the case of dysfunction in the cell division process. Micronucleus tests are suitable for the detection of chromosome mutations. In mutagen-treated broad beans (*Vicia faba),* after the treatment micronuclei are detected in the secondary root tips, and the incidence of micronuclei is given compared to the control root tip cells.

5.2.2 Ecotoxicological Measurements at Individual, Population and Ecosystem Level

Individual-level tests study primarily the adverse physiological effects of a given chemical, the pathological changes in the affected organs, and attitudinal and behavioural disorders. In the past decades more and more chemicals have polluted our environment, and from them xenobiotics, which are foreign to the environment, can be regarded as particularly hazardous. Based on their origin, they are divided into physical, chemical and biological xenobiotics (Photo 7).

Photo 7: The most common chemical xenobiotics (food additives, drugs, polymers, plastics)

Chemical xenobiotics include:

- food additives (flavour enhancers, emulsifiers, preservatives)
- drugs
- chemicals from industrial and agricultural activities

Xenobiotics are man-made substances, to the degradation of which wildlife has not been able to adapt yet.

The fate of xenobiotics in the environment:

1) If in its chemical structure and properties a given xenobiotic is very similar to a substance already known by members of the ecosystem, biodegradation is possible.
2) If in its structure and properties a given xenobiotic is entirely unknown to living organisms, it accumulates in the environment and the tissues of living organisms as an undegradable chemical (this includes the persistent compounds).
3) If a given xenobiotic gets involved in the biochemical degradation processes, but does not provide usable energy, that is called a cometabolic effect.
4) If during the degradation of a given xenobiotic a more toxic intermediate or end product is generated than the original one, then predicting the damage to

ecological systems, and assessing the real threat presents serious difficulties (e.g. in the case of heavy metals, pesticides).

Today hundreds of chemicals put a load on our environmental systems, the threat to aquatic and terrestrial ecosystems is particularly striking. In ecotoxicological tests one of the main difficulties is that it is impossible to draw conclusions and relationships with respect to whole ecosystems. The normal behaviour of healthy and intact biological communities is not yet known in detail. Although some international research projects have been initiated, trying to explore the structural and functional role of every species in the trophic chain, these tests are very time-consuming and expensive. And the fact that pollutants occur in environmental systems not in themselves, but they can interact with each other and other substances in the given system, makes it even more complicated to draw real conclusions. As a result of combined exposure, their toxic effects can be summed (addition), decreased (antagonism), or enhanced (synergism). At ecosystem level the exploration of interactions between groups belonging to different feeding types, the mapping of food webs seems an almost impossible task. Based on our present knowledge, field tests are the most suitable for the assessment of the ecosystem-level impact, environmental risk of a given chemical.

Test Questions

Match the letters to the correct numbers! (letters may be used more than once)

a) acute toxicity tests b) probit method c) addition d) xenobiotic

1) toxic effects are summed

2) flavour enhancers

3) short-term

4) drugs

5) can be used for the statistical evaluation of toxicological results

6) detect the presence of a toxic substance

7) plastics

Decide which of the following statements are true, and which are false (mark with T or F)!

8) Wildlife has not been able to adapt yet to the degradation of xenobiotics.

9) The AMES-reverse mutation test is suitable for the detection of genotoxicity.

10) In vivo tests are conducted not in a living organism, but on cells, cell cultures taken from a living organism, then maintained under laboratory conditions.

6. Widely Used Test-Organisms, Common Testing Methods

In toxicological testing the selection of the test-organism to be used is determined primarily by the test objective, practical feasibility, and the reliability of the results. There is no universal test-organism, that is no such living organism, or group of living organisms exists in our environment that would indicate the adverse effect of any chemical with the same sensitivity. Living organisms respond differently to the negative effects affecting them, or use different adaptation mechanisms to reduce the degree of anatomical and physiological damage. In recent years attempts have been made to compile lists defining the range of the most appropriate test-organisms for detecting the toxicity of certain chemical compounds, or group of compounds. Because of the difficulties outlined earlier, and the complexity of the problem, these attempts have not led to any actually useful result in practical ecotoxicology.

The living organisms most commonly used for testing represent different stages of evolutionary development: from prokaryotic bacteria to mammals numerous species are used for testing. The accurate testing methodology, the strict criteria for standardization are specified in standards, and in the countries of the European Union the OECD guidelines are also taken into consideration. Full compliance with these guidelines ensures that on the territory of the EU test results obtained in any laboratory are comparable to test results measured in other laboratories.

6.1 Bacterial Biotests

Bacterial tests are very widely used in toxicological practice, as they can be quickly performed, easily implemented under laboratory conditions, and they represent well the given ecosystem.

Pseudomonas fluorescens tests are commonly used for the testing of soil extracts and surface waters (MSZ 21 470-88, OECD 301). This species of bacteria requires inorganic nitrogen for growth, therefore it can be used primarily for the testing of samples where nitrification plays a key role in nitrogen circulation (Photo 8). The *Pseudomonas* test is based on the production of acid during the breakdown of glucose. If there is no toxic substance in the tested environmental sample, the pH value of the medium decreases due to the acids produced during the breakdown of glucose. In the presence of a toxic substance the breakdown of glucose is inhibited, the production of acid is prevented, and the pH value does not change, or only slowly and slightly.

Photo 8: The *Pseudomonas fluorescens*-test

The presence of a toxic substance can significantly inhibit bacterial cell division, the growth of bacterial colonies. As a result of a toxic substance diffusing into the medium, an inhibition zone is formed in the culture. The size of the inhibition zone is proportional to the toxicity (Photo 9).

Photo 9: An inhibition zone formed around a bacterial colony (EKF – Laboratory of Microbiology)

6.2 Plant Tests

In ecotoxicological practice, numerous species are used in plant tests, from simpler organisms, such as algae, to more complex ones. In higher plants destruction, or the degree of it, growth rate, photosynthetic activity, and metabolic enzyme activity are used to express the toxicity of a given chemical. Standards specifying the methods of testing recommend primarily easy to handle annual plants and grasses.

6.2.1 Algal Tests

Algal-tests are used mainly for aquatic toxicity testing. Similarly to bacterial biotests, tests performed on microalgae are also very widely used, as most of the species belonging to them are easily cultured under laboratory conditions, and are perfectly suitable for microcosm tests. In a medium of appropriate composition, and by ensuring the optimum conditions required for photosynthesis (appropriate temperature and lighting, adjustment of the pH value), significant biomass can be produced in a short time. When cultured in bioreactors, microalgae can be obtained on a large scale in a short time for tests (Photo 10).

Photo 10: A bioreactor (Eva Decker University Freiburg 2008)

In toxicological testing the detection of algal growth inhibition is used for assessing the toxicity of a given substance. The algal species recommended for testing are specified in OECD Guideline 201. From the cyanobacteria (blue-green algae) *Anabaena flos-aquae* and *Microcystis aeruginosa*, from the diatoms (Bacillariophyceae) *Navicula pelliculosa*, and from the green algae (Chlorophyta) *Pseudokirchneriella subcapitata* and *Chlorella vulgaris* are recommended as test-organisms (Photo 11).

Photo 11: Species used in algal tests

In ecotoxicological testing algal tests are based on the detection of growth inhibition in the test-organisms. The effect of the given chemical is monitored in an algal culture in exponential growth phase. The duration of testing is usually 96 hours. In 3 replicates per test series the change in algal cell concentration is determined daily by cell counting by microscope, chlorophyll-content measurement, or the gravimetric method. The growth rate compared to the control, or growth inhibition is determined for assessing toxicity. In algal tests the EC_{50}, NOEC and LOEC values are recorded as endpoints. In the case of soil samples, algal growth tests can be performed only with soil extracts. For aquatic ecosystems algal tests are widely used, and the type of the tested chemical determines which algal species is the most suitable as a test-organism, and what should be the test period (Table 4).

Table 4: Freshwater algae used in ecotoxicology (Calow 1993)

6.2.2 Seedling Tests

Seedling-tests use the sensitivity of germinating seeds for the assessment of the disposability of wastewater or hazardous waste. The great advantage of the test is that the seeds used in the test are easily stored and available at any time throughout the year. The test is performed most commonly with white mustard (*Sinapis alba*) seeds (Photo 12). *Sinapis alba* is sensitive to a wide range of pollutants, therefore it is used in many ecotoxicological tests. The obtained results assist in the decision-making concerning the protection of water resources and the preservation of the quality of groundwater.

Photo 12: Seedling-test with white mustard (*Sinapis alba)* seeds

The accurate testing methodology is specified in standard MSZ 21 976-17: 1993. In seedling tests the germination of given seeds is observed for 72 hours under laboratory conditions. Mustard seeds are germinated at a temperature of 20 C^o in darkness. After 24 hours the germinated seeds are counted for the first time and the length of the shoot is measured. The next counting is performed after 48 hours, when the length of the stem below the seed leaf (hypocotyl) and the root is also measured. The last measurement is performed after 72 hours. If in the control samples high germination is obtained, but seeds in the samples treated with the tested substance show no or low germination, the germination inhibition effect of the tested chemical can be clearly determined. The degree of germination inhibition is recorded as the endpoint of the test, expressed as a percentage of the control samples, that is the inhibition of stem and root growth is compared to the control samples. The ED_{50} value can also be determined during the test.

$$\mathbf{X= (K - M/K\ ) \times 100}$$

where X – is root growth %, or stem growth %

K – is the root, or stem length of the control seeds (mm)

M – is the root, or stem length of the treated seeds (mm)

6.2.3 Elodea (Pondweed) Tests

From flowering plants the pondweed species *Elodea canadensis* originating from North-America can be used very well for testing purposes (Photo 13).

Photo 13: *Elodea canadensis*, pondweed

It can be found everywhere in standing and slowly flowing waters, it is a common aquarium plant. For the testing 10 cm long shoot tips of pondweed are used. If they are collected outdoors, they should be allowed to get accustomed to the artificial laboratory conditions for a few days before testing. In addition to the control samples, 10 cm long shoot tips are placed into glass cylinders filled with solutions containing the substance to be tested, kept in light, and the efficiency of photosynthetic activity is monitored for 48 hours.

Evaluation of the results:

- a concentration of the given substance at which the green colour of the plant and its photosynthetic ability is lost during the test period, and in many cases even plant tissue breakdown occurs, can be regarded as lethal
- a concentration of the tested substance at which the lower leaves of the plant turn yellow, but the shoot tip survives, can be regarded as harmful
- a concentration at which the whole plant remains healthy can be regarded as harmless

The fluorometric method is also suitable for monitoring photosynthetic activity, as chlorophyll fluorescence is a good indicator of environmental stress effects.

6.2.4 Lemna (Duckweed) Tests

Different species of duckweed are common all over the world, they are widely used as test-organisms, because they are small, simple and easily cultured. They are characterized by rapid growth, with a leaf doubling time of 0.35 – 2.8 days. In toxicological tests the species *Lemna gibba* and *Lemna minor* are the most commonly used. The testing methodology is specified in OECD Guideleine 221. From heavy metals, Lemna species are particularly sensitive to chromium(VI) pollution, and strong growth inhibition can also be observed as a result of herbicides (Photo 14).

Photo 14: Lemna test used for detecting the growth inhibition effect of herbicides (based on K. Gruiz, BME)

6.3 Animal Test-Organisms

In the case of animals, the organisms used in ecotoxicological tests are also at different stages of evolutionary development, implementing different adaptation mechanisms. The selection of the test-organism, in addition to the test objective, is determined by the ease of maintenance under laboratory conditions. It is usually a simpler task to ensure the appropriate environmental factors for organisms belonging to invertebrates, and with them in many cases the effect of a given pollutant can be monitored over several generations. Some of the used test-organisms can be used for both aquatic and sediment samples, as well as soil samples.

6.3.1 Protozoa (Single-Celled Organism) Tests

Species belonging to protozoa are used in the ecotoxicological testing of both aquatic ecosystems and soil samples. In the case of soils, single-celled organisms live in the pores of the soil and are very sensitive to the toxic effects affecting them. From protozoa three species are used most commonly for testing: *Tetrahymena pyriformis* (SAUVENT et al. 1999), *Colpoda culus,* and *Paramecium aurelia* (Photo 15).

Photo 15: Single-celled organisms used most commonly in ecotoxicological tests

For aquatic samples in acute tests the % survival value, the EC_{20}, EC_{50}, and LD_{50} values are determined as endpoints.

6.3.2 Daphnia Acute and Chronic Tests

Daphnia species belong to the Cladocera. In our waters they are important elements of the zooplankton, they also feel comfortable in waters with higher trophic levels, but their sensitivity to toxic substances is high. In water bodies they are capable of daily vertical movement, therefore they often appear in swarms in an inhomogeneous form. In aquatic ecosystems they play an important role as primary consumers, and serve as food for fry. In toxicological testing primarily two species are used: *Daphnia magna* and *Daphnia pulex*. In the USA Ceriodaphnia species are also used as test-organisms (Photo 16).

Photo 16: Daphnia species used for testing

Daphnia acute test: the duration of testing is 48 hours, the methodological criteria are specified in OECD Guideline 202. For the test the appropriate water quality is ensured, as an indispensable condition, and 10 one-day-old Daphnia are placed in 100 ml of test solution. During the test period the animals are not fed. The responses of the test-organisms are recorded at different concentrations of the tested chemical. The immobilization of the tested individual or individuals is regarded as the endpoint of the test. Acute Daphnia tests can be used well for determining the toxicity of „pure" chemicals, wastewaters, hazardous wastes. Previously third generation four-day-old individuals were used for Daphnia tests, now the tests should be performed on one-day-old specimens. This modification was necessary because the younger the individuals used for the testing, the lower the chance of their getting accustomed to the given chemical, and as a consequence, the higher their sensibility to the tested substance. Daphnia tests can be easily implemented, are repeatable under laboratory conditions, have appropriate sensitivity and are cost-effective.

Daphnia chronic test: the duration of testing is 21 days. This is a long-term test, in addition to survival, during this period the growth rate and reproduction rate can also be observed. The methodological criteria for Daphnia reproduction tests are specified in OECD Guideline 211. Daphnia species can be regarded as atypical species, as they use an asexual reproductive strategy and reproduce by parthenogenesis (virgin birth) under laboratory conditions. In the reproduction tests therefore, in the absence of males, mating habits cannot be observed. During chronic testing the test-organisms should be fed, primarily with algae, but a yeast suspension, or other additives can also be used. Survival, growth and reproduction are recorded as endpoints.

6.3.3 Collembola (Springtails) Tests

Springtails can be regarded as members of an ancient order of insects, species belonging to them are used both in acute and chronic toxicity tests. The results of Collembola-tests can be taken into account primarily in the ecotoxicological classification of soils (SUBAGJA & SNIDER 1981). The most commonly used species is *Folsomia candida*, which is suitable for determining the toxicity of organic pollutants (Photo 17).

Photo 17: *Collembola* (Springtails), as ecotoxicological test-organisms (based on T. Tully, 2008)

The methodology for Collembolan reproduction tests is specified in OECD Guideline 232. During laboratory testing the animals are kept on an artificial soil made of plaster and charcoal. The OECD standard soil can be used as control. During the test period it can be observed to what extent is the survival rate (ED_{50}) affected by the polluted samples, compared to the control, and in 14-day chronic tests the reproduction rate can also be monitored.

6.3.4 Eisenia foetida (Earthworm) Tests

Earthworm, as a test-organism, is suitable for testing the toxicity of soil. The method is also suitable for the detection of sublethal concentrations (VAN GESTEL et al. 1989). In acute and chronic tests the survival rate, and the LC_{20}, LC_{50} and NOEC values are determined as endpoints (Figure 15).

Figure 15: Evaluation of the results of *Eisenia foetida* ecotoxicological tests (based on B. Papp, BME)

In chronic tests the reproduction rate can also be determined (OECD 222). Bioaccumulation tests measure the tissue concentration of the tested substance, and in that case the tested individuals are analyzed after starvation.

6.3.5 Acute and Chronic Fish Tests

Fish tests are indispensable for the assessment of the risk of harmful effects affecting aquatic ecosystems, as fish are of central importance in the aquatic food chain from both a structural and a functional point of view. The degree of the bioconcentration and bioaccumulation of hazardous chemicals, the bioavailability of the tested substance can be monitored well with fish tests, and the obtained results and the conclusions drawn from them cannot be left out of consideration from the human health point of view either. The list of species that can be used in fish-tests is very wide-ranging. The OECD Guidelines recommend small-size tropical and subtropical fish species with short life-cycles that are easily acclimated to laboratory conditions and are available at any time of the year (e.g. *Brachydanio rerio* – zebrafish, *Lebistes reticulata* – guppy). However, ecotoxicologists always call attention to the fact that in order to determine the actual toxicity of a given chemical it is also absolutely necessary to perform the tests on one or more fish species native to the given area (Table 5).

Table 5: Fish species recommended for fish-tests

The methodology for acute 96-hour fish tests is specified in OECD Guideline 203, while the criteria for chronic 14-day or longer tests are included in OECD Guideline 204.
From the recommended fish species zebrafish originates from Peninsular India, but it is a common and popular aquarium fish in Hungary as well. Its average body length is 4-4.5 cm, the female is more robust, the male is slightly slimmer. It is named for the darker stripes running along the body. For this species the optimum water temperature is 20 - 24 C^{o}, it prefers moderately hard water, and has a lower oxygen demand (MILLS 1993, PASARÉTI et al. 2005).

Guppy is native to Venezuela, Barbados, and Guyana. It is a very popular ornamental fish, now bred in many varieties. The primary objective of breeding is to achieve a more impressive tail fin. Guppy also prefers moderately hard water, for it the optimum water temperature is 24 - 26 C^o, and it is more sensitive to oxygen deficiency (Photo 18).

Photo 18: *Brachydanio rerio* – zebrafish, *Lebistes reticulata* – guppy

Test methodology: 7 days before the testing the tested individuals should be acclimated, and the optimum water temperature, the dissolved oxygen content, the appropriate pH and water hardness should be ensured. In acute tests the animals are not fed during the test period, in chronic tests appropriate food should be provided.

The selection of the test-organism, the determination of the test conditions requires sufficient information on the tested substance: its water solubility, chemical stability, and biodegradability are important in the selection of the test method. The above data determines whether a static, a semi-static, or a flow-through system should be used.

Static test: is a toxicity test in which no flow of the test solution occurs.

Semi-static test: is a toxicity test without flow of the test solution, but with occasional batchwise renewal of the test solution after prolonged periods, e.g. 24 hours.

Flow-through test: is a toxicity test in which the test solution is continually renewed in the test chamber.

There are strict conditions for the validity of fish-tests:

- the mortality in the control sample should not exceed 10% at the end of the test
- constant conditions should be maintained throughout the test
- the dissolved oxygen content should be at least 60% of the air saturation value throughout the test, aeration can be used, if necessary, provided that it does not lead to a significant loss of the tested substance
- the concentration of the tested substance should be maintained at minimum

80% of the nominal concentration

- at least 7 individuals should be used in each sample (including the control)
- the recommended loading in static and semi-static systems is 1 g fish/l, in flow-through systems higher loading can be accepted
- during the test period factors disturbing the behaviour of the fish should be avoided

In fish tests the acute and chronic test endpoints are different: in acute tests the mortality rate and survival rate are determined. In chronic tests nutritional, attitudinal and behavioural changes, other physiological abnormalities, and in longer-term tests reproductive disorders can also be detected. On the basis of this it can be clearly stated that the risk of the hazard of a given chemical, the consequences of its effect can be assessed more realistically, and the actual role of sublethal concentrations in physiological abnormalities can be understood better by using chronic tests. The results of ecotoxicogenetic tests have shown that sublethal concentrations of a given pollutant can significantly reduce the genetic variability of the tested population in the long-term. Chronic tests are also suitable for the detection of the bioaccumulation and biomagnification of certain chemical substances.

Both acute and chronic tests can be single-species and multi-species tests. The use of multiple species allows the simplified modelling of the food chain, the understanding of the biological interactions and the toxicity of a given chemical.

Test Questions

Circle the correct answer!

1) Which fish-test is used when no flow of the test solution occurs?

a) flow-through test

b) static test

c) microcosm test

d) field test

2) Which fish species should be used for laboratory testing?

a) species with long life spans

b) large-size species

c) species not easily acclimated to laboratory conditions

d) species with short life spans

3) The bacterial Pseudomonas test is based on what process?

a) the biosynthesis of fats

b) the breakdown of proteins

c) the biosynthesis of vitamins

d) the production of acid during the breakdown of glucose

4) Which species is used in seedling-tests?

a) pondweed

b) white mustard seeds

c) watermilfoil

d) duckweed

5) Collembola-tests are suitable for the testing of which environmental element?

a) surface water

b) air

c) soil

d) groundwater

6) Lemna species are particularly sensitive to the presence of which heavy metal?

a) copper

b) chromium(III)

c) cadmium

d) chromium(VI)

Match the letters to the correct numbers!

a) algal-test b) OECD 202 c) protozoa-test d) lethal concentration

7) Paramecium aurelia

8) the photosynthetic ability of the plant is lost

9) Daphnia acute test

10) Chlorella vulgaris

7. The Fate of Toxic Substances in Environmental Systems

Toxic substances released into the environment can cause the pollution of air, surface and groundwater resources, and soil. In a given environmental system different chemicals undergo different transformation processes, as a result of which their toxic effect can change. Various chemical effects (hydrolysis, oxidation, reduction) can eliminate them from the system, or as a result of physical processes (adsorption, sedimentation) they can appear in the sediment in orders of magnitude higher concentrations.

For the detection of anthropogenic pollution in environmental systems, for decades primarily physical and chemical parameters have been determined, supplemented with some biological observations. Attempts to place the emphasis on biological parameters in the assessment of the ecological condition of a tested environment try to eliminate this methodological deficiency. In the assessment of the effects of hazardous substances, today bioconcentration and bioaccumulation measurements play an important role within the biological tests. The fate of xenobiotics released into ecological systems is particularly important from the point of view of the response of wildlife. These substances foreign to the environment can be either used by living organisms in the synthesis of their own materials, or converted into water-soluble substances and excreted from the organism with the urine. The efficiency of excretion is often not appropriate in the case of xenobiotics (DARVAS & SZÉKÁCS 2006). Less water-soluble persistent compounds can accumulate in the tissues of living organisms, and by becoming mobilized at any time, they can cause physiological, as well as attitudinal and behavioural disorders, or in serious cases the destruction, death of the living organism.

7.1 Bioindication, Bioaccumulation, Bioconcentration and Biomagnification

The measurement of the concentration of chemicals appearing and accumulating in living organisms can provide adequate information on anthropogenic pollution impacts, and can be suitable for the implementation of biomonitoring systems.

In practice biomonitoring can be divided into three levels:

- passive biomonitoring

- active biomonitoring
- ecological biomonitoring

Passive biomonitoring is used more commonly in ecotoxicology. A conclusion can be drawn on the anthropogenic pollution of the tested habitat from the concentration of the pollutant accumulated in the tissues of organisms living there.

In **active biomonitoring** organisms that have a high tendency for accumulation are moved from a relatively unpolluted habitat to a selected environment, and the tissue accumulation of the given pollutant is measured after a specified period of time.

In the monitoring of toxicity, the physiological responses of the observed individuals to the effects of toxic substances are monitored by means of static „bioassays”, or dynamic biological early warning systems - „BEWS”. Biological early warning systems, through physiological or behavioural changes in indicator organisms, can record any deterioration occurring suddenly in the environmental conditions (OERTEL et al. 2001).

In **ecological monitoring** the integrity of ecosystems is tested by taking into account the environmental impacts (e.g. diversity, the incidence of indicator organisms). Thus in the detection of anthropogenic pollution the selection of the appropriate indicator organisms is an important factor, and in practice the use of so called „accumulator organisms” having a high bioaccumulation potential proved very effective (SALÁNKI 1989, OLIVIERA RIBEIRO et al. 2005).

Bioindication: the method is based on the fact that living organisms have different tolerances to environmental changes (DÉVAI et al. 1993). In aquatic ecosystems, from macroinvertebrates the groups with longer life-cycles and limited locomotion ability are suitable for bioindication test, e.g. amphipods. They are capable of active locomotion, but incapable of avoiding effects harmful to the environment (ALIKHAM et al. 1990). Amphipods, as fish food organisms, also play an important role in transport through the food chain.

Organisms belonging to molluscs, due to their filter feeding lifestyle and relative immobility, can also be good indicators of anthropogenic pollution (FARKAS & SALÁNKI 2000).

In aquatic ecosystems, from vertebrates in many respects fish are the most suitable for bioindication tests. They play a central role in the food chain, they have a high bioconcentration factor, and because of their long life-cycle they preserve the traces of changes in the habitat for a long time (FARKAS et al. 1999, MILINKI & MURÁNYI 2001).

Bioaccumulation: the accumulation of substances, or pollutants in the tissues of living organisms, irrespective of the method of absorption. The bioaccumulation of pollutants is the manifestation of the defence mechanisms of organisms forming the given ecosystem, as living organisms try to get rid of toxic substances by either excreting them, or „removing" them from circulation in an insoluble form. The latter can occur e.g. by crystal formation, or often they are stored in the tissues, organs of the living organisms, bound to insoluble proteins. The concentration of the toxic substance accumulated in the organism can be several times the pollutant concentration found in the environment. Bioaccumulation in this sense can be regarded as a biologically enhanced selective signal (GRUIZ 2007).

Unnatural xenobiotics released into environmental systems cannot be broken down by living organisms, or only to a limited extent, thus they can accumulate in the tissues and cause acute or chronic damage. The amount of pollutants accumulating in living organisms depends largely on the size, age and health of the individual. Tissue accumulation is also greatly influenced by the bioavailability and absorbability of the given chemical. In the dynamics of uptake and depuration species differences can be observed (FÖRSTNER 1993). In bioaccumulation tests, from the amount of the pollutant enriched in the test-organism a conclusion can be drawn on the extent of environmental damage, and good information can be obtained on the effect of the given substance and its method of entry into the food chain.

Bioconcentration: means the net result of uptake from the environment, that is in living organisms it can be determined from the difference of uptake and depuration. Bioconcentration tests can be divided into two phases (uptake and depuration phase) and from the dynamics of these the toxicokinetics of the given pollutant can be determined (SANCHO et al. 1998).

The bioconcentration factor (BCF) expresses the ratio of the tissue concentration measured in the tested living organism and the concentration detected in the environmental system (GRUIZ et al. 2001).

$$\mathbf{BCF = C_{\text{in the given organism}} / C_{\text{in the given environmental system}}}$$

In aquatic ecosystems, fish having an average lipid content of 4.8% are suitable for bioconcentration tests. The BCF value is determined by the place of the test-organism in the food chain and the proportion of its fatty tissue. The higher an organism is in the food chain, and the higher the proportion of fatty tissue in its body, the higher the BCF value (FALANDYSZ et al. 2004).

The bioconcentration factor of organic compounds can be characterized well by the octanol-water partition coefficient (K_{ow}). Naturally, the relationship between the BCF and the K_{ow} values can be modified by several biological factors (the kinetics of uptake-depuration, cross-membrane transport, cellular metabolic processes). Based on the BCF value, different compounds can be characterized by different tendencies for accumulation (Table 6).

Tendency for bioaccumulation	BCF value
high	> 3
moderate	1.5 - 3
low	< 1.5

Table 6: The relationship between the tendency for bioaccumulation and the BCF value (based on K. Gruiz, 2006)

In bioaccumulation tests the following factors can be determined for a given chemical:

- the BCF value
- the time of excretion from the organism
- the characteristics of metabolic pathways, biotransformation methods
- the degree of organ-specific accumulation
- the non-excreted residue and its potential availability

Biomagnification: means the transport of a given element or compound through the food chain, that is its accumulation at successive levels, thus increasing the risk of secondary poisoning (NENDZA et al. 1997). The degree of enrichment in the food chain can be so high that symptoms of poisoning can occur in living organisms even when the concentration of the tested substance in the surrounding medium is below the toxic threshold (BARANOWSKA et al. 2005). In the evaluation of the results of toxicological tests, it is important therefore to take into account the enrichment in the food chain as well. For assessing biomagnification the concentration of the tested pollutant should be monitored at least at three trophic levels, e.g. phytoplankton, zooplankton, fish (Figure 16).

Figure 16: The biomagnification of DDT in aquatic ecosystems (based on G. Miller, 2007)

The results of biomagnification tests can be used well with respect to human health (CORDIER 2004, CID et al. 2007, GRANDJEAN 2008). They allow the assessment of the health risk of highly hazardous persistent compounds entering the human organism through the food chain.

7.2 The Measurement of Biodegradation in Ecotoxicological Tests, Biodegradation Tests

Biodegradation is an aerobic or anaerobic process in which the microorganisms of the soil/sediment mineralize, that is convert into a form that can be absorbed by plants, the biogenic elements participating in the production, transport of organic substances, and the storage of energy. Biodegradation thus determines the efficiency, rate of the circulation of elements in a given ecosystem, and plays an important role in degrading and rendering harmless pollutants.

7.2.1 The Process of Biodegradation, its Applicability in Practice

In the process of biodegradation chemicals released into the environment are converted, during the process their physical and chemical properties can change. The efficiency of biodegradation is determined by the composition of the microflora of the soil/sediment, and the degree of its adaptation. Biodegradation potential is a natural inherent property of ecosystems, as microbial communities living in the soil/sediment have learned over time the utilization of almost all substances, and the possibilities of gaining energy. They have adapted to most of the pollutants released into environmental systems, and thus the microflora plays an important role in their removal, the neutralization of their toxic effects.

The objectives of biodegradation research are:

- to study the biodegradation potential of a given environmental element
- to determine the biodegradability and persistence of a given pollutant
- to assess the environmental risk

The rate and degree of biodegradation can be influenced by several environmental factors (e.g. the number and species composition of microorganisms present in the medium, the pH, the temperature, the period of exposure).

In the case of organic compounds the biodegradability of the pollutant is significantly influenced by the volatility, the water solubility, the molecular weight, the octanol-water partition coefficient, and the nature of the chemical bonds of the given substance. The water solubility and molecular weight of the given chemical determines the possibility and type of transport through the cell membrane. With respect to chemical bonds, compounds containing ester bonds are more easily hydrolizable, and thus more readily biodegradable. From persistent compounds, chlorinated substances and those having a polycyclic aromatic structure are not readily biodegradable (LEI et al. 2005).

The environmental risk of a chemical is always inversely proportional to its biodegradability. The longer a given pollutant stays in an environmental system, the longer it exerts its harmful effect, and the more biodegradable a substance is, the shorter the time it stays in the environment.

7.2.2 Biodegradation Test Methods, Biodegradation Tests

The selection of the biodegradation test method is determined by the test objective, the physical-chemical properties of the tested substance, and the practical possibilities of standardization.

Biodegradation tests can be divided into three levels:

- readily biodegradable pollutants
- not readily biodegradable pollutants
- simulation tests

For **readily biodegradable pollutants** the so called „ready” biodegradability test should be performed: by using aerobic microorganisms, the rates of oxygen consumption and carbon dioxide production, and dissolved organic carbon (DOC) removal are measured. For readily degradable substances it is sufficient to enrich some bacterial strains, to use a small amount of inoculum.

For **not readily degradable pollutants** the so called „inherent” biodegradability test is performed: a larger amount of inoculum is used, and conditions similar to those of wastewater treatment are provided.

In **simulation** tests the biodegradation rate constant is estimated from the results of standard tests for the characterization of biodegradability in soil/sediment. The rate constant and half-life can show significant differences between the readily and not readily degradable pollutants (Table 7). Half-life means the period of time it takes for the tested substance to degrade by 50%.

Test result	Rate constant (k) l/day	Half-life (day)
readily biodegradable	4.7×10^{-2}	15
readily, but not within 10 days	1.4×10^{-2}	50
not readily biodegradable	4.7×10^{-3}	150
not biodegradable	0	∞

Table 7: The relationship between the rate constant and half-life in an aquatic ecosystem (K. Gruiz 2006)

The most commonly used biodegradation test methods:

1) Respiration rate measurement: a method based on the measurement of $C0_2$ production and 0_2 consumption (not compound-specific, the amount of oxygen consumed and carbon dioxide produced during microbial breakdown is measured).
2) Mineralization monitoring using a C^{14}-labelled substrate.
3) Nitrogen metabolism test:
   - nitrification test (studies ammonia oxidation using specific bacteria - *Nitrosomas, Nitrobacter* species)
     $NH_4^+ \rightarrow N0_2^- \rightarrow N0_3^-$
   - denitrification test (studies $N0_3 \rightarrow N_2$ conversion under anaerobic conditions)
   - atmospheric nitrogen fixation test (studies nitrogen fixation by *Rhizobium* species)
4) Soil/sediment enzyme activity test (measurement of urease, phosphatase, dehydrogenase, and cellulase activity):
   - determination of the number of cells showing enzyme activity
   - detection of intermediate or end products appearing as a result of enzyme activity
5) Use of genetic engineering technologies
   - Biotechnologists inject a soil or sediment sample with so called genetically modified bacterial strains. Genetic engineering can produce bacterial strains capable of degrading not readily biodegradable substances as well. Genes adapted to a given pollutant are often linked to mobile genetic elements in cells, e.g. plasmids, transposons. These mobile genetic elements, if necessary, can spread very quickly in a microbial population, and thus it is able to adapt to the given pollutant in a short time. There is a close relationship between the adaptation and the resistance of the microflora of the soil/sediment. The presence of resistance proves that pollution has been in existence for a longer time, or in the case of

persistent substances resistance means that the immobilization of the toxic substance is achieved by storage. This immobilization is the basis of bioaccumulation.

- Detection of coding genes responsible for enzyme activity and their incidence by using a hybridization technique, or by means of a polymerase chain reaction (PCR). The hybridization technique can be used directly, without isolation and culturing from soil and sediment samples. The PCR technique, due to its higher sensitivity, is suitable for detecting the presence of even 1-2 genes, by exponentiation.

The practical significance of biodegradation tests: they make possible the removal of pollutants from environmental systems, and on the basis of the results obtained from biodegradation tests the appropriate remediation technology can be selected. As a result of progress in this field of research, today the removal of chlorinated hydrocarbons from soil and sediment, the elimination of oil pollution can be achieved in practice, and answers can be given to questions about whether the tested soil/sediment is polluted, whether the given pollutant is toxic, whether microbial functions are inhibited, and whether the microflora has adapted to the tested chemical.

Test Questions:

Match the letters to the correct numbers! (more than one letter may be correct for each number)

a) tissue accumulation b) mineralization process c) not readily biodegradable pollutants d) neutralization of toxic effects e) detection of readily biodegradable substances f) BCF value

1) biodegradation

2) microbial communities

3) persistent compounds

4) „ready” biodegradability test

5) can be determined on the basis of bioaccumulation tests

Decide which of the following statements are true, and which are false (mark with T or F)!

6) On the basis of biodegradation tests the appropriate remediation technology can be determined.
7) The environmental risk of a chemical is directly proportional to its biodegradability.
8) The biodegradability of organic compounds is not influenced by their water solubility.
9) Microbial communities can adapt to the degradation of pollutants.
10) For assessing biomagnification the concentration of the given pollutant should be tested at least at two trophic levels.

8. Ecotoxicology and Risk Assessment, Types of Early Warning Systems

The quantification of the effect, risk of chemicals released into the environment is indispensable from both the ecological and the human health point of view. The determination of the degree of risk is important in practical environmental protection, and greatly contributes to making the appropriate environmental policy decision. With the predictions that can be made on the basis of the results of environmental impact assessments and ecotoxicological risk assessments, many environmental disasters could be prevented. And more efficient prevention would lead to much more positive gains in the long-term from an ecological, economic and health point of view.

8.1 Environmental Impact Assessment, Risk Assessment of Chemicals

Environmental impact assessment (EIA) is a prediction method, the task of which is to determine, estimate the expected changes in environmental conditions as a result of planned human activity (FÖLDI & HALÁSZ 2009). EIA tests greatly contribute to a sound foundation for environmental decisions (MAGYAR et al. 1997).

The main steps of environmental impact assessment:

- determination of the impact factors (direct and indirect factors)
- description of the processes of impact (exploration of the possible interactions)
- determination of the area of impact
- assessment of the environmental conditions (estimation of changes in the environmental conditions, exploration of interactions between environmental elements, environmental responses to the given impact factors, and determination of the sensitivity of the system)

By exploring the above factors, the risk of a given human activity can be quantified, and the negative effects can be reduced to the minimum.

Risk means the probability of the occurrence of a future adverse event. The assessment of the risk of chemicals released into the environment as a result of human activity is one of the main segments of ecotoxicological tests. Chemicals released into ecological systems cause changes that are difficult to estimate both locally and globally. This is particularly true for xenobiotics, to which the metabolism, enzyme system of living organisms has not been able to adapt yet, the biological communities of the ecosystem have not had sufficient time to prepare for the biodegradation of these substances. The above factors can result in irreversible damage to our environment, causing dramatic changes in the species composition.

Risk of chemicals: the RQ (Risk Quotient) value is used for the quantitative characterization of ERA (Environmental Risk Assessment). The objective of environmental risk assessment is to determine whether the concentration of a given pollutant poses an acceptable or unacceptable risk to environmental systems.

For the calculation of the RQ value two factors need to be known, which can be determined by means of ecotoxicological tests:

- PEC value (Predicted Environmental Concentration – the predictable environmental concentration of the given chemical). For its calculation the physical and chemical properties of the given pollutant, its biodegradability and tendency for bioaccumulation, the effect of the polluted environmental system, the mobility of the tested chemical, and the distance from the site of release should be taken into account.

  The distribution of the given pollutant is determined by using propagation models, which can be simulated by computer programs.
- PNEC value (Predicted No Effect Concentration – the concentration of the given chemical with no adverse effect). By means of ecotoxicological tests the concentration of the given pollutant with no adverse effect can be determined for some representative species, and on the basis of the obtained results it can be extrapolated to the whole ecosystem. For the determination of the PNEC value a sufficient number of tests should be performed, and the obtained results should be multiplied by a safety factor (Table 8).

Harmless concentrations in the toxicological testing of members of aquatic ecosystems	Safety factor

LC_{50} at three different trophic levels (acute test: algae, Daphnia, fish)	1000
Measurement of at least one long-term NOEC (fish, or Daphnia)	100
Measurement of NOEC at two different trophic levels (algae and Daphnia, or Daphnia and fish)	50
NOEC value from chronic tests at three trophic levels	10
Field data, or mesocosm experiments	1

Table 8: Safety factors for the determination of PNEC values by extrapolation from the results of ecotoxicological tests in the case of aquatic ecosystems (LC – lethal concentration, NOEC - the highest concentration at which no effects are observed (based on K. Gruiz, 2007).

The RQ value is given by the quotient of PEC and PNEC:

RQ = PEC / PNEC – the higher the quotient, the higher the risk (Table 9).

RQ value	level of hazard to the given ecosystem
< 0.001	negligible
0.001 – 0.1	low
0.1 - 1	moderate
1 - 10	high
> 10	very high

Table 9: The relationship between the RQ value and the hazard of the given chemical

In ecotoxicological testing and in the assessment of human health risk different characteristics are taken into account for determining the degree of the harmful effect. The HQ (Human Risk Quotient) value is used for assessing the human risk of a given chemical.

$$HQ = ADD / TDI$$

ADD – Average Daily Dose (the hazardous substance entering the organism on a per-unit-body-weight basis (mg/kg x day)).
TDI – Tolerable Daily Intake (a value determined by extrapolation from results obtained in animal experiments).

In the case of the combined presence of several pollutants, the RQ and HQ values should be calculated for all pollutants and all routes of exposure, and the sum of these gives the degree of risk for the whole population.
The more data are available, the more accurate the estimate of risk will be. When there is insufficient information, or poor data on a pollutant, the most pessimistic scenario should be taken into account.

8.2 The Applicability of Early Warning Systems (EWSs)

Early warning systems try to predict the occurrence of a hazardous pollution impact on the basis of physical, chemical, biological and ecological tests, and thus can play an important role in the control and protection of environmental systems. Monitoring systems are suitable for determining the environmental risk of chemicals by using mathematical models.
Environmental risks can be assessed at four levels (GRUIZ, 2007):

- general
- local
- regional
- global

General level: deals with the general problems of predicting the impact of pollutants.
Local level: the tests cover the whole life cycle of a given chemical from production to use, and pollution impacts are separated (agricultural, industrial, household or other pollution). At local level point sources, wastewater dischargers can be identified.
Regional level: pollutants are identified, transport routes are determined in the whole catchment area.

Global level: on the basis of primarily satellite images covering the whole Earth, global trends can be determined, e.g. deforestation caused by acid rain (Figure 17).

Figure 17: Early warning systems at local, regional and global levels (Gruiz, 2007)

Early warning systems can be set up at various points:

- At the sources of pollution (which can be point or diffuse sources) – they can be monitored by means of aerial photographs, the degree of risk, and the urgency of intervention can be usually determined.
- Along transport routes – the spread of a polluting agent in the given environmental element can be detected by physical, chemical and biological test methods. The concentration of the given chemical can decrease along the transport route as a result of dilution, or the adaptation of living organisms, the bioaccumulation and biomagnification of the pollutant can occur. In addition to the primary effects, the appearance of secondary effects should also be taken into account.
- At receptor level (ecosystem, man) – receptors are often located far away from the source of pollution, therefore the direct detection of a given chemical is usually not possible. In that case primarily biological and ecological tests are effective. The appearance of a polluting agent can cause a change in biodiversity, which is a good indicator of damage to the ecosystem. At receptor level the determination of bioaccumulation, genetic variability, tolerance and the degree of biodegradation can also be used efficiently in early warning systems.

The conditions for setting up early warning systems:

1) an appropriate risk model (transport models, exposure models)

   Transport model: the transport routes are determined by the properties of the given chemical and the environmental elements getting into contact with it.

Exposure model: it determines primarily the extent to which receptors (ecosystem, man) are exposed to the effect of the given pollution impact.

2) a relevant indicator (it should be sensitive to and selective for the given pollutant)

   There are indicator species that have a high tendency for bioaccumulation, therefore they are suitable for the detection of persistent pollutants (e.g. *Potamogeton pectinatus* – sago pondweed, *Dreissena polymorpha* – zebra mussel can be used well for testing the accumulation of heavy metals and pesticides). These organisms are introduced into pollution hot spots, and the physiological effect of the pollutant, and its accumulation in the tissues of the given indicator is monitored.

3) an appropriate measurement technique
4) the use of appropriate data evaluation and interpretation methods

Biological methods are often much more effective than chemical tests that record a momentary condition. Biological methods are suitable for the detection of a toxic effect even when the pollutant is present in an environmental system at a low concentration, and by detecting the selective responses of biological systems, they can provide early warning of hazards. Biological tests are also the most suitable for the detection of chronic toxicity, and for cases when synergistic or additive effects occur as a result of interactions between pollutants. In such cases, as the toxic effects enhance each other, chemical analytical methods prove ineffective.

In early warning monitoring systems the following techniques based on biological responses are in widespread use:

1) biomarkers
2) biosensors
3) bioindicators
4) remote sensor systems

Biomarkers: indicator molecules (biochemical, or cellular level responses), produced as a result of a pollutant. Their amount is proportional to the concentration of the substance causing the adverse effect and the degree of exposure. Certain enzymes, metallothioneins, different cytogenetic structures and the genetic macromolecules themselves can function as biomarkers.
The concentration of metallothioneins (MTs), as multifunctional proteins, significantly increases in organisms exposed to toxic metals, as these proteins are capable of binding metal cations, therefore they are very important in the detoxification of harmful metals. Above a certain threshold, heavy metal concentrations prevent the activation of enzymes functioning as antioxidants, and the level of antioxidants in the liver (GSH-glutathione, SOD-superoxide dismutase) detectably decreases.
A clear relationship can be shown between the appearance of certain chromosome aberrations and exposure to a given chemical, and qualitative and quantitative changes can be detected even at DNA level as a result of an adverse environmental impact.

Biosensors: by means of a converter unit the response of a biological system is transformed into an electrical signal. Biosensors are used increasingly in practice due to their ease of use, low cost and quick implementation. Primarily cells or enzymes can be taken into account as biosensors. Bacteria have proved to be excellent selective biosensors for testing certain pollutants. Microorganisms respond immediately to chemicals released into environmental systems, e.g. with a change in diversity, with the appearance and spread of specific genes. These biological response signals can be transformed into electrical signals and transmitted to a processing unit.
The so called „shellfish monitoring” system can be regarded as biosensor monitoring. The essence of the method is that the opening and closing of shells is greatly influenced by pollution impacts. In the presence of toxic substances the degree of opening is less, the system transmits the shell movement pattern changed by the pollution in the form of electrical signals to a data processing unit. The change in shell movement can be interpreted as an early warning sign, and the setting up of a „shellfish monitoring” system can play an important role in the prevention of pollution impacts.

Bioindicators: biomonitoring systems often use bioindicator organisms. Living organisms have different sensitivities to certain environmental impacts, and thus they are suitable for use as indicators in the assessment of environmental conditions. The incidence of such indicator organisms changes as a result of stress (HOLT & MILLER 2011). They respond to differences occurring in the environmental parameters with an increase or decrease in the number of individuals, and the species composition of the habitat can also be greatly modified. Many species suitable for bioindication can also be used as monitoring species.

Types of species used for monitoring:

- Guarding species – highly sensitive species that are introduced into the tested area, a decrease in the number of individuals, or their destruction can be interpreted as an early warning sign. Such species are used by early warning systems in so called hot spots along pollution transport routes.
- Detector species – species that live in the given area and show a change in behaviour, in the number of individuals in the presence of a pollutant.
- Exploiting species – after pollution their number of individuals increases, as they become resistant to the pollution. In the case of pollution the number of individuals of species that can adapt to the given chemical increases, that is there is a close relationship between resistance and adaptation.
- Accumulating species – they absorb and accumulate in their tissues the tested pollutant, thus the presence of harmful chemicals can be detected even when they occur in abiotic environmental elements in a chemically undetectable amount. Species having a high bioaccumulation potential are e.g. in aquatic ecosystems shellfish species, amphipods, and pondweeds.

Remote sensor systems

Today the use of remote sensor systems plays an increasingly important role in the mapping of pollution sources, in the assessment of the spread and impact of pollution, and in taking timely actions. Early detection can help in making environmental decisions early, therefore the development of the remote sensor technique can be a tool for preventing many environmental disasters. Remote sensing is an indirect means of obtaining information, on the basis of the analysis of electromagnetic radiation and light reflectance spectra very useful information can be obtained on the condition of our environmental systems.

The most common fields of application:

- monitoring of changes in biodiversity over large areas
- assessment of the amount of biomass
- evaluation of soil condition
- assessment of vegetation condition
- monitoring of the spread of pollution, assessment of the impact of pollution

Remote sensor systems can be grouped as follows:

1) on the basis of the used carrier
   - satellite remote sensor systems (Photo 19)
   - aircraft-mounted remote sensor systems

Photo 19: Satellite image of the Earth taken by Spot-5 (2003)

Satellite images of the surface of the Earth can be used well to monitor the scope, size of environmental elements, the trends in environmental changes, the spread of pollution and its negative effects observable in the biosphere.

2) on the basis of the detected electromagnetic wavelength bandwidths and band numbers
   - multispectral remote sensor systems: 2-20 spectral bands with 20-100 nm bandwidths
   - hyperspectral – hundreds of spectral bands with 1-10 nm bandwidths

Hyperspectral aerial images, in addition to recording spatial and temporal changes in vegetation, allow the separation and identification of plant species. Aerial images are taken of the tested area with a hyperspectral camera in the wavelength range of the infrared reflectance spectrum. For the identification of individual tree species the spectra seen in the taken image are compared to a reference spectrum taken of the given plant species. The method has been improved by a Japanese company, by creating multiple reference spectra for the identification of each given plant species. This method allows identification with more precision (Figure 18).

Figure 18: Hyperspectral images (FUJITSU, 2011)

With this method it is possible to produce the complete vegetation map of an area, and to indicate changes in biodiversity.

The advantages of remote sensor systems:

- there is no direct contact between the measuring instrument and the tested object, a biological response can be detected from a longer distance (e.g. in the case of „shellfish monitoring")
- changes in condition can be observed continuously
- measurements can be planned and controlled spatially and temporally
- phenomena invisible to man can also be detected

The assessment, estimate of the ecotoxic risk of pollutants released into our environment, the use of integrated risk models, and the setting up of various early warning systems can significantly reduce the further deterioration of the quality and condition of environmental elements.

Test Questions

Circle the correct answer!

1) What are the characteristics of „guarding" species?

a) they are less sensitive species

b) their gene composition does not change

c) they are highly sensitive species

d) they do not change their habitat

2) What are biomarkers?

a) organisms living in peripheral areas

b) indicator molecules

c) dominant species of plant communities

d) once living, but now extinct organisms

3) What are biosensors?

a) instruments measuring the intensity of solar radiation

b) tiny creatures capable of independent existence

c) a group of bacteria

d) they transform a biological response into an electrical signal

4) Which group of organisms has a high tendency for accumulation?

a) snails

b) termites

c) shellfish

d) ants

Match the letters to the correct numbers!

a) exploiting species b) amphipods c) hyperspectral imaging d) multispectral remote sensor e) exposure model f) suitable for the identification of plant species

5) uses the infrared reflectance spectrum

6) is characterized by 2-20 spectral bands

7) have a high tendency for bioaccumulation

8) hyperspectral aerial image

9) develop a resistance to the pollutant

10) determines the extent to which receptors are exposed to a pollutant

9. Microcosm, Mesocosm, Field Experiments and Bioremediation Technologies

The pollution of our environment in the past decades has made it necessary to take environmental measures, and to develop relevant technologies capable of removing some or all of the hazardous substances from the ecological systems. The results obtained from laboratory and field micro- and mesocosm models have allowed the development of several bioremediation technologies. Although a method capable of removing all anthropogenic pollutants has not been developed yet, with the intensive development of biotechnological research, the solutions to many of our current problems will be found in the future.

9.1 Microcosm Models

Microcosm models can be regarded as multi-species ecological test systems that are suitable for ecotoxicological tests, and under certain conditions for determining the degree of bioaccumulation and biodegradation as well.

The main characteristics of microcosm experiments:

- they are able to model the ecosystem, their environmental reality is much higher compared to single-species tests
- they are artificially created multi-species ecological systems
- the physical, chemical and biological interactions between the tested pollutant and the environmental elements can be well tested
- they are not faithful imitations of ecosystems, but cause-and-effect relationships can be determined under controlled experimental conditions
- interactions between species can be interpreted (e.g. prey-predator relationships, competition)
- microcosm models usually mean small-size experiments that are performed mainly in a laboratory

The types of microcosm tests:

By environmental element they can be

a) aquatic microcosm experiments (laboratory and field models)

b) terrestrial microcosm experiments (laboratory and field models)

For terrestrial microcosms such standardized methods as those for aquatic ecosystems have not been developed yet. Because of the high degree of heterogeneity of soils it is difficult to measure the effect of the tested pollutant, the pollution of the soil is usually concluded from the pollution of groundwater and soil gas.

Aquatic microcosm tests: SAM – standardized aquatic microcosm

FIFRA – field microcosm

Standardized aquatic microcosm (SAM)

A multi-species laboratory test. The primary consideration in the selection of species is that they should play a functionally central role in the aquatic food chain (algae, single-celled animals, water fleas, ostracods, rotifers). The type and number of species forming the microcosm, and the environmental conditions (temperature, pH, intensity of lighting) during the experiment are explicitly specified. During a 64-day laboratory observation period the chemical to be tested is added weekly to the microcosm system, and samples are also taken on a weekly basis for chemical and biological measurements. Changes in the concentration of the tested pollutant are detected, the value of dissolved oxygen and pH is measured, and variations in the number of algae and the distribution of species are determined.

Field microcosm (FIFRA)

Based on its size, it is between the micro and mesocosm models. It is used primarily for testing the biological effects of pesticides. The number of trophic levels is higher than in SAM tests, therefore it is suitable for simulating the ecological processes occurring in artificial ponds. In the experiment aquatic plants, phyto- and zooplankton species, macroinvertebrate organisms and fish are introduced into the modelled system. Because of its relatively small size (ca. 6 m^3) parallel tests and a sufficient number of repetitions are possible, however it is less controllable. In laboratory microcosm experiments the test conditions can be controlled, while in field models the environmental impacts, weather changes, and hydrogeological factors can greatly influence the obtained results.

Soil microcosm (SCM – soil core microcosm)

It is usually used for testing the interactions between terrestrial agricultural ecosystems and xenobiotics entering the soil. The soil sample collected from the site is treated with a solution of the pollutant to be tested. Physical, chemical and biological tests are performed on the leachate passing through the soil, and the whole soil sample is processed. Changes occurring in the number of individuals and the distribution of species as a result of the tested pollutant are monitored, and the degree of bioaccumulation, biotransformation and biodegradation can also be determined.

9.2 Mesocosm Models

They are able to model real ecosystems, they are more complex than microcosms.

The main characteristics of mesocosm experiments:

- their environmental reality is higher than that of microcosm models
- they include all trophic levels, therefore the obtained results can be used well in ecosystem related decisions
- their size varies (ranging from a few litres to smaller artificial ponds)
- they are usually set up outdoors (exposed to external environmental impacts)
- evolutionary processes occur in them (new metabolic pathways, adaptation mechanisms can develop as a result of the selection pressure of pollutants of different chemical structures entering the system)
- they allow the detection of structural and functional interactions in ecosystems
- they are suitable for integrated monitoring tests

Compared to microcosm models, mesocosm models allow more complex ecological and ecotoxicological observations, making possible the more thorough exploration of bioaccumulation and biodegradation mechanisms. They can be used for determining the conditions for the biodegradability of xenobiotics, persistent compounds released into the environment, therefore results obtained from mesocosm experiments are important starting points for the development of remediation techniques.

In summary it can be stated that micro- and mesocosm models are not completely able to faithfully imitate real ecosystems. However, the effects that can be observed in their dynamics are similar to those occurring under natural conditions, therefore they are suitable for the simulation of ecosystem-level processes and interactions.
Field experiments: they usually follow laboratory tests and can either confirm or disprove the extrapolatability of the obtained results to real ecosystems. Field models are able to explore more realistically the actual interactions between the tested pollutants and the abiotic and biotic factors of the environment, thus they play an important role in the development of bioremediation methods.

9.3 The Applicability of Remediation and Bioremediation Technologies

9.3.1 The Possibilities of Reducing the Environmental Risks of Pollutants by Bioremediation

Remediation: is the activity of reducing the concentration of a pollutant in soil, wastewater to an extent where its environmental risk can be regarded as acceptable. The methods used in remediation can be physical and chemical methods. The development of technologies suitable for the effective remediation of various pollutants is a tough challenge for environmental protection. In addition to the feasibility and efficiency of technical solutions, cost optimization is also an important consideration. In view of this, today research is shifting towards the development of bioremediation technologies, as they are usually quick and cost-effective solutions.
Bioremediation: is the reduction of the environmental risk of polluted soil, groundwater, surface water or sediment with biological methods. Living cells, organisms, or their metabolic products perform the degradation of organic substances, and the chemical and biochemical conversion of inorganic substances (LAKATOS 2000). Bioremediation techniques use primarily microorganisms for the biodegradation of a given chemical,

while ensuring the optimum conditions required for the process (appropriate temperature, oxygen supply and pH, sufficient nutrient, adequate amount of active biological communities). The living organisms used in bioremediation can come from the own communities of the ecosystem, but can also be artificially bred microbes or plants.

During remediation the clean-up of the tested environmental element can be performed in two ways:

- with in situ technology
- with ex situ technology

In situ technology: one of the ways of remediating polluted environmental elements, when the polluted soil, sediment, surface water or groundwater is treated on the original site, by exploiting its own self-healing capacity and supporting its natural bioremediation activity. The great advantage of the in situ remediation technology is that it is low cost, suitable for the treatment of large areas, and gentle with wildlife in the environmental element to be cleaned up. Its disadvantage is that residual pollution can be expected after the treatment, and the duration of the treatment can be very long.

For the treatment of soil it is indispensable to ensure the optimum conditions: the degradability of a given pollutant is influenced by the oxygen content, temperature, soil moisture content, nutrient supply, and pH. By finding the optimum conditions, the parameters of the clean-up technology can be changed to make the biodegradability of the hazardous chemical the most effective.

Bioventilation: it is used in soil treatment technologies, when sufficient oxygen is not available for microbial breakdown. Aerobic microbes break down primarily organic substances in the presence of oxygen, and their activity can be maintained at an adequate level only if sufficient oxygen is present for them in the medium. In the case of low oxygen content, by means of ventilation fans or by air blowers air flow is generated in venting wells or pipes introduced into the soil from the outside.

Thus by bioventilation sufficient oxygen supply can be provided for the aerobic microflora, and by increasing the temperature the biodegradation activity of the microorganisms can be increased, and so called „intensified biodegradation” can be achieved.

The degradability of a given pollutant can also be modified by the type of the soil itself, as in different soils the availability of the given chemical, and the activity of the microbial communities can be different. Availability can be increased by various additives (enzymes, vitamins, other activating agents).

Microbial composition, microbial activity: in the case of readily degradable pollutants it can be sufficient to maintain the efficient activity of the own microflora by ensuring the optimum environmental conditions. In the case of not readily degradable substances, or chemicals with a structure foreign to living organisms, enrichment with new microbial strains, or a microbial mixture can be effective.

A microbial community can adapt to compounds with a chemical structure foreign to it by responding to the changes in the environmental factors with changes in diversity (selection pressure), or with the spread of specific genes. Through gene transfer the individuals of the species/strain adapted to the pollutant proliferate, an increase in microbiological diversity occurs. Thus new microbial strains adapted to the utilization of a given pollutant can develop naturally, but by means of genetic engineering it is possible to create specific strains performing the degradation of a given chemical under laboratory conditions as well.

Ex situ technology: the other way of treating polluted environmental elements, when the polluted soil, sediment, surface water or groundwater is removed from the original site and treated in biobeds, tanks, prisms or reactors. The method used can be based on the mobilization or immobilization of the pollutant.

In the case of mobilization, the risk of hazard can be reduced by dissolving, and thus removing the pollutant. This method can be used during remediation e.g. for the removal of toxic metals. For soils with high metal content, by ensuring the solvent concentration required for optimum metal dissolution and the appropriate pH, an efficient treatment procedure can be implemented.

In the case of immobilization, the pollutant to be removed is bound to the surface of a carrier, or converted into an insoluble chemical form. With this method the environmental spread and bioavailability of a given chemical can be prevented, or

significantly reduced. This technique can also be used for the treatment of environmental elements (soil, sediment) polluted with toxic metals and persistent organic compounds.
The advantage of the ex situ remediation method is that it is easier to select the most appropriate technology, the duration of the treatment is shorter, but the treatment is more expensive.

9.3.2 Phytoremediation Technology and its Applicability in Practice

In bioremediation the emergence of phytoremediation technologies is a promising field for the future. As it is evident from the name of the method, phytoremediation uses plants to reduce the concentration of a given hazardous chemical, and thus its environmental risk to an acceptable level (Simon 2004).
The „healing” of our environment with plants can be an energy-saving, environmentally sound and also inexpensive solution. For phytoremediation larger-size, fast-growing species should be selected, because even then an effective result can only be achieved over years (Photo 20).

Photo 20: Plant species used for the phytoremediation of polluted soil

The possibilities, types of reducing pollution impacts through phytoremediation:

- phytoextraction
- rhizofiltration
- phytostabilization
- phytodegradation

Phytoextraction: a process for reducing the concentration of pollutants, or completely removing them from soil/sediment by means of different plant species. Phytoextraction treatment is performed primarily with plant species able to hyperaccumulate heavy metals. Hyperaccumulation is defined as a metal concentration exceeding 1000 mg/kg of dry matter in given plant organs. The degree of metal accumulation is species-specific and varies by metal. In the temperate zone members of the family Brassicaceae (e.g. rape), in the tropic zone species belonging to the family Euphorbiaceae have the ability to hyperaccumulate metals (Figure 19).

Figure 19: Processes involved in the phytoremediation of polluted soil (based on Cunningham, 1995)

In Hungary, in addition to rape, sunflower can also be used well for removing heavy metals from the soil, and by adding chelating agents to the soil, the degree of extraction can even be significantly enhanced. Chelates form highly stable complexes with metal ions, and can easily bring metals into aqueous solution. By this chelates can increase the mobility of metals in the soil, and thus their absorbability by plants (Photo 21). It is important that the extracted toxic metals should be enriched in the above-ground organs of the plant.

Photo 21: The use of chelates in the case of heavy metal pollution (based on A. Farsang, 2007)

The plant biomass with high heavy metal content is collected from the treated area, processed, recycled, or deposited at a waste disposal site. The removal of metals from heavily polluted soils can take decades, and this process cannot be accelerated significantly even by using fast-growing species.

Rhizofiltration: a process for removing a hazardous substance from wastewater or groundwater (Figure 20). During phytoremediation by rhizofiltration the pollutant is removed by means of a dense network of plant roots in the soil, or by rhizosphere immersed in water. Plants suitable for rhizofiltration are those that have a large mass of roots, but transport little hazardous substance to the shoot, e.g. grasses, sunflower.

Figure 20: The process of rhizofiltration (based on J. Benedek, 2011)

In the case of both phytoextraction and rhizofiltration, the phytoremediation technology can be implemented in two steps:

a) the selection and planting of the appropriate plant
b) after the absorption and binding of the harmful substance, the removal of the produced polluted biomass (in the case of incineration by depositing the ash at a hazardous waste disposal site, or in the case of metal pollution by recovering and recycling the metal)

Polluted plant biomass should under no circumstances be used for feeding purposes.

Phytostabilization: a process using pollution-tolerant plant species (tolerant primarily to heavy metals) to reduce to a minimum the transport of a hazardous environmental pollutant in the area through wind erosion, water erosion, or run-off. With this bioremediation method pollution in environmental systems is not prevented, but it is immobilized. In the phytostabilization process the used plant species do not accumulate the harmful substance (Figure 21).

Figure 21: The phytostabilization process (Phytotechnology Technical and Regulatory Guidance Document, 2001)

Phytodegradation: a process using certain plant species, or microorganisms living together with plants, to render harmless hazardous organic pollutants enzymatically. Experiments have proved that poplar roots are capable of degrading trichlorethylene. From petroleum derivatives the alkanes and aromatic hydrocarbons with 10 to 22 carbon atoms are relatively well-degradable. Today oil-degrading bacterial strains are already known and used in practice (*Pseudomonas, Bacillus, Micrococcus, Corynebacterium, Arthrobacter*). Polycyclic aromatic compounds with a high number of carbon atoms are the most resistant to biodegradation, although in recent years the number of bacterial varieties capable of utilizing man-made substances foreign to the environment has been increasing.

It clearly follows from the above that the development of bioremediation technologies can be the most efficient means of „cleaning up” environmental systems that have become polluted as a result of human activity. Genetic engineering can create plants that are capable of phytodegradation, with genes for degrading a wide variety of organic pollutants. It remains a question for the future, however, whether scientific development will keep pace with global production worldwide, and the associated global pollution.

Test Questions

Circle the correct answer!

1) Which of the following abbreviations stands for the standardized aquatic microcosm test?

   a) FIFRA

   b) SCM

   c) SAM

   d) RQ

2) Which statement is true for the in situ technology?

   a) it is suitable for the treatment of small areas
   b) the treatment of the polluted environmental element is performed on-site
   c) there is no residual pollution
   e) it significantly harms wildlife in the environmental element

3) What is the essence of the phytoremediation process?

   a) it uses chemical methods to reduce the concentration of a pollutant
   b) it is suitable only for the removal of heavy metals
   c) it can be used only in aquatic ecosystems
   d) it uses plants to reduce the concentration of a pollutant

4) What are called hyperaccumulator plants?

   a) plants with a metal concentration below 1000 mg/kg of dry matter in the plant organs
   b) plants with a very high potassium concentration in the plant organs

c) plants with a metal concentration exceeding 1000 mg/kg of dry matter in the plant organs
d) plants capable of producing large quantities of starch

5) Poplar roots are capable of degrading which compound?
   a) trichlorethylene
   b) acetic acid
   c) benzene
   d) methylmercury
6) What is characteristic of mesocosm models?
   a) they can be performed only under laboratory conditions
   b) they include all trophic levels
   c) their ecological reality is negligible
   d) they are single-species models

Decide which of the following statements are true, and which are false (mark with T or F)!

7) Phytostabilization can prevent the spread of a pollutant in a given environmental element.
8) Rhizofiltration is a method for removing a pollutant from soil.
9) The Rosaceae include many hyperaccumulator organisms.
10) In the case of the ex situ remediation technology the clean-up of the polluted environmental element is performed on-site.

10. Thresholds of Toxicological Concern, National and International Standard Systems, Approval Procedures

The harmful levels of toxic substances accumulating in environmental systems have made it necessary to set commonly accepted allowable thresholds both internationally and nationally. In practice the development of these thresholds has been greatly facilitated by the results of ecotoxicological tests, but the wide range of pollutants, and their combined effect in our environment presents numerous difficulties. Thresholds can be set individually for one given pollutant, or jointly for several polluting compounds in combination. In the latter case the tests require the use of more complicated and expensive methods, however the conclusions that can be drawn from the obtained results are more realistic.

10.1 Legislation on Thresholds of Toxicological Concern

The pollution of environmental systems became apparent on a European scale from the 1970s. Particularly hazardous environmental pollutants include the following elements and compounds:

- micropollutants (heavy metals are inorganic micropollutants, pesticides are organic micropollutants)
- macropollutants (nitrogen fertilizers are inorganic macropollutants, mineral oil and mineral oil products are organic macropollutants)

The first Environmental Action Programme of the European Union was developed for the period from 1973 to 1976. The fundamental principles of that were included in the Treaty of Rome, which already created legal obligations in the environmental chapter. From the environmental systems, the Soil Charter adopted in 1990 deals with the protection of soil, and states that soil, as a limited, fragile natural resource, is under legal protection.
In Hungary the general rules of environmental protection were specified in 1995 by the provisions of Act No. LIII of 1995, taking into consideration the environmental standards

of the EU. The National Environmental Remediation Programme was launched in 1996 with the objective to assess and inventory polluted sites in Hungary. The Remediation Programme was also incorporated into the National Environmental Health Action Program (NEHAP). As a new element, computer models used for human risk assessment were taken into account in the development of thresholds for hazardous substances polluting the environmental systems.

Today the harmonization, alignment of ecotoxicological test results and environmental health requirements is an indispensable task for the experts. In the future it will become increasingly urgent to develop a system of ecological and environmental health thresholds, and to ensure the conditions for its applicability in practice.

On the basis of the Water Framework Directive (WFD) of the EU in force since 2000, primarily the results of biological tests should be taken into account in determining the effect, hazard of a given pollutant. In the WFD numerous heavy metals and pesticides classified as micropollutants are listed as so called „priority substances" that are particularly hazardous due to their mutagenic, teratogenic and carcinogenic effect (MONGE 2007).

Gov. decree No. 219/2004 (21st July) on the protection of groundwater and the geological medium, and KvVM – EüM – FVM – KHVH joint ministerial decree No. 6/2009 (14th Apr.) on the implementation thereof, set the thresholds for various pollutants with respect to groundwater and the geological medium.

The decree specifies five threshold categories:

1. (A) background concentration: the concentration of the given element or compound under natural or close to natural conditions.
2. (Ab) verified background concentration: the actual concentration of the given substance in groundwater and in the geological medium, specific for the given region, and determined by measurement.
3. (B) pollution threshold: the pollutant concentration in groundwater and in the geological medium established by legislation, at which the given groundwater or geological medium is qualified as polluted.
4. (C) remediation target threshold: the concentration prescribed in an official

ruling, to be achieved as a result of remediation.

5. (D) site-specific pollution threshold: it is used on sites where the background concentration exceeds the pollution threshold (B).

On the basis of the ministerial decree, in order to protect the quality of groundwater and the geological medium, in official actions the pollution threshold (B) shall be considered and enforced.

In compliance with Government decree No. 219/2004, the pollution sensitivity map of Hungary was prepared (Figure 22).

Figure 22: The pollution sensitivity map of Hungary

From the thresholds established by the decree, the thresholds for some heavy metals and pesticides that are the most common pollutants in soil are shown below (Tables 10-11).

Table 10: Thresholds for some heavy metals in soil (mg/kg of dry matter)

Table 11: Pollution thresholds (B) (mg/kg of dry matter) for some pesticides

In ecotoxicological testing both the individual countries and the international organizations seek to harmonize the methods and principles of testing. Harmonization allows the comparability and mutual acceptance of the obtained results.

In Europe the most widely accepted methodological guide is the OECD Guidelines for Testing Chemicals issued in Paris in the 1980s. The OECD guidelines specify methodological criteria for all accepted toxicological tests in a standard-like manner. However, they are only guidelines, they cannot be regarded as standards, as the steps of testing are not described in such detail. Compliance with the criteria specified in the OECD guidelines is nevertheless indispensable for the correct evaluation of the results. The OECD guidelines were the basis for the development of the EU directives that are accepted in Hungary as well, and serve as a basis for the development of the standards used in Hungary. The standards in force are periodically reviewed and adjusted to the latest scientific findings.

10.2 Approval Procedures for Chemical Substances

As they can cause the pollution of environmental systems, the production process and transportation of chemicals, and the conditions for the treatment and disposal of the generated wastes are strictly regulated by legislation. Today the toxic substances generated through industrial and agricultural activity pose a series of ecotoxicological and human toxicological problems, therefore now, in addition to the assessment of the environmental risk, the human health consequences should also be taken into account. Most of the chemicals appear in the tissues of living organisms through the food chain, and by bioaccumulation they can exert their harmful effects for years, or even decades. More and more experts explain with this negative trend the increase in the number of several neurological and immune system diseases, or in the incidence of cancerous tissue degeneration in the human population.

The conditions for the production process and the waste disposal of different chemicals are specified in approval procedures. Detailed approval procedures are in place for the production and use of agricultural chemicals (fertilizers, pesticides), and for veterinary and pharmaceutical products.

Ecotoxicological and human toxicological tests play an important role in the approval process. On the basis of the results of these tests the potential acute, subacute or chronic toxicity, and the mutagenic, carcinogenic, teratogenic, or allergenic effect of a given chemical can be determined.

10.2.1 Approval Procedure for Yield Enhancers and Pesticides

Fertilizers and pesticides released into the environment cause primarily the pollution of soil, and through that surface and groundwater.

Act No. XXXV of 2000 defines the main guidelines for integrated plant protection, the scope of measures for preserving human health, preventing and eliminating hazards related to plant protection. Yield enhancers have a positive effect on soil fertility, the approval, marketing and use of products belonging to this category is regulated by FVM decree No. 36/2006 (18^{th} May). From the fertilizers applied to arable lands, the environmental and health impacts of nitrogen- and phosphorus-based fertilizers should be mentioned. Both nitrogen and phosphorus are indispensable plant nutrients, but when they are applied to the soil in a larger amount than required, the excess amount gets into the groundwater or surface waters. The plant nutrient enrichment in water bodies can start an eutrophication process. The nutrient surpluses lead to the excessive proliferation of plants.

Nitrogen fertilizers: their large-scale use resulted in a significant increase in the nitrate content of waters. The allowable nitrate concentration in drinking water is 50 mg/l. Drinking water with high nitrate content causes „blue baby syndrome" in infants. The nitrate entering the organism is reduced to nitrite by bacteria, the nitrite is absorbed through the intestinal wall and converts the hemoglobin in the red blood cells to methemoglobin. Methemoglobin cannot carry oxygen, therefore infants can develop severe respiratory distress and cyanotic symptoms. From sodium nitrate, a food additive used as a preservative (E251), nitrosamines can form in the organism, which are carcinogenic.

The marketing, use, packaging, storage and transportation of pesticides is regulated by FVM decree No. 89/2004 (15^{th} May). It determines the scope of necessary requirements and measures on the basis of the physical and chemical properties, biological mechanism of action, harmful dose and method of application of the given agent. By means of animal experiments the acute, subchronic or chronic toxicity of the given agent can be determined, and tissue accumulation can also be monitored. Most of the pesticides are damaging to health at concentrations above a certain threshold, they have a clearly demonstrated role in carcinogenic, mutagenic and teratogenic changes, and their immune system, nervous system and endocrine system modulating function has also been shown. Their tissue concentration in living organisms can increase by orders of magnitude by transport through the food chain, pesticide residues can be detected in 75% of the food supply.

10.2.2 Approval Procedure for Veterinary Agents

Substances produced for the purpose of influencing the physiological and pathological conditions in living animals are classified as veterinary agents (veterinary drugs and veterinary preparations). The improper and inappropriate use of these agents poses primarily food hygienic problems, but if they are released into environmental systems, they can also cause physiological, attitudinal and behavioural changes in the organisms living there. The production and use of veterinary preparations is regulated by FVM decree No. 128/2009 (6th Oct.), the withdrawal periods for food hygiene purposes are specified in regulation No. 470/2009/EC. The above regulations also cover the risk of the accumulation of the residues of veterinary preparations. A thorough multi-step approval procedure can provide sufficient guarantee that drug residues, toxic metabolites, substances leading to allergic reactions, effects causing bacterial resistance, anabolic steroids are not present above a certain threshold in meat products. In the case of food, strict compliance with the appropriate withdrawal period and the thresholds can provide adequate safety.

From the factors listed above, the followings can cause health problems:

- **antibiotic residues** (they accumulate mainly in the kidneys and the liver)

  Today in industrial-scale animal husbandry an increasing amount of antibiotics is used, and some of them enter the human organism through food, resulting in an increase in the number of resistant bacterial strains (MRSA, ESBL).

  MRSA – methicillin-resistant *Staphylococcus aureus* – is a flesh-eating bacterium, ESBL – is a collective name for multidrug resistant pathogens (e.g. mutant forms of *E. coli* and *Klebsiella* strains are capable of producing enzymes that inactivate the antibiotics used in the therapy of the disease).
- **high nitrite content in meat products** (can be formed in large amounts during curing and grilling)

  In the organism nitrite is converted into nitrosamines that are carcinogenic (causing cancer).

- **tranquilizers, growth-promoting substances, hormonal preparations**
  Different drug residues, hormonal preparations can enter the human organism directly, through food, or indirectly, upon release into environmental systems. Agent residues released into our environment can significantly change the functioning of populations forming the ecosystem. Research has shown that as a result of hormonal agents released into waters, a shift in sex ratio has occurred in aquatic populations, and by monitoring the process over several generations, disorders of reproductive and sexual-behaviour could be observed.
  As a consequence of water withdrawal from surface waters, drinking water can also contain hormonal substances, tranquilizers in different concentrations. Some experts attribute to this the increase in the number of patients with infertility, impotence and mental disorders in the human population in the past decade.

Test Questions

Circle the correct answer!

1) How many threshold categories are specified for groundwater and the geological medium?

   a) two

   b) ten

   c) five

   d) three

2) What is MRSA?

   a) a virus

   b) a type of toxicological tests

   c) a single-celled animal

   d) a flesh-eating bacterium

3) What disease does drinking water with high nitrate content cause in infants?

a) methemoglobinemia

b) leukemia

c) anaemia

d) proteinuria

Decide which of the following statements are true, and which are false (mark with T or F)!

4) On the basis of ecotoxicological tests the allowable thresholds for various pollutants can be developed.

5) Residues of hormonal preparations released into waters have no effect on the organisms living there.

6) Antibiotic residues in food promote the emergence of resistant bacterial strains.

7) The run-off of nitrogen and phosphorus fertilizers does not contribute to the eutrophication of waters.

8) The Soil Charter states that soil, as a limited, fragile natural resource, is under legal protection.

9) On the basis of the Water Framework Directive (WFD), the results of biological tests should be taken into account in determining the hazard of a given pollutant.

10) On the basis of a ministerial decree, in order to protect the quality of groundwater and the geological medium, in official actions the pollution threshold (B) shall be considered and enforced.

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