1 Chapter

ECOTOXICOLOGY AS A BRANCH OF SCIENCE

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1.1. GENERAL CONSIDERATIONS

The beginning of the development of a new branch of science – ecotoxicology – was the book *Silent Spring,* written by Rachel Carson and published in 1962, in which the author describes the cases of mass death of birds and fish due to the uncontrolled use of pesticides. R. Carson concluded that the harmful effects of pollutants on wildlife foreshadow an impending disaster for humans (Carson, 1962). This book has attracted the widespread attention of the public and the environmental protection societies, governments, which has intensified the drafting of several governmental laws regulating the xenobiotic emissions in the living environment.

The term „ecotoxicology" as a branch of science was designated by Renne Truhaut in 1961 – ***„Ecotoxicology is a branch of science that studies the interactions that determine the distribution and abundance of organisms" while toxicology is „the science that deals with the study of the adverse effects of substances on living organisms".*** The author brought together two completely different topics: ecology and toxicology. In fact, ecotoxicology, in addition to ecology and toxicology, also includes elements of other sciences (Truhaut, 1977).

For ecotoxicological research thorough knowledge is required of organic and inorganic chemistry, biochemistry, analytical chemistry, physical chemistry, toxicology, ecology, biology, medicine. It is also necessary to know some methods of statistical calculation.

The definition of ecotoxicology has been changed by several researchers.

Francois Ramade published in 1977 a textbook in which he emphasizes that: ***„Ecotoxicology studies the ways of contamination of the environment by natural and artificial pollutants produced by human activity, the mechanisms of action and the effects on the living creatures that populate the biosphere"*** (Ramade,1977). This definition was later clarified at the SCOPE (International Scientific Committee on Environmental Issues) conference in 1978. Here, Butler G.C. presented and then published the main objectives of ecotoxicology – ***„Ecotoxicology – is the branch of toxicology that studies the toxic effects of natural or artificial substances on living organisms that constitute the biosphere (animal, plant, terrestrial, aquatic), including the interaction of toxic substances with the physical environment in which living organisms exist"*** (Butler, 1984).

Valery E. Forbes & Thomas L. Forbes in 1984, then in the book *Ecotoxicology in theory and practice* edited in 1995, asserted that ***„Ecotoxicology is the field of study that integrates the ecological and toxicological effects of chemical pollutants on populations, ecosystem communities with their „fate" in the environment (transport, disintegration)"*** (Forbes & Forbes, 1984,1995).

P. Calow, in 1998, by conducting several investigations and editing textbooks on the ecotoxicology and protection of ecosystems, began to unite these two directions – ***„Ecotoxicology is the branch of science that deals with the protection of ecological systems against the adverse effects of synthetic chemicals”*** (Calow, 1998).

C.H. Walker, R.M. Sibly, S.P. Hopkin & D.B. Peakall, in their 1998 *Principles of Ecotoxicology,* wrote that: ***„Ecotoxicology is the science that studies the harmful effects of chemicals on ecosystems”*** (Walker et al.,1998).

With the development of the concept of *„ecotoxicology”*, it has undergone a certain evolution. Walker et al. (2001) again found that ***„Ecotoxicology is a science that studies the toxic effects of chemical agents on living organisms, especially in populations and communities, in certain ecosystems”***.

The topic of ecotoxicology is represented by organisms and biological systems of different levels (community, population, ecosystem, etc.), subject to anthropogenic pollution. Therefore, the fundamental principles of the functioning and structure of the natural population systems and biocenotic rank, actively developed by modern theoretical ecology, serve as a theoretical basis for ecotoxicology.

Interdisciplinary research areas related to ecotoxicology

The expansion and deepening of the concept of „ecology” led to the emergence of new independent scientific fields. These directions are in contact with each other: they have a common methodology, a conceptual apparatus, research objects and they use the same scientific results to solve their own specific problems.

As an example, *the chemical ecology* (Bogdanovsk, 1994; Egorov, 2009) studies the chemical changes under the influence of environmental factors. The central task is to study the behavior of anthropogenic substances: concentration in the environment, accumulation processes in the environment. The sphere of interest includes chemical processes in the environment due to changes caused by human activity. The close connection between ecological chemistry and ecotoxicologists is expressed in the fact that both fields use methods and techniques of chemicaland physico-chemical analysis (Korte, 1997).

The subject of ecotoxicological research is the establishment of the link between the dynamics of chemicals and the development of communities of living organisms in ecosystems. Unlike ecological chemistry, the objects of ecotoxicological research refer not only to anthropogenic substances, but also to natural substances, through which their interaction on living communities takes place, and to deciphering the role and importance of organisms in transport processes, circuitry and reducing or increasing the toxicity of chemicals.

A common feature of toxicologyi and ecotoxicology is that both fields of research use the methodology, the toxicological conceptual apparatus and the fundamental bases regarding the toxicity (Ecological Bulletins No. 36, 1984).

Toxicology is mostly a compartment of medicine that studies the physical, chemical properties of poisons (harmful and toxic substances), the mechanisms of their action on the human body and develops methods of diagnosis, treatment and prevention of poisoning (Golikov, 1972).

Toxicology is the study of toxicity and the toxic process. Toxicity and the toxic process are two basic concepts of modern toxicology. Toxicity is a property (capacity) of chemicals, which act on biological systems and cause damage (dysfunction) or death. Thus, toxicity is the ability to cause a disruption, illness or death. Chemicals that, under certain conditions, may present this property, are called

toxics (toxic substances) (Kutsenko, 2006; Lysenko & Dogadina, 2015; Oprea, 2007).

The action of a substance, which leads to disruption of the functions of biological systems, is called a ***toxic effect***. The basis of toxic effects is the interaction of a substance with a biological object at the molecular level.

The chemistry of the interaction of a toxic agent and a biological object (at the molecular level) is called the ***mechanism of toxic action***. ***Toxicant*** = poison = poisonous, harmful or dangerous substance (Kutsenko, 2006).

Ecotoxicology and toxicology, when toxicity and toxic processes are taken into account, use a common apparatus, models and conceptual methodology; these are their common features. However, toxicology and ecotoxicology study the toxicity of substances and the manifestation of the toxic process at different levels of organization of a biological object (Bazerman, Charles & René Agustin De los Santos, 2006).

Ecotoxicology treats a toxic material as a toxic component in the ecological processes of a living ecosystem or community, and toxicology – to an organ or organism. This is the fundamental difference between ecotoxicology and toxicology (Relyea & Hoverman, 2006).

In toxicology, the intake, metabolism and elimination of the toxic substance from a biological object and the toxic process are studied without an ecological perspective or ecological context (without taking into account the behavior in the ecosystem and the impact on populations and communities) (Lysenko & Dogadina, 2015; Oprea, 2007).

Ecotoxicology studies toxic effects above different communities of organisms up to the ecosystem level and thus the value of the environment becomes an active factor in development; in addition, a specific feature of ecotoxicology is the evaluation of migration processes and the circuit of toxic substances and the environmental consequences of the combined action of anthropogenic and natural factors on biological systems.

Ecotoxicology has the most interferences with the general toxicology and the toxicology of the environment. Drawing a parallel between these disciplines and ecotoxicology, it is necessary to highlight the following:

Toxicology deals with the takeover, absorption, diffusion, excretion of toxic substances by individual organisms. Ecotoxicology deals with the kinetics of pollutants in the abiotic environment (air, water, soil/sediment) and the biotic environment (trophic chain), the study of the transformations and biotransformations of chemicals at the level of communities of organisms and the ecosystem.

Toxicology aims to protect human/animals (viewed as an individual) from exposure to toxic substances. Ecotoxicology aims to protect the populations and the communities of different species, as well as ecosystems, from the harmful effects of exposure to toxic substances (Kutsenko, 2006).

A major importance for both toxicology and ecotoxicology is the relationship between the amount of chemical substance which the body is exposed to and the nature, degree of subsequent harmful effects (dose/response relationship, which would be the basis for estimating the hazard and risk posed by chemicals in the environment).

Ecotoxicology (Straalen, 2002) was for a while assimilated with environmental toxicology. Environmental toxicology addresses pollution problems in a limited way, namely:

- the way in which it exerts its physiopathological action on target organisms, in particular invertebrates, mammals (humans);
- the mechanism of action on the cellular and molecular level.

In the process of studying the effects of chemicals present in the environment on humans and human communities, environmental toxicology acts with established categories and concepts of classical toxicology and, as a rule, applies the traditional experimental, clinical and epidemiological methodology. The objective of the research in this case are the mechanisms, the dynamics of development, the manifestation of the adverse effects of the action of toxic substances and the products of their transformation in the environment on humans.

Ecotoxicology, different from Environmental Toxicology (Robinson & Thorn, 2005; Frumin, 2013), mostly addresses the effects of pollution directly on populations and ecosystems as a whole, in order to protect them completely and not on isolated components.

The basic concept of ecotoxicology, is also ***toxicity***, which in general terms can be defined as a property (capacity) of chemicals, which act on biological systems causing their damage or death, or the ability to cause degradation of biocenosis or the entire ecosystem.

The consequence of the toxic effect of substances on biological systems and the reaction of a biosystem to the action of a toxic agent that leads to the deterioration (*i.e.* impairment of its functions, viability) or death is called a ***toxic process***. External signs, recorded by toxic processes (toxic action), represent its manifestation (toxic effect) (Forbes & Calow, 1999).

The toxic process at the population level is manifested by (Bezel et al.,1994)***:***

- death of the population;
- increased morbidity, mortality, number of birth defects;
- decrease of the multiplication potential;
- violation of structural features (gender, age, space, size, mass structure, etc.);
- decrease of the average living possibilities and the degradation of the population composition.

The toxic process at the level of biocenoses is manifested by:

- changes in the population spectrum of cenosis, up to the disappearance of certain species and the emergence of new ones, which are not typical of this biocenosis (this can change the dominant species and, as a rule, diminish biodiversity);
- violation of interspecific relationships, reduced productivity of biocenoses as a whole system;
- possible degradation and disappearance of this ecosystem.

Methodological differences between ecotoxicology and environmental toxicology are completely erased when a researcher is asked to assess the indirect effect of pollutants on human populations (e.g. due to toxic changes in biota) or, on the contrary, to find out the mechanisms of action of chemicals in the environment on representatives of the one or other individual (Bezel et al.,1994).

In this sense, from a theoretical point of view, the „toxicology of the environment" as a science is only a special problem of „ecotoxicology", while the methodology, conceptual apparatus and structure of the sciences are the same (Robinson & Thorn, 2005).

The difficulties of establishing the ecotoxicology as an independent direction are today related to the absence of a sufficiently strict theoretical basis for unifying the accumulated experimental material and explain it.

The ambiguity of the initial data obtained under different natural conditions and under various influences, the lack of an explicit relationship between the field observations and experiments, the isolation of theoretical positions from their specific application to the solving of practical problems, are all signs of a novelty and the first steps of the new scientific direction.

BASIC OBJECTIVES OF ECOTOXICOLOGY

The content of the discipline „Ecotoxicology" is the study of the ecotoxicity and the main characteristics of the profile of the xenobiotic habitat, ecotoxicokinetics, ecotoxicodynamics and ecotoxicometry of ecotoxicants and xenobionts, the evaluation of biological communities of different rank subject to pollution of the living environment.

The most important tasks of ecotoxicology include: the study on fluctuations in species composition as well as in the relations of species at a trophic level. As far as ecotoxicology is concerned, the factor of reducing species diversity is particularly important (diversification), since a decrease in the number of species may lead to the disappearance in the first place of their previously existing indicator organisms. The changes of growth parameters and metabolism of organisms lead to the structural modification of the communities of species and cause pertrubations in the functioning of the entire ecosystem (Forbes & Calow, 1999; Panin & Bezel, 2008).

If epidemiological studies are carried out, the loss or reduction of the number of main species of organisms shall require an analysis of trends in the development of biological communities. Such studies are another task of ecotoxicology, which can help identify the degree of risk and prevent dangerous phenomena (Kaplin, 2006).

Thus, ecotoxicology presents a deep study of ecosystems, and the most important parts of it should be the problems of conservation (for undisturbed ecosystems) and restoration (for already affected systems). Both goals can only be achieved in functional modeling mode. However, due to the fact that there is no universal model, the development of such models, as well as the creation of laboratory and mathematical models or similar studies in natural conditions, are carried out on the basis ofthe objective set for solving a particular problem (Walker et al., 2002; Robinson & Thorn, 2005).

The nature and extent of the influence of chemical pollutants on the general ecological situation, biogeocenoses and biosphere components vary in different natural areas and even in relation to some species of animals and plants, and as a result of causing dangerous toxic-ecological situations local environmental disturbances often occur (Katrin Franke Bader, 2012).

Natural ecosystems have the ability to withstand both fluctuations in ordinary natural factors and changes in living conditions under anthropogenic influence. Therefore, the rational use and conservation of nature, as well as the properties that can detect negative or potentially dangerous environmental changes in the early stages become essential. Consequently, effective methods for early detection of certain changes are also needed (Israel, 1984).

Any transformation of the environment as a result of human activity can be called anthropogenic (or human factor). An anthropogenic impact that leads to a change in the chemical composition of one or more natural components of the environment is geochemical, as it inevitably involves this change in natural systems that interact with each other at different speeds and intensities (Panin & Bezel VS, 2008).

The works of several researchers (Truhaut, 1977; Forbes & Forbes, 1995; Schueuermann & Markert,1997; Connell et al., 1999; Forbes & Calow, 1999; Walker et al., 1998, 2001) have become classics in the development of ecotoxicology as a *scientific branch* in which the main notions, objectives, tasks, ecotoxicological research directions are stipulated and argued, and which refers to all the compartments of this chapter, as follows.

THE MAJOR OBJECTIVES OF ECOTOXICOLOGY ARE:

1. Obtaining the ecological and toxic data for risk assessment and environmental management;
2. Knowledge and establishment of chemicals dispersion laws in the environment;
3. Elaboration of the bases (empirical or theoretical) to enrich and improve knowledge about the behavior regarding the toxic effects of chemicals in the living system.

The following main issues are addressed in the above objectives:

- distribution of pollutants in the environment by identifying the disposal of chemical substances in the different compartments of the environment (air, water, soils, food);
- kinetics of pollutants (penetration modalities, storage in the environment, diffusion, their transformations into the environment);
- the effects of pollutants on living organisms, respectively the definition of the picture of harmful effects at the *individual* level *(disruptions of the biochemical, molecular and physiological structure and* function), as well as at the *population level (change in the number of individuals, change in the frequency of genes, modification of the entire ecosystem function);*
- recording the individual toxicity of toxic substances: dose, concentrations with standard tests or through complex systems;
- hazard or risk assessment for a particular toxic (based on toxicity data);
- assessment of the overall potential for disorders (changes) in the ecosystem;
- elaboration of „therapy" measures *(recovery);* taking preventive measures regarding the risk and analyzing them.

XENOBIOTIC PROFILE

The foreign substances in the environment (water, soil, air and living organisms) in the form (state of aggregation) allowing them to enter into physico-chemical and chemical interactions with biological objects of ecosystems constitute the xenobiotic profile of its biocenosis. In this list of substances, radioactive materials are also included.

The xenobiotic profile should be considered as one of the most important environmental factors (along with temperature, light, humidity, trophicity, etc., qualitative and quantitative characteristics) (Katrin Franke Bader, 2012).

The important elements of the xenobiotic profile are also substances that are xenobionts in the organs and tissues of living organisms, because all of them are consumed by others, sooner or later.

In contrast, chemicals that are fixed in solid, non-dispersed in objects in air and water, insoluble (stone, various solids, glass, solid plastic, etc.), cannot be considered as components of the xenobiotic profile.

Only bioavailable substances, which interact non-mechanically with the living organisms, are of interest. These are in the gaseous or liquid state, in the form of aqueous solutions, adsorbed on soil particles, solids, and in the form of finely dispersed dust (< 50 microns).

THE MOST COMMON COMPONENTS OF THE XENOBIOTIC PROFILE AND THEIR ROLE

➢ ***Oxygen*** is practically not a pollutant in the true sense of the word, but it can influence health by decreasing its concentration in the air and by decreasing the atmospheric pressure, the effect being determined by the decrease of the partial pressure at the level of the pulmonary alveoli and the alteration of the gas exchange. ***Oxygen and carbon dioxide*** (O_2 and CO_2) are determinants of the process of blood oxygenation. The specific phenomena that occur are phenomena of hypoxia or anoxia, their severity being dependent on the degree of decrease of the partial pressure.

- ***Ozone*** is naturally found in the composition of the atmosphere forming, due to the increased concentration, a real layer in the Earth's stratosphere. This layer or ***„ozone shield"*** has an important ecological role at a global level due to its ability to shield radiation from the ultraviolet sector of the spectrum of sunlight.

The concentration of ozone in the breathable atmosphere is normally quite low and is variable depending on the geographical conditions that determine a different incidence and intensity of ultraviolet radiation. Thus, in arid areas, the average concentration of ozone is about 15 parts per million (ppm), while in areas at higher altitudes it can reach values above 100 ppm.

Recent researches have shown that exposure of the human body to increased ozone concentrations leads to damage to the eyes, respiratory diseases and the overall breathing process. The effects are more strongly felt by young organisms.

- ***Carbon dioxide*** does not cause disturbances of the human body, only in situations where the passage of gas from the venous blood into the pulmonary alveole and its elimination through the exhaled air is prevented. In fact, toxic phenomena occur when the partial pressure of CO_2 in the air increases so much that they prevent it from being eliminated.

Initially, there is an increase in CO_2 in the blood less due to its penetration from the outside air, but because of the body's autointoxication. As the concentration in atmospheric air increases, its solubilization in the blood plasma also intervenes due to the increased partial pressure.

The first disorders occur around the concentration of 3%, being manifested by respiratory disorders (acceleration of breathing, cyanosis occuring, followed by respiratory and circulatory disorders).

- ***Sulphur dioxide*** **(SO_2)** causes mucosal irritation and bronchioles dilation. In contact with the blood forms sulfhemoglobin which imprints a reddish-brown color on the blood. Also, sulfur dioxide can disturb the synthesis activity of nucleic acids resulting in chromosomal aberrations, decrease in the growth rate. The harmful effects that occur on humans in connection with the concentration of SO_2 in atmospheric air are well known, regarding the impairment of the respiratory process.

Sulphur dioxide is naturally found in the atmosphere in the extremely low concentrations up to 0.2 ppm, the volcanic activity being the main source of sulphur dioxide. The main sources of sulphur dioxide pollution are dependent on human activity and are mainly represented by activities involving the combustion of fossil fuels. Estimates show that SO_2 emissions in the atmosphere can reach the figure of 145 million tonnes annually, which includes quantities due to the combustion of coal (70%) and other fuels (16%), the remaining percentages being provided by metallurgical industrial activities (processing of 1000 t of copper ore is equivalent to the release of 600 t of sulphur dioxide).

The concentrations of SO_2 in the atmosphere of industrialised cities vary from case to case, with an average value of 0.17 to 2 ppm, which must become of concern, as long-term exposure of homeothermic organisms, including humans, to concentrations higher than 0.1 ppm of sulphur dioxide in the air can induce serious damage.

- ***Hydrogen sulphide (H_2S)*** normally enters the composition of the atmosphere as a result of fermentation processes produced in the absence of oxygen (anaerobiosis) in which microorganisms specific to the terrestrial and aquatic environment are involved.

The quantity of hydrogen sulphide produced in this way by the bacteria is estimated annually at values of 68 million tons for the terrestrial environment and 30 million tons in the aquatic environmentc. Also, naturally, but as a result of volcanic activity, a significant amount of H_2S is permanently eliminated in the atmosphere.

It has been estimated that as a result of the industrial activities involved in the production of sulphur derivatives or the processing of ores containing sulphur, about 3 million tonnes of sulphur equivalent are released into the air annually.

It is known that in the geographical areas considered unpolluted the concentration considered normal of this gas in the atmosphere is about 2 ppm. The elimination of this highly toxic pollutant from the atmosphere occurs naturally through complicated chemical processes that determine the oxidation of hydrogen sulfide by producing sulfur dioxide.

This gas enters the body through the the respiratory tract and its effects are felt both at the short-term exposure and at medium- or long-term exposure, being linked to impaired breathing.

There is a great variability of the sensitivity of human individuals to the action of hydrogen sulfide but, in principle, depending on the amount inhaled, the effects are represented by constriction of the bronchi, the appearance of bronchitis and even of the bronchial spasm. The effect is more severe in young organisms.

➢ ***Nitrates and nitrites*** can cause harmful effects on humans through intoxication, a phenomenon that occurs through food and drinking water. According to the hygienic norms of the Republic of Moldova, the drinking water can have a nitrates' content of no more than 45 mg/liter and the presence of nitrites is allowed only exceptionally in the amount of 0.3 mg/liter in groundwater up to 60 m depth. Water becomes suspicious at concentrations of 50 mg nitrates/litre and toxic at 1 g/litre. The most common route of intoxication of man is the digestive one. In the digestive tract, nitrates undergo a series of successive transformations up to ammonia. These transformations are performed under the influence of bacterial flora and some enzymes.

The toxicity of nitrates and compounds resulting from their metabolism is initially manifested in the digestive tract through irritating and congestive effects on the digestive mucosa. Then, the irritating action is manifested on the kidney through congestion and hemorrhage.

After entering the bloodstream, nitrates and nitrites act paralyzingly on the vasomotor centers and veins, especially on small ones, causing vasodilation and hypotension.

These nitrogen compounds have negative influences on the endocrine glands, on the pituitary gland, but especially on the thyroid. Its nitrates and metabolites induce a state of hypothyroidism.

➢ ***Phosphates*** can cause polluting effects that manifest directly on humans through the appearance of intoxications. Some „impurities" contained in superphosphates, the most important of which is fluorine, also contribute to the increase of the toxicity. Phosphorus along with fluorine have an irritating effect on the mucous membrane of the digestive tract.

➢ ***Pesticides*** - are the largest group of xenobiotic substances (Rathore & Nollet, 2012; http://npic.orst.edu/factsheets/ ecotox.pdf) which, according to the way they penetrate the body and the character of the harmful action, are divided into:

- *ingestion products* (which penetrate the body with food),

- *contact products* (which act by touch with the skin),
- *breathing* products (which act through the respiratory apparatus).

➢ ***Organophosphorus insecticides*** have generally action by contact and ingestion and less often through breathing. It has a strong toxic action on the *nervous system, by altering the transmission of the nervous influx, causing spasms, convulsions and death*.

➢ ***Nematocides and molluscicides*** are pesticides with high toxicity that are applied to the soil. They act by ingestion and contact. The chronic effects of pesticide poisoning are most often observed on the liver and nervous system. Since the liver is the most important organ that metabolizes pesticides, it is the most injured (traumatized, affected).

➢ ***Metals-macroelements and trace metals*** are the most conservative and persistent ecotoxicants, the role of which is multilateral from those essential for life to those extremely toxic. The influence of macro- and micro-metals is dependent on their physico-chemical properties, quantity, form of migration, environmental factors (temperature, humidity, pH, oxidation-reduction processes, etc.) and, last but not least, the state and metabolic processes in the impacted living organisms.

CHANGING THE XENOBIOTIC PROFILE

In recent years, human activities have often significantly altered the natural xenobiotic profile in many regions (especially urban and industrial).

Chemicals that accumulate in the environment, in unusual amounts, contaminate the xenobiotic profile in which the so-called ecopollutants and xenibiotics are concentrated. (Katrin Franke Bader, 2012).

Changes in the xenobiotic profile may occur as a result of excessive accumulation of one or more pollutants in the environment, but this does not always lead to disastrous consequences for life.

Only ecopollutants accumulated in an amount sufficient to initiate a toxic process in the biocenosis (at any level of organization of living matter) can be designated as ecotoxicants (Harris et al., 2014).

One of the biggest practical problems of ecotoxicology is the determination of quantitative parameters for which the ecopollutant turns into an ecotoxicant.

THE BASIC COMPONENTS OF THE XENOBIOTIC PROFILE

Ecotoxicants – naturally occurring toxic substances that accumulate in organisms up to toxic amounts or are harmful to life (petroleum products, heavy metals, microbial biotoxins, etc.).

Xenobionts - substances that do not exist in nature and are synthesized by humans, which cause inhibition of vital processes or may become the cause of intoxication of living organisms and, ultimately, of man (pesticides, pharmaceuticals, detergents, dyes and other synthetic substances, including medicines obtained from plants and organisms).

Main pollutants

There are many classifications of pollutants, depending on toxicity, availability, chemical composition, etc. (Ciubotaru, 2003; Yufit, 2002).

Biogeochemical cycles now include a large list of synthetic compounds unknown to the virgin natural environment. These include, in particular, a large group of substances united by the general term „pesticides”, „phenols and their derivatives”, „freons”, „dioxins”, „benzopyrenes”, etc.

From classical geochemical positions, the notion of ***pollution*** means a change in the chemical properties of the environment, which are not associated with natural processes. When using this term, the biomedical meaning is most often introduced when ***pollution*** is taken into account from the point of view of the impairment of human health (Hoffman et al., 2003).

In this case, pollution means any change in the air, water, soil and food that has undesirable effects on human health, survival or activity.

The severity (degree) of exposure to pollutants is determined by three factors:

The first factor is the nature of the toxic pollutants, *i.e.* how active and harmful the substances tested for humans, plants and animals are;

A second factor is the concentration of pollutants, *i.e.* the content of air, water or soil per volume or mass unit;

The third factor is the durability/persistence of the substance, or the duration of its existence in air, water and soil.

The scale of pollution is divided by location:

- according to *the source of pollution* (around industrial enterprises, livestock farms, oil deposits, etc.);
- corresponding to *the geographical and state regions* (in the region, the basin of a region, republic, state);
- in compliance with *space* (in outer space – for example, the level of aircraft debris, etc.).

Pollution sources are divided according to the availability of pollutants. All sources of industrial emissions and wastewater discharge are *focal points* (point sources) of pollution, while *diffuse sources* are associated with agriculture, chemicalization, surface leakage from polluted areas, etc.

When assessing the impact of pollution on the natural environment, it is necessary to distinguish between *primary, secondary* and *indirect* effects (*e.g.* the direct impact of emissions from a metallurgical or chemical plant causes the primary effect of the vegetation loss in the immediate vicinity, up to the complete destruction of the soil (indirect effect)).

By the nature of the formation, the sources of pollution are divided into *natural* and *products formed by human activity*. Natural pollution is caused by the existence of the pollutant in unpolluted rocks and soils (e.g. fluorine, selenium in groundwater, or mercury, molybdenum in the rocks of Central Asia). Endemic areas of increased concentrations of some or other ecotoxicants in the environment are formed here.

In the process of natural pollution, in a narrower concept, natural catastrophic processes are also included – a strong volcanic eruption, landslides, etc., as well as geochemical pollution, which appeared in the process of formation and development of our planet. These processes can be both positive and negative. In the first case, there is an excess of element in a certain locality, and in the second, there is a deficiency (as an example – the excess of fluor, selenium and the deficiency of iodine in the groundwater of Moldova).

Pollution caused by human activities, called anthropogenic

Human actions are divided into ***industrial*** (metallurgy, coxochemistry, chemical, pharmaceutical, food, etc., caused by a single enterprise or the whole industry), ***agricultural*** (resulting from the application of fertilizers, pesticides, herbicides and other toxic substances, waste of animals and agricultural production) and ***military*** (chemical, radioactive).

By their nature, all types of pollution are reflected by physical (including mechanical, radioactive, magnetic), physico-chemical, chemical and biological effects (Panin & Bezel, 2008; Kaplin, 2006).

Physical pollution is associated with changes in physical environmental factors, such as: temperature – thermal pollution; wave parameters – electromagnetic pollution; noise – noise pollution; radiation parameters – radioactive contamination.

The only form of **physico-chemical** pollution is aerosol pollution, that is, air pollution by fine and solid liquid substances; an example of this form of pollution is the industrial smog or just the smoke.

The penetration of chemicals into the environment, which are initially lacking in this media or which are altering the natural concentration to a level above the normal rate, is called **chemical pollution** (heavy metals, pesticides, salts, detergents, polychlorinated biphenyls, dioxins, dyes, pharmaceuticals, etc.).

Biological pollution is associated with the introduction into and reproduction in the environment of allogeneic organisms, as well as with substances eliminated by microorganisms (they are the most toxic, known thousands of years ago), plants (during flowering – allergenic substances), products of decomposition of living organisms, various viruses and pathogenic microorganisms.

Natural sources of bioavailable xenobiotics, according to the WHO, include: wind-borne dust particles, sea salt, aerosols, volcanic activity, forest fires, biogenic particles of volatile nutrients (WHO. Promotion of Chemical Safety Unit & International Programme on Chemical Safety, 1992).

Most of the waste is generated in cities, where most of the world's population lives, and most of the various industrial sectors are concentrated. The anthropogenic flows of matter formed during the production activities of the urban population are extremely diverse, containing high concentrations of a wide range of chemical elements, including toxics (Ballschmiter & Zell, 1980).

By engaging in cycles of natural migration, anthropogenic flows lead to the rapid spread of pollutants in the components of the urban landscape, where their interaction with humans is inevitable (Izrael,1984).

The military actions have a significant impact on the environmental pollution process. As a result of the Second World War, thousands of tons of metals were stored on the battlefields. In1999,following a NATO military operation (ECETOC, 2021; https://moldova.europalibera.org/a/dou%C4%83-decenii-de-la-decizia-nato-de-a-interveni-militar-%C3%AEn-serbia/29508829.html).

Thus, on a relatively small scale in Yugoslavia, it has been observed that air, water and soil pollution has increased in Serbia both through the consumption of these toxic elements – lead (Pb), cadmium (Cd), arsenic (As) and mercury (Hg) – but also due to the inadequate quality of food products imported or received for humanitarian purposes. According to the overall effect of forces on the environment in peacetime, this is comparable to the effect of a medium-sized industry (about 4% of all wastewater discharges, and 1.2% of emissions).

Ecotoxicology is targeted in three compartments:

- **Ecotoxicokinetics** investigates the fate of ecotoxicants and xenobiotic substances in the environment around (source/origin, distribution of abiotic and biotic elements of the environment, transformation and elimination from the environment around the world);
- **Ecotoxicodynamics** examines the specific mechanisms of development and forms of manifestation of the toxic process caused by the action of ecotoxicants and xenobiotic substances on biocenoses and/or the population, as well as on its components;
- **Ecotoxicometry** analyzes the methodological techniques for evaluating the ecotoxicity of pollutants.

1.2. ECOTOXICOKINETICS

Ecotoxicokinetics is a basic direction of ecotoxicology that investigates the processes that determine the diffusion of xenobiotics and ecopollutants in the ambient environment, namely:

- sources of their occurrence;
- distribution in the abiotic and biotic environmental elements;
- conversion/transformation of xenobiotics into medium;
- their elimination from the environment.

Among the ***sources of bioavailable ecotoxicants***, according to WHO (WHO, 1992) are: *dust particles, sea salts brought by the wind, volcanic activity, forest fires, biogenic particles, biogenic volatile substances*. However, the most *dangerous* source of ***xenobiotics in the environment***, the magnitude of which is constantly increasing, is human activity.

Among the substances persistent in the environment are also included the ecotoxicants, of which heavy metals and trace elements – in concentrations at the level of parts per million (ppm) – form one of the most numerous groups of ecotoxicants (lead, copper, zinc, nickel, cadmium, cobalt, antimony, mercury, arsenic, chromium, vanadium, molybdenum, selenium, strontium, boron, bismuth, beryllium, etc.), and xenobiotic substances – polycyclic hydrocarbons, dioxines and benzofurans, polychlorinated biphenyls, pesticides (in particular organochlorine pesticides – DDT, HCH, aldrin, lindane, etc.), pharmaceutical products, detergents, dyes, and many other substances.

Substances obtained by chemical synthesis, which as a rule are quite resistant to destruction, being persistent in the environment, are the most dangerous substances (Table 1.1).

The release of persistent pollutants into the environment leads to their accumulation, being the most vulnerable (sensitive) part of the ecosystems. After the cessation of their persistent toxic release, these remain in the environment for a long time. Thus, in the waters of Lake Ontario, high concentrations of the pesticide Mirex were determined in the 1990s, whose use was stopped in the late 1970s.

Table 1.1. The half-life of some xenobiotics (Kutsenko, 2002)

Xenobionts	Half-life	Substrate
DDT	10 years	Sol
TCDD	9 years	Sol
Atrazine	25 months	Apă (pH 7.0)
Phenantrene	138 days	Sol
Carbofuran	45 days	Apă (pH 7.0)
Phosphorous thiazoline	21 days	Sol (t + 15^0)
Yperite	7 days	Sol (t + 15^0)
Sarin gas	4 hours	Sol (t + 15^0)

In aquatic test ecosystems in Florida, in the period 1962-1964 it was sprayed for research purposes the Agent Orange, which for 10 years was observed in amounts of 10-35 ng/kg (CLA in the USA being of 0.1 pg/kg) (Lysenko & Dogadina, 2015).

PROCESSES OF POLLUTANT ELIMINATION BY NON-DESTRUCTIVE PROCESSES

Evaporation and dilution processes. Some processes occurring in the environment contribute to the elimination of xenobiotics in the region, changing their distribution in environmental components. A pollutant with a high vapour pressure can easily evaporate from water and soil, and then migrate in other regions under the action of air currents. This phenomenon highlights the ubiquitous distribution of relatively volatile organochlorinated insecticides such as lindane and hexachlorobenzene (Katrin Franke Bader, 2012; Panin & Bezel, 2008).

Elimination under the influence of wind and atmospheric currents. The entrainment by wind and atmospheric currents of the toxic or soil particles on which the substances are adsorbed is also an important way of redistributing the pollutants into the environment.

In this regard, the example of polycyclic aromatic hydrocarbons (benzo[a]pyrene, dibenzopyrene, benz[a]anthracene, dibenz[a,h]anthracene, etc.) is characteristic. Benzo[a]pyrene and related compounds, both of natural (mostly volcanic) and anthropogenic origin (emissions from the metallurgical industry, the oil refining industry, thermal power plants, etc.), are actively involved in the biosphere circulation of substances, moving from one environment to another. In this case, as a rule, they are associated with solid particles of atmospheric dust.

Dust (1-10 μm) is preserved in the air for the long term and larger dust particles settle quickly enough on the soil and in the water at the site of formation. Moreover, the more intense the emission, the more dispersed the pollutants are.

The absorption of substances on particulate matter in water, followed by precipitation, leads to their elimination from the layers of water but increases their accumulation or level in sediments.

The redistribution of water-soluble substances depends on the nature of the rains and the circulation of groundwater. For example, herbicide atrazine, used to protect forest plants in rural farms and parks in the United States, is practically detected permanently in the surface waters. According to some investigations, up to 92% of the water bodies studied in the USA contain this pesticide. Since the substance is sufficiently stable and easily soluble in water, it migrates into groundwater and accumulates there.

ABIOTIC TRANSFORMATION AND DESTRUCTION

A large number of processes affect the persistence of some substances in the environment. The main processes are photolysis (destruction under the influence of light), hydrolysis (destruction under the influence of water) and oxidation (destruction under the influence of oxidation-reduction processes) (Vergeichik, 2009).

Photolysis. Light (especially ultraviolet rays) destroys chemical bonds and thus chemicals are decomposed. Photolysis occurs mainly in the atmosphere and on the surface of the soil and water. The rate of photolysis depends on the intensity of the light and the ability of the substance to absorb it. Unsaturated aromatic compounds, such as polycyclic aromatic hydrocarbons (PAHs), are increasingly sensitive to photolysis because they absorb light energy. Light accelerates other processes of substance degradation – hydrolysis and oxidation. In turn, the presence of photooxidants in the environment, such as ozone, nitrogen oxides, formaldehyde, acrolein, organic peroxides, significantly accelerates the process of photolysis of other pollutants.

Hydrolysis. Water, especially when heated, quickly destroys many substances. Essential bonds, for example in molecules of organophosphorus compounds, are very sensitive to the action of water, which determines the moderate resistance of these compounds in the environment.

The hydrolysis rate is largely dependent on pH values. As a result of the transformation of chemicals in the environment, new substances are formed. However, their toxicity may sometimes be higher than that of the original agent. Photochemical transformations in the environment of 2,4,5-trichlorophenoxiacetic acid, a well-known herbicide, can lead to the formation of an even more dangerous pollutant - 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD).

Another well-known example is ***the formation of nitroso compounds*** (Vergeichik, 2009). Thus, according to scientists, in the soil (in an acidic environment), a number of pesticides are easily associated with nitrites. Among them are dialkyl thiocarbamates, thiocarbamoyl disulphides, phenoxyacetic acid salts, etc. The resulting nitroso compounds are currently considered to be potential carcinogenic substances.

Oxidation. The presence of photooxidants in environment, such as ozone, nitrogen oxides, formaldehyde, acrolein, organic peroxides, significantly accelerates the photolysis process. The decomposition of some xenobiotics in the process of oxidation diminishes their toxicity in aquatic ecosystems.

BIOTIC TRANSFORMATION

Abiotic destruction of chemicals usually occurs at low speed. Xenobiotics degrade significantly faster with the participation of biota, especially microorganisms (mainly bacteria and fungi), which use them as nutrients.

The process of biotic destruction occurs with the participation of enzymes. Biological transformations of substances are based on the processes of oxidation, hydrolysis, dehalogenation, cleaving of the cyclic structures of the molecule, cleaving of alkyl radicals (dealkylation), etc.

The degradation of a compound can be complete by the complete destruction, that is, mineralization (the formation of water, carbon dioxide and other simple compounds). It is also possible to form intermediate products of biotransformation of substances with a toxicity sometimes higher than the original agent (Perminova et al., 2020).

Thus, the transformation of inorganic mercury compounds can lead to the formation of more toxic organo-mercury compounds, in particular methylmercury. A similar phenomenon occurred between 1950 and 1960 in Japan, on the shores of the Minamata Bay, considered as one of the worst environmental catastrophes having human causes.

BIOACCUMULATION

Many toxic substances accumulate in the body of animals and plants, especially in aquatic organisms. The process by which organisms accumulate toxins, eliminating them from the abiotic phase (water, soil, air) and from nutrition (through the foodchain), is called bioaccumulation. Bioaccumulation has negative consequences both for the organism itself (reaching a harmful concentration in critical tissues) and for organisms that use this species as food.

The aquatic environment provides the best conditions for the bioaccumulation of compounds. Here there are myriads of aquatic organisms, which filter the water and accumulate a huge amount of substances diluted in the water up to the toxic level. Hydrobionts accumulate substances in concentrations sometimes thousands of times higher than those normally contained in water (Zubcov et al., 2012, 2013 a, b).

An example of an aquatic food chain, in the direction of increasing body size, is the following: dissolved substances – phytoplankton – planktonic invertebrates – benthic invertebrates – fish – birds of prey – poikilothermic animals that feed on fish.

If foreign substances are consumed, and if these substances cannot be „digested" or simply eliminated from the body, they begin to accumulate along the foodchain, especially if the substance has a long biological half-life.

The rate of accumulation of poisons that in most cases do not decompose, is about 10 for each stage of the food chain. In addition, the accumulation of toxic substances in trophic chains is often increased due to the slower response and limited mobility of animals accumulating these substances, since more poisonous individuals become more easily prey for predators than all other components of the food chain.

As a result, in the food chain of the aquatic ecosystem, the highest content of toxic substances is often observed in detritophagous and predatory fish. Poisonous substances in fish get into the body of predatory birds, pinnipeds, as well as, last but not least, through fish products in the human body.

Factors affecting bioaccumulation

The bioaccumulative tendency of ecotoxicants depends on a number of factors. The first is the persistence of xenobiotics in the environment. The degree of accumulation of a substance in the body is ultimately determined by its content in the environment. Substances which are quickly eliminated, generally are insufficiently accumulated in the body. An exception is the conditions in which the pollutant is constantly introduced into the environment (regions near industries, etc.). For example, cyanuric acid, which is a highly toxic compound due to its high volatility, is not a potentially dangerous substance, in the opinion of many experts. However, to date it has not been possible to completely exclude the fact that certain types of diseases, pregnancy disorders in women living near gold mining enterprises, where cyanides are eliminated in large quantities, are not associated with the chronic action of this substance. After the substances enter the body, their fate is determined by ***toxicokinetic*** processes. Soluble (lipophilic) substances, slowly metabolized in the body, have the highest bioaccumulation capacity.

The fatty tissue, as a rule, is the main place of long-term deposition of the xenobiotics. Thus, many years after exposure, high levels of Orange Agent were found in the adipose tissue and blood plasma of veterans of the U.S. Army and participants in the Vietnam War.

Many lipophilic substances are prone to sorption on the surfaces of various precipitated particles in water and air, which reduces their bioavailability. For example, benzopyrene sorption with humic acids reduces the ability to bioaccumulate in fish tissues and diminishes the toxicity three times. Fish in ponds with a low content of particles suspended in water accumulate more DDT than fish in eutrophic ponds with a high content of substances in suspension.

Substances that are metabolized in the body accumulate in smaller quantities than might be expected, based on their physicochemical properties. Inter-specific differences in the values of bioaccumulation factors of xenobiotics are largely determined by the particularity of their metabolism.

The importance of bioaccumulation. Bioaccumulation can be the basis not only of chronic acute toxic effects, but also of delayed ones. Thus, the rapid loss of fat, in which a large amount of the substance accumulates, leads to the release of a toxic product into the blood stream. Mobilization of adipose tissue in animals is often observed during the breeding season. In environmentally unfavorable regions, this can be accompanied by the mass death of animals when they reach the sexual maturity.

Persistent accumulated pollutants can also be transmitted to baby birds – with the contents of the yolk sac, to fish – by the content of roe, and in mammals – through breast milk. In this case, the offspring may develop effects that do not manifest in the parents (Katagi, 2010).

BIOAMPLIFICATION

Chemicals can circulate through chains and from victim organisms to consumer organisms. For substances with a high degree of lipophilia, this migration may be accompanied by an increase in the toxic concentration in the tissues of each subsequent organism of the trophic chain. This phenomenon is called **biomagnification** or **bioamplification** (Gobas et al. 1999; Kutsenko, 2002).

It is known that DDT was used to kill mosquitoes on one of the lakes in California. After processing, the pesticide content in the water was 0.02 ppm. After a certain period of time in plankton, DDT was determined at a concentration of 10 ppm, in the tissues of fish that consume plankton – 900 ppm, in predatory fish – 2700 ppm, and the birds that consumed fish accumulated 21000 ppm. The DDT content in the bird tissues that were not directly affected by pesticides was 1,000,000 times higher than in water and 20 times higher than in the first step of the food chain.

In the book „Silent Spring", Rachel Carson (1962) gives such an example. To combat the carrier of the „Dutch disease" affecting the elms – the European elm bark beetle *Scolytes multistriatus* – the trees were treated with DDT. Part of the pesticide ended up in the soil, where it was absorbed by earthworms and accumulated in the tissues. Migratory birds, which eat mostly earthworms, have accumulated this pesticide. One of them died, and at the other ones the reproductive function was affected and they laid sterile eggs. As a result, the fight against tree diseases led to the almost complete disappearance of the birds in numerous regions of the USA.

1.3. ECOTOXOCODYNAMICS

Ecotoxicodynamics is a compartment of ecotoxicology focused on deciphering specific mechanisms and toxic processes caused by the action of toxins on the communities of organisms and/or on biocenosis. The mechanisms by which substances can cause adverse effects in biogeocenosis are numerous but unique in each case. However, they can be classified. Thus, we can highlight the direct, indirect and mixed action of ecotoxicants.

- **Direct action – is the direct loss of the organisms of one or more populations due to some or more ecotoxicants or xenobionts of a xenobiotic profile.**

The mechanism of the direct action of the toxic substances leads to the death of representatives of sensitive species. The use of effective pesticides leads to the mass death of pests: insects (insecticides) or weeds (herbicides). This ecotoxic effect creates a strategy for the use of chemicals. However, in some cases there are associated negative phenomena. For example, in Sweden in the 1950s and 1960s, the methylmercidicyanamide was widely used to treat crop seeds. The mercury concentration in the cereals was higher than 10 mg/kg. The periodic storage of the treated seeds by the birds led to the fact that, after a few years, it was observed the massive death of pheasants, pigeons, partridges and other birds that had eaten cereal seeds and had been chronically intoxicated with mercury.

When assessing the environmental situation, it is necessary to take into account the fundamental law of ecotoxicology: ***the sensitivity of different types of living organisms to chemicals is always different.*** Therefore, the appearance of the pollutant in the environment, even in small quantities, can be detrimental to representatives of the most sensitive species. As an example, lead chloride kills daphnia during the day when it is contained in water in a concentration of about 0.01 mg/l, but these amounts pose a low risk to other species (Kutsenko, 2002).

- **The indirect action – represents the situation in which the xenobiotic profile or some abiotic components of the environment are no longer optimal for the existence of the population and, as a result, it no longer develops.**

The indirect action of the xenobiotics leads to the development of allobiotic states and special forms of the toxic process. In the late 1980s, about 18 thousand seals died in the Baltic Sea, North Sea and Ireland as a result of viral infections. In the tissues of dead animals, a raised content of polychlorinated biphenyls (PCBs) was found. It is known that PCBs, like other chlorine-containing compounds such as DDT, hexachlorobenzene, dieldrin, have an immunosuppressive effect on mammals. Their accumulation in the organisms led to a decrease in the resistance of seals to infections. Thus, without having direct action, such as the death of animals, the pollutant significantly increased their sensitivity to the action of other unfavorable environmental factors (Kutsenko, 2002; Panin & Bezel, 2008).

A classic example of this form of ecotoxic effect is the increase in the number of neoplasms and the decrease in reproductive abilities in the populations of people living in regions polluted by toxicants (the effect of dioxins in South Vietnam).

➢ **Mixed action**

Several toxic substances especially xenobionts have an effect both direct and indirect, that is, their influence is reflected by ***mixed action***. An example is that of the herbicides 2,4,5-T and 2,4-D which contain as an impurity a small amount of 2,3,7,8-tetrachloro-p-dioxin. The widespread use of these substances by the U.S. military in Vietnam caused direct damage to the country's flora and fauna and, indirectly, also affected human health (Panin & Bezel, 2008).

➢ ***The action of chemicals depends on their toxic capacity and persistence***

Ecotoxicity represents the ability of a xenobiotic profile of the environment to cause adverse effects in the corresponding biocenose. In cases where the natural xenobiotic profile is associated with the excessive accumulation of a single pollutant in the environment, it is possible to conditionally speak of the ecotoxicity of only this substance.

According to Bezel et al. (1994), the adverse ecotoxic effects can be evaluated at different levels:

- ***at the level of the body*** – it is manifested by a decrease in resistance to other active environmental factors, a decrease in activity, the incidence of diseases, the death of the body, carcinogenesis, impaired reproductive functions, etc.
- ***at the level of the population*** – we observe the obvious loss of the population, the increase of morbidity, mortality, the decrease in the birth rate, an increase in the number of congenital defects, the violation of the demographic characteristics (age, sex, etc.), changes in life expectancy, cultural degradation;
- ***at the level of biogeocenosis*** – we observe obvious changes in the composition of the population until the disappearance of certain species and the appearance of new ones, which are not characteristic of this biocenosis, by destroying the interspecific relations.

In the case of the ecotoxicity assessment for only one substance in relation to representatives of only one species of living communities, the full qualitative and quantitative characteristics are considered, as well as in the classical toxicology (acute and subacute value, chronic toxicity, dose and the concentration causing mutagenic, carcinogenic, teratogenic effects, etc.).

However, in more complex systems, ecotoxicity is not measured by numbers (*quantitative*) and is characterized by a number of *qualitative* or semi-quantitative indicators, by the notions of „*danger*" or „*environmental risk*" (Hoffman et al., 2003).

Depending on the duration of the action of ecotoxicants ***on the ecosystem***, we can speak of acute and chronic ecotoxicity.

➢ **Acute ecotoxicity**

The acute toxic effects of substances on biocenoses can be the result of accidents and catastrophes, accompanied by the release into the environment of a large number of relatively unstable toxic substances or improper use of chemicals.

They are already well known different events. Thus, in 1984, in the city of Bhopal

(India), there was an accident at the factory of the American company Union Carbide of chemicals for the production of pesticides. As a result, a large amount of pulmonotropic metil isocyanate ended up in the atmosphere. Being a volatile liquid, the substance formed an unstable source of infection. However, about 200 thousand people were poisoned and 3 thousand of them died. The main cause of death was acutely developed pulmonary edema. Experts point out that in Bhopal there were unusually many children born with defects or who have developmental deficiencies, as well as various forms of cancer, diabetes and other chronical diseases (Kutsenko, 2002).

Another known case of acute toxicological and ecological disaster occurred in Iraq. The government of this state bought a large batch of grainseeds. Cereals sown for the purpose of pest control were treated with a fungicide (methyl mercury). However, this batch of cereals accidentally went on sale and was used for baking bread. As a result of this ecological disaster, more than 6.5 thousand people were poisoned, of which about 500 died. In 2000, in Romania, in one of the companies for the extraction of precious metals, as a result of the accident, there was a leakage of hydrocyanic acid and cyanide-containing products. Toxicants in large quantities entered the waters of the Danube, poisoning all living organisms hundreds of kilometers downstream (Kutsenko, 2002; Oprea, 2007).

The biggest environmental disaster was the use of highly toxic chemicals for military purposes. During the First World War, the conflicting countries used about 120 thousand tons of poisonous substances on the battlefields. As a result, more than 1.3 million people were poisoned, which can be considered one of the greatest environmental disasters in the history of mankind.

Acute ecotoxic effects do not always lead to the death or acute diseases of humans or other affected species. Thus, among the poisonous substances used in the First World War, there were also carcinogenic substances, which caused late death due to the formed neoplasms (Kutsenko, 2002; Lysenko & Dogadina, 2015).

➢ Chronic ecotoxicity

Chronic damage occurs with prolonged exposure to low concentrations. Sublethal effects are usually associated with chronic toxicity of substances. Often this implies a violation of reproductive functions, changes in the immune system, endocrine pathology, malformations, allergies, etc. However, chronic exposure to a toxic agent can also lead to deaths among individuals depending on the level of individual resistance.

The effect of long-term exposure on sulfur dioxide can be very noticeable. Vegetation studies in the area of metallurgical furnaces in Ontario (Canada) showed that at a distance of 16 km from them, 25 species of plants grew in a normal state and, as they approached the furnaces, their number decreased. At a distance of less than 1.6 km, no plant grew (Lysenko & Dogadina, 2015).

➢ Embryotoxic effect

It is well established that DDT, which accumulates in the tissues of birds such as wild duck, angler eagle, bald eagle, etc., leads to a thinning of the eggshell and, consequently, to the hitting and/or death of chickens. This is accompanied by a decrease in the number of birds (Frumin, 2013).

Examples of the toxic effects of various xenobiotics (including drugs) on human and mammalian embryos are well known.

➢ *Direct action of the product of biotransformation of pollutants with an unusual effect*

Field observations of fish in the state of Florida made it possible to identify populations with a large number of females with obvious signs of masculinization (strange behavior, alteration of the fin, etc.). These populations were found in a river downstream of an effluent and it was assumed that the effluent contained masculinizing agents.

However, studies have shown that in emissions there are no such substances: wastewater did not cause masculinization. It was also found that phytosterone was contained in wastewater (formed during the processing of raw materials), which, in the river water, was exposed to the influence of bacteria that converted the given substance into androgen, which caused an adverse effect (Kutsenko, 2002).

Thus, the interaction between xenobiotics and the biotic component of the environment (microorganisms) can cause significant effects on the population in the biocenosis.

➢ ***Action mediated by reducing the food resources of habitats***

In one of the Canadian regions, an organophosphate pesticide with rapidly degradation in the environment has been used to combat pests (spruce worms) in the forestry industry. As a result of a sharp decrease in the number of caterpillars, about 12 million birds died from starvation.

The explosion of population size can occur due to the destruction of a competing species. In the United States, after the start of the use of synthetic pesticides to control certain types of plant pests, a small number of the mat butterfly caterpillar began to multiply rapidly, their number increasing from 6 to 16. This phenomenon is explained by the fact that in the world of insects, there is a complex system of relationships, and the number of species in a population of herbivorous insects is often controlled by other species, which are predators of these insects. The effects of pesticides may be more pronounced on predatory species. It is easy to see that the mechanisms of the ecotoxic action of substances on animals, under other conditions, can be used to estimate the impact on humans (Kutsenko, 2002).

➢ ***The usual mechanisms and diseases of toxics entering the body***

The absorption of toxics represents their passage from the external environment into the blood or circulating lymph. The absorption of toxic substances, with all their structural variety, is based on common laws that involve transport through the membrane. The absorption of toxic substances is differentiated, depending on the segment of the digestive tract. Substances that are absorbed by this route (*e.g. cocaine, nicotine, potassium cyanide)* avoid the liver barrier and their detoxification is limited. Absorption is done by simple diffusion (passive transport) and active transport (Robinson &Thorn, 2005).

The active transport is carried out against the concentration gradient, with energy consumption. In clinical toxicology, active transport is present only for a limited number of toxics absorbable by digestive route and exceptionally, for those that penetrate transcutaneously or by lung route. By active transport are absorbed: *fluorides, chlorine, nitrites, nitrates, antimony, lead, copper, chromium.*

Simple diffusion is based on the selective permeability of the membrane and is generally produced without energy consumption, representing the most frequent mechanism of transport through biological membranes (Katrin Franke Bader, 2012).

Among the toxics that are absorbed by the mechanism of simple diffusion we can mention: *halogens, gaseous derivatives of sulfur, nitrogen, phosphorus, arsenic, carbon sulfide, ethyl alcohol, ethyl ether, phenol, carbon tetrachloride, etc.*

➢ ***The main ways of penetration of toxics into the body are:***

- ❖ ***DIGESTIVE ROUTE:*** consumption of toxic plants, water containing toxic substances, fodder contaminated with toxics or toxins;
- ❖ ***RESPIRATORY ROUTE:*** it is characteristic for volatile toxics, gaseous toxics or toxics in the form of aerosols (Katagi, 2010).

The respiratory route is a severe route of intoxication, because the absorption of toxics is done quickly, detoxification is diminished, toxics bypassing the liver barrier. Exceptions are liver-activated toxics (*e.g.* parathion). The respiratory route absorbsn the **volatile toxics** *(chloroform, ether),* **gaseous toxics** *(carbon oxide, hydrocyanic acid, sulfur dioxide, hydrogen sulfide, ammonia),* sublimating products, fine aerosol particles (less than 5 μ), electrically charged metal powders. **The mucous membranes** of the anterior airways (nasal, tracheal, bronchial) are of little importance for absorption. In general, toxics are expelled by coughing, sneezing. However, repeated contact can lead to intoxication (for example, alkaline salts such as sodium iodide, sodium salicylate can be absorbed). **The pulmonary system** has a high absorption capacity due to its large area, structure and vascularization.

- ❖ ***SKIN PATHWAY*** and through apparent mucous membranes: skin treatment of potentially toxic substances, accidental contact (Gobas et al., 1999).

The absorption of toxics through intact skin is difficult due to its structure and the hydrolipidic layer. The skin is considered a physiological barrier to foreign particles and chemical molecules. There are, however, two possibilities for the penetration of toxics into the capillaries in the dermis and from here into the general circulation, namely **transepidermic** (for fat-soluble substances with high coefficient of sharing) and **transfolicular** (from sebum into the sebaceous glands or hair follicle and from here to the dermis). ***Transcutaneous*** can be absorbed the gaseous and volatile substances (*hydrogen sulfide, carbon oxide, carbon dioxide, formic aldehyde, hydrocyanic acid),* a series of organic substances (liquid alyphatic hydrocarbons from C_6 to C_{10}; *cyclic, terpene, aromatic hydrocarbons; alcohols, esters: acetates, butyrations; phenols; chlorinated solvents; organophosphorus insecticides, organochlorinated insecticides; liquid alkaloids: nicotine,* etc.), mineral substances (directly or after transformation in contact with the fatty acids in sebum – *thallium salts, some mercury salts, lead and bismuth salts, alkaline iodines).* In general, substances with high molecular weight hardly cross the skin, while toxics with low molecular weight, fat-soluble, non-ionic and non-pollar, penetrate the skin more easily. ***The transcutaneous absorption*** is favored by massage (by compressing the follicles), by sweating (by dissolving the toxics on the skin), by erosions (by discovering the lymphatic circulation of the chorith), by organic solvents, keratolytic substances, detergents.

- ❖ ***PARENTERAL PATHS*** are rarer ways of toxics penetration. Poisoning can occur in these ways by overdosing medicines, administration in unusual ways or fraudulent injection (Hoffman et al., 2003). Most of the poisoning is due to penetration in **multiple ways.**

The intravenous route allows the rapid absorption of toxics, the substances avoiding gastric and intestinal barriers.

The subcutanate path. The substance being introduced into the hypodermic tissue, by diffusion, reaches the circulation and exerts its toxic effect.

The intramuscular route ensures faster absorption of substances than the subcutaneous one. From the muscles, due to the rich vascularity, the substances reach the circulation by diffusion.

Water-soluble substances are most rapidly absorbed. The amount of toxic bound depends on the concentration of the toxic, the affinity for the places of fixation, the available coupling capacity of the plasma proteins. **There was a competitiveness** for the places of coupling between the toxics from the same group: the weak acids between them, the weak bases between them. Basic toxics have more coupling places than acidic ones.

1.4. ECOTOXICOMETRY

Ecotoxicometry is a compartment of ecotoxicology, within which the methodological techniques that make it possible to evaluate the ecotoxicity of toxic substances prospectively or retrospectively are taken into account. All the types of classical quantitative toxicological studies are fully used to determine ecotoxicity.

The acute toxicity of ecotoxicants is determined experimentally on several species representing different levels of trophic organization in an ecosystem (algae, plants, invertebrates, fish, birds, mammals).

The US Environmental Protection Agency (2011) calls for toxicity to be determined in at least 8 different freshwater types and marine organisms (16 tests) in determining criteria for the quality of water containing a toxic agent (EPA U.S., Clean Water Act Policy and Guidance Documents, 2018).

Repeated attempts have been made to classify the species of living beings by their sensitivity to toxic substances. However, for various toxic substances, the ratio of the sensitivity of living organisms to them is different. In addition, the ecotoxicological use of the 'standard species' of representatives of certain levels of ecological organisation to determine ecotoxicity from a scientific point of view is incorrect because the sensitivity of animals, even of species in these families, sometimes differs very significantly.

TYPES OF POISONINGS

Accidental poisoning can occur under **natural** conditions or in **man-made** conditions.

Poisoning in natural conditions occurs with toxic substances that are commonly found in nature, such as toxic minerals in drinking water (*fluorides, nitrates, iron)* or accumulated in plants *(molybdenum, selenium, cadmium),* from toxic plants (especially *in drought conditions, affecting young animals at the beginning of grazing),* venomous insects (*bumblebees, wasps, spiders)* and venomous snakes.

Poisoning in conditions created by human activity are the most common. The causes are: industrial pollutants, pesticides, chemical fertilizers (nitrate, phosphate, potassium), medicines, feed additives, mycotoxins. The poisonings caused are those made for criminal or euthanasic purposes.

Factors influencing toxicity. Toxicity factors are the totality of the conditions on which the toxicity of a substance or its degree of harmfulness depends (climatic conditions, water and soil temperature values, oxidation-reduction processes, etc.).

Toxicity depends on the physico-chemical properties of the toxic factor and the organisms, populations or ecosystems subjected to their influence (species, age, body weight, sex, degree of fullness of the digestive tract, diet, state of maintenance, state of health, predisposition, individual sensitivity, ratio between different species, genera).

PHYSICO-CHEMICAL PROPERTIES IN THE CHEMICAL SIDE, AGGREGATION SALT AND PARTICLE SIZE

Liquid salt promotes absorption and increases toxicity. Gaseous substances are rapidly absorbed and have a high degree of toxicity. The amorphous forms of solid toxics, being more soluble, are more easily absorbable and, as a result, more toxic, having an increased degree of toxicity.

Vegetable toxics. Their toxcity is influenced by the pedoclimateric and meteorological factors (in drought conditions, in the steppe regions, in some plants are concentrated cyanogenetic glycosides), the plant part (toxics are concentrated in the roots, seeds, pollen), the vegetation stage (toxics are found in larger quantities in certain phases of vegetation, for example glycosides and cyanogenetics – in maize, in the milk phase); in some plants the most frequent cases are found in pollen and flowers.

Combinations of substances may alter their activity in the sense of increasing or reducing toxicity. The associations may be synergistic (increase toxicity) or antagonistic (reduce toxicity).

COMBINATION OF TOXIC EFFECTS

Additing (1+1=2) (e.g. cocaine and adrenaline): the result represents a sum of the toxic effects of several toxicants;

Synergism (**1+1>2)**: enhancing the toxic effects of a mixture of substances more than the sum of their toxic effects (Liess et al., 2016);

Antagonism (**1+1<2)**: it treats toxicity through physical, chemical or pharmacodynamic effects. Antagonism can be reversible or irreversible. A particular case is antidotism. For example: tannins precipitate alkaloids; copper poisoning can be prevented by molybdenum and vice versa; potassium cyanide in combination with glucose is converted into cyanhydrin, a non-toxic product.

Clasification of the pollutants after their action

- ✓ **IRRITATING POLLUTANTS** cause irritative effects on the ocular mucosa and especially on the respiratory system. This group includes non-toxic powders, as well as a sum of gases and vapors such as *sulfur dioxide, nitrogen dioxide, ozone and oxidizing substances, chlorine, ammonia, etc*. (Katrin Franke Bader, 2012).

Irritating pollution is the most widespread of the types of pollution, resulting primarily from the processes of fuel combustion, but also from other sources.

- ✓ **FIBROSING POLLUTANTS** produce fibrous changes in the respiratory system. Among the most common are *silicon dioxide, asbestos and iron oxides, cobalt compounds, barium, etc*. They are much more aggressive in the industrial environment, determining specific illnesses. Intense dust pollution can lead to fibrous pulmonary changes (Katagi, 2010). **Powders** resulting especially from the building materials industry (*cement, asbestos, gypsum, magnesite)* can cause different symptoms to the human body, generally having ***irritating, toxic, allergic and carcinogenic action***. The mode of action of powders on the body depends on their physical and chemical structure. *The irritating action* is the consequence of the sharp shape or of a certain hardness of the particles that trigger a defense reaction from the exposed tissues or organs. Powders cause discomfort and specific diseases called coniosis. In the affected organ or tissue we observe different ***dermo-, ophthalmo-, entero-*** or ***pneumopathologies***.
- ✓ **ASPHYXIATING POLLUTANTS** are those that prevent the supply of oxygen to the body's tissues. Among the most important are *carbon oxide, nitrates, nitrites,* which form with hemoglobin a relatively stable compound *(carboxyhemoglobin)* and derivatives incapable of transporting oxygen and thus preventing blood oxygenation and the transport of oxygen to tissues (Gobas et al., 1999).

Depending on the concentration in the air and the exposure time, a certain proportion of carboxyhemoglobin is achieved, which can exceed 60% of the total hemoglobin, blockage of cellular respiratory enzymes *(cyanides)*, damage to hematoforming organs *(radioactive substances)*.

Acute *carbon oxide* poisoning is relatively rare, appearing practically only in enclosed spaces in the presence of important sources of CO (in rooms where the heating systems are malfunctioning, garages, underground passages for motor vehicles, in closed vehicles, etc.)

✓ **ALLERGENIC POLLUTANTS** in the atmosphere have been known for a long time. We can mention here the case **of natural pollutants *(pollen)*** as well as ***dust in the house*,** responsible for a very large number of respiratory or skin allergies (Hoffman et al., 2003).

Besides these, there are added pollutants from artificial sources - especially industrial - which can emit into the atmosphere a sum of complete or incomplete allergens. On the first place from this point of view, there is the*chemical industry* (plastics industry, pharmaceutical industry, insecticide factories, etc.). There are also reported situations with the occurrence of mass allergic phenomena, an example may be the one produced in New Orleans in 1958 in which the allergen was *identified in dust from industrial waste* (Kutsenko, 2002).

Allergens are substances that activate the immune system. Some allergens act directly as antigens, being recognized as foreign substances by white blood cells and stimulate the production of specific antibodies (proteins that recognize and bind to cells or chemicals foreign to the body). Other allergens act indirectly by binding and chemically modifying foreign substances so that they become antigens and cause an immune response.

✓ **TERATOGENIC SUBSTANCES** are chemical substances or factors that produce abnormalities during embryonic development and growth. Some compounds, which are otherwise not dangerous, can cause tragic problems at this sensitive stage of life. It is considered that the most widespread teratogenic in the world is alcohol. The consumption of alcohol during pregnancy can lead to a syndrome that involves the appearance of several symptoms: craniofacial abnormalities, mental retardation, behavioral problems.

✓ **CORROSIVE SUBSTANCES** act on the contact areas and on the excretory organs *(mineral toxics);*

✓ **PROTOPLASMIC AND PARENCHYMATIC TOXIC SUBSTANCES** cause fat degeneration of the liver and a tendency to hemorrhage *(carbon tetrachloride, phosphorus)*.

✓ **CARCINOGENIC SUBSTANCES** are substances that cause cancer - invasive cells with uncontrolled growth that lead to the appearance of malignant tumors. The rate of cancer increased in most industrialized countries in the twentieth century, in the USA being now the second leading cause of death (Chapman, 2002). In the year 2000, for example, more than half a million people died. However, the increase in the frequency of cancer, especially in urban areas, has required the consideration of air pollutants as possible causative agents, all the more so as carcinogenic substances have been identified in the air in polluted areas.

We can classify the present substances in *organic* substances and *inorganic* substances. Among the inorganic carcinogenic pollutants we mention *asbestos, arsenic, chromium, cobalt, berylium, nickel and selenium*. Among the more widespread organic ones are polycyclic aromatic hydrocarbons such as *benzopyrene, benzoanthracene, benzofluoranthene,* etc. The last carcinogenic substance has been known for a long time, and the presence in the air indicates an increased risk of lung cancer.

Carcinogenic effects are also attributed to *organochlorine insecticides*, as well as to some *monomers* used in the manufacture of plastic masses.

- ✓ **NEUROTOXIC SUBSTANCES.** Neurotoxins are a special class of substances that specifically attack nerve cells – neurons. The nervous system is so important in coordinating and regulating the activity of organs, that the interruption of these activities causes numerous damages (Katrin Franke Bader, 2012).

The mode of action of neurotoxins may differ. Substances such as **lead** and **mercury** kill nerve cells and cause permanent neurological damage.

Anesthetics *(ether, chloroform)* and chlorinated pesticides *(DDT, dieldrin, aldrin)* break the nerve cell membrane necessary for nerve activity. Organophosphorus substances *(malathion, paration)* and carbamates *(carbaryl, zineb, maneb)* inhibit the enzyme acetylcholinesterase which regulates the transmission of the signal between the nevous cells and tissues or organs.

Most neurotoxic substances have acute and very high toxicity. Currently more than 850 compounds are considered neurotoxic.

The number of potential neurotoxics in the environment is constantly increasing and poses a great risk to humans and the environment. The neurotoxicity assessment is currently carried out to the greatest extent to predict and prevent the disease of human populations.

However, knowledge of the neurotoxic potential of environmental contaminants in ecosystems is very limited, as the neurotoxicity assessment is currently mostly focused on human exposure to individual chemicals.

Well-known human neurotoxic or neuroactive compounds, such as pesticides, pharmaceuticals and heavy metals, occur in the environment along with thousands of chemicals with neurotoxic potential unknown to different species and life stages. It has been estimated that up to 30% of all commercially used chemicals (~30,000 chemicals) may have neurotoxic potential.

In addition, in most aquatic ecosystems are recorded organic contaminants such as pesticides *(DDT, dieldrin, aldrin)*, heavy metals (Hg, Pb, Cd, As), etc. that are neurotoxic ones, but which can be determined by bioanalytical equipment and instruments capable of identifying these substances.

Unfortunately, we are not aware of any regulatory guidance available to identify neurotoxic risks in vertebrates or invertebrate animals. In addition, to date there is no European regulatory framework for the assessment of eco-neurotoxicity.

In the risk assessment and risk management of eco-neurotoxic substances, pesticides are a category of substances of particular interest. Some pesticides kill pests through neurotoxic mechanisms.

The European Food Safety Agency (EFSA) is responsible for the registration of pesticides and all other substances that may come into contact with or occur in food and are not evaluated in accordance with the REACH legislation – Regulation for Registration, Evaluation, Authorisation and Restriction of Chemicals No. 1907/2006.

Water Framework Directive (WFD) (Directive 2000/60/EC of the European Parliament and Council Establishing a Framework for Community Action in the Field of Water Policy; OJ:L327, Dec. 22, 2000), aims at integrating biological and chemical information to obtain an overview of the quality of individual water bodies. According to the WFD, the chemical status of a body of water is determined by analyzing the concentrations of 45 priority substances.

A good chemical status is defined by the concentrations of all these substances below the average and maximum permissible environmental quality standards, which are defined to protect the environment and human health. As a result, regular chemical monitoring of water quality is carried out almost exclusively through targeted chemical analysis of a limited set of compounds (indicators).

However, there are some serious limitations related to the use of target chemical analyses of high volume samples to monitor the overall chemical status of a water body. First of all, since only a limited number of target substances are analyzed, the risk of other unknown substances present in the aquatic environment remains uninvestigated. Moreover, products of the transformation of micropollutants formed in the environment or through biological metabolism are not always known or recorded and may be more toxic and persistent than basic compounds (Katagi, 2010; Katrin Franke Bader, 2012; Vergeichik, 2009).

These limitations can thus lead to an incomplete assessment of chemical hazards and risks, requiring the exploration of alternative approaches. There is an urgent necessity to provide rapid evaluations of substances with unknown toxicological potential in order to prevent possible negative effects on consumers by the water suppliers and public health departments supervising the process.

THE ELIMINATION OF TOXICS FROM THE ORGANISM

The metabolized products and the untransformed fractions are eliminated from the body. In general, toxic substances have selectiveness for a route of elimination, but they can also be eliminated simultaneously in several ways.

A link between the route of penetration of the toxic and the organ of elimination cannot be established (for example, the atropine administered subcutaneously can be found in the stomach; mercury applied to the skin is found in the intestine).

The rate of elimination of toxics depends on the route of administration, the physico-chemical properties, the fixation on plasma and tissue proteins, the metabolizations suffered, the route of elimination and the functional state of the route of administration (Chapman, 2002).

Elimination is the removal of the toxics and/or polar and ionized metabolites from the blood, lymph, interstitial fluid, cells or tissues. The route, speed, duration and degree of elimination vary in dependence on a number of physico-chemical and biological factors.

The harmfulness of a toxic is as greater as its elimination is slower.

In general, elimination is achieved through the metabolic processes of several organs, by renal, digestive, pulmonary and transcutaneous pathway.

The digestive tract is of little importance. A distinction should be made between the expulsion by vomit and faeces of insoluble or less soluble substances or those that have not been absorbed, and the elimination of substances that have entered the general circulation.

The elimination can be done by: saliva (alkaloids: quinine, strychnine; metals: mercury, lead, bismuth; iodine), gastric juice (morphine, halogens), bile (only products with molecular weight over 400 kDa, salts of heavy metals: lead, mercury, chromium, nickel; arsenic; alkaloids).

The kidney pathway is the major route for most toxics. This way eliminates toxic substances with a molecular weight lower than 150 kDa. The elimination is achieved by glomerular filtration, tubular resorption or tubular secretion.

Glomerular filtration is a process of passive ultrafiltration of a quantity of water and solvit substances with low molecular weight, polar, untethered to proteins. It depends on the hydrostatic pressure of the blood and the pressure of the blood proteins.

Tubular resorption represents the return to the renal tubules from circulation, by passive process, of large quantities of water, ionized inorganic substances, some fat-soluble non-ionized substances, undissociated acids and weak bases.

Tubular secretion consists of the active transport from blood capillaries to tubules against the concentration gradient. It has specificity and is made with energy consumption.

The kidneys eliminate most of the metal salts, iodides, nitrates, nitrites, alkaloids, pesticides. The determination of concentrations in the urine has diagnostic value for some toxic ones.

The pulmonary pathway is the fastest way of elimination. The elimination is made through the alveolar epithelium, by bronchial or nasal secretion. The speed of elimination depends on the degree of volatility. In this way, gaseous substances (hydrogen sulfide, carbon oxide), gaseous metabolites, volatile substances (alcohol, ether, chloroform, vegetal essences) are eliminated.

The skin is a route of elimination for gaseous, volatile toxics and some solids. Thus, in this way are eliminated the alkaloids, arsenic compounds, heavy metals, iodides, bromides, camphor.

The mammary gland is a route of elimination for volatile compounds (alcohol, ether), organic bases (alkaloids: caffeine, morphine, quinine, antipyrine), acids (acetylsalicylic acid, barbiturate acid), pesticides, aflatoxins, mineral ions (lead, mercury, arsenic, iodine, bromine), chloralhydrate.

The activity of the human society causes multilateral changes in the environment. Before the development of industrial civilization, before the beginning of the XIX century, pollution was extremely limited in nature, quantity and distribution. Nowadays new problems have been added to the old problems – the presence in the environment of non-physical chemical compounds, to which biological systems cannot adapt.

Some terminological notions concerning the examination and assessment of toxicity

Toxics (poisons) are organic or inorganic substances with strong harmful activity on the body, capable of producing disorders even in small quantities. ***The poison can also be defined as a chemical substance that, introduced or formed into the body, produces disturbances or leads to death***.

Toxic – is a word with controversial etymology; it derives from the Greek „*toxon*" (bow with arrows with *poisoned tips*), or from the Egyptian word „*tako*" *(destruction, precipice, death)*. The toxic word derives from the Latin „*toxicus*" (*venomous, poisonous*) which derives by alteration from „taxicus" which, in turn, derives from the name of the plant *Taxus bacata* (yew), which contains a very toxic alkaloid. ***Poison*** – derives from the Slavonic verb „*otrăviti*" (to grieve deeply), a word that refers to the psychic background. Both terms can be used. In other languages, two terms are used for the same notion – so the French use *toxique* and *poison;* the Germans, *toxin* and *gift;* the English, *toxic* and *poison*.

Intoxication presents a morbid state produced under the action of an organic or inorganic substance of vegetal, mineral or synthetic origin, which causes profound disorders of cellular metabolism and which results in the disease of the body.

Toxins are organic substances with protein structure, produced by plant or animal organisms, which cause poisoning phenomena.

Toxicosis is a morbid state produced by toxins.

The dose is one of the most important indicators of toxicity:

- **maximum tolerated dose (DMT)** - the highest amount of substance that is borne by the body free of toxic phenomena;
- **lethal dose (DL)** - the minimum amount of substance causing the death of an adult animal;
- **zero lethal dose (DL0)** - the amount of substance that causes serious, but not fatal, toxic phenomena;
- **lethal dose 50 (LD50)** - the amount of substance that produces lethal effects in 50 out of 100 experienced animals within 24-48 hours;
- **lethal dose 100 (LD100)** - the amount of substance that produces lethal effects in all animals used in the acute toxicity experiment;
- **minimum lethal dose (DML)** - the minimum amount of substance that kills only one animal in the batch;
- **certain lethal dose (DLC)** - the amount of substance that causes death to animals in any situation.

Toxic substances that penetrate the respiratory route are characterized by:

- lethal concentration of toxic in the atmosphere (CL) - corresponds approximately to the lethal dose and is expressed in ppm (mg/m^3);
- lethal concentration 50 (CL50) - the amount of toxic (mg/m^3 air) that causes the death of 50% of the animals experienced within 4 hours;
- lethal concentration 100 (CL100) - the minimum amount of toxic (mg/m3 air) in the air breathed by experimental animals causing 100% mortality.

In the case of medicinal products, the **lethal dose DL50** is reported to the **effective therapeutic dose DE50** and the **therapeutic index (IT)** is obtained. If the therapeutic index is greater than or equal to **10**, the respective medicine does not present a danger at usual doses, and if it is less than **10**, it should be used with caution.

Due to the large differences in toxic doses, Hodge & Sterner (2005) have developed a classification whereby all substances are identified in 6 categories according to the DL50 value (expressed in mg/kg), a classification which is also accepted at the moment:

- extremely toxic (less than 1 mg/kg);
- very toxic (1-50 mg/kg);
- moderately toxic (50-100 mg/kg);
- slightly toxic (500-5000 mg/kg)
- practically non-toxic (5000-15000 mg/kg);
- relatively non-toxic (over 15000 mg/kg).

In toxicology, the spectra of the toxic process are based on the properties of the toxic product; toxic substances, biological substances, radiation and other harmful factors are considered harmful agents (ECETOC, 2021). Since the effects can be considered on the basis of a variety of signs, the conceptual dose (the affectable dose) is used to indicate the amount of substance acting on a biological object. The dose of effect can be measured directly using technical means and expressed in appropriate units (mg/kg, mg/m^3, Gray, cells/kg, etc.).

The dose-effect relationship in most cases will be recorded by a general pattern: with increasing the dose increases the degree of damage to the system. In ecotoxicology, in determining the dose-effect relationship, the main aspect arises from the intraspecific variety of communities, organisms and ecosystems. In this regard, the dosage of a particular substance that causes damage often differs significantly (Hughes, 1996).

Accordingly, the dose-effect relationship reflects the properties not only of the toxic substance, but also of the organism on which it acts. In practice, this means that the quantitative assessment of toxicity, based on the study of the dose-effect relationship, must be carried out by different modelling with various biological objects and by correctitudingthe non-useof methods for processing the data obtained. In general, the graph is an S-shaped curve of the normal log-distribution, symmetrical to the central point, used in the experimental ia toxicologist. The question of estimating the range of effective doses for biological systems is complex and inextricably linked to the concept of the norm. The theory of the norm on biological systems is not sufficiently developed at present. The most characteristic indicator of the norm of biological systems is the ability to thus change its functional parameters in changing the conditions of existence in order to keep the system in optimal conditions. The population as a system of interconnected individuals, due totheinitial heterogeneity of its individual ecological-functional groups, is characterized by the diversity of their response to any external influence (Ostroumov, 2004).

For ecotoxicological regulation it is necessary to optimize the interaction by limiting the anthropic impact on environmental capacities and this aims at optimizing the use of resources or renewables. The main criterion for determining the permissible environmental load is the absence of a decrease in productivity, stability and ecosystem diversity. The death of some organisms in this case is not estimated as critical.

For each ecosystem, it should be identified its own environmental quality criteria, depending on the ecological potential of the ecosystem and the ecological capacities of the region. The basis of the development of environmental standards is the theoretical concept of a systematic approach to regulating the quality of the natural environment.

Yurie Israel (Israel, 1984) has developed ideas about the maximum allowable capacity for the environment (CMA) on this ecosystem. The significance of the CMA is based on the concept of the sustainability of ecosystems, since their normal functioning under anthropogenic pressure is possible only if the CMA is not exceeded, which is derived taking into account the combined and complex action of all the impact factors on a particular ecosystem. To date, the general principles for justifying environmental regulations are implemented by developing the concept of assimilative capacity of ecosystems.

A common approach in defining their critical points is based on the assumption that the criterion for the normal state of an ecosystem is the normal distribution of its variables. In this case, the maximum and minimum critical values are found in the functional link of normal statistical distribution.

An achievement of this approach for the evaluation of biogeochemical CAM of the chemical elements (especially their metals), which are microcomponents of waters of origin of both natural and anthropogenic origin. Each of these components of the environment must be within the limits of those biologically tolerant of aquatic organisms, communities and populations with an ability to achieve its optimal physiological functions.

For stability of the ecosystem or aquatic functioning, the main criterion is the self-protection capacity and the balance between the quality of the aquatic environment and the diversity of hydrobionts. Indeed, between the biological structure of the ecosystem and its capacity for self-purification, there is an interdependent functional relationship.

Therefore, the regulation of water quality should be done by determining the size of the self-treatment capacity, the tolerance of hydrobiocenoses, the values of which should serve as water quality standards (Zubcov et al., 2015; Moiseenko, 2008). Self-purification is an integrated process of the operation of three components – water column, underwater sediments, aquatic organisms – organically related to the ecosystems of waterbodies, so that it is an indicator not only of chemical pollution but also of the physico-geographical changes (hydrological, climatic).

1.5. ENVIRONMENTAL RISK ASSESSMENT

Risk is a probabilistic feature of the threat that arises for the environment in terms of possible anthropogenic influences or other phenomena. In a system of environmental risk assessment, any impact (be it a chemical factor or an energy field) that causes changes in biological systems (both positive and negative) is called a *stressor* (Hoffman et al., 2003). In this sense, any ecotoxic agent is undoubtedly a stress factor (Liess et al., 2016).

The concept of risk assessment includes two elements: risk assessment and risk management (Negrei & Hat, 2013; Oprea, 2007).

Risk assessment is a scientific analysis of its origin, including its identification, and the determination of the degree of danger in a given situation. In ecotoxicology, the concept of risk is associated with sources of danger to the ecological systems and processes that occur in them (Calow, 1998). Environmental indicators of damage (environmental risk) in this case include: destruction of the biota, harmful, sometimes irreversible effects on ecosystems, deterioration of the quality of the environment due to pollution, increased likelihood of specific diseases, land erosion, loss of forests and water bodies (*e.g.* Aral Sea), etc.

Risk management is an analysis of the risk situation itself, the development and justification of a management decision, usually in the form of a regulatory act aimed at reducing the risk, finding ways to reduce the risk.

The development of risk theory has led to the consistent formation of principles characterizing the attitude of society to ensuring the smooth operation of anthropogenic objects – sources of danger to the environment:

- the *zero* risk principle, which reflects the confidence that the risk will not be applied; is the principle of the successive approximation of absolute security, *i.e.* at zero risk, involving the study of certain combinations of structures, alternative technologies, etc.;
- the principle of *minimum* risk, according to which the level of danger is set at as low a level as possible, based on the justification of any costs for the protection of a person;
- the principle of *balanced* risk, according to which the different natural risks and anthropogenic impact are taken into account; the degree of risk of each event and the conditions under which people are at risk are studied;
- the *principle of acceptable* risk, based on the analysis of the ratio „cost-risk", „benefit-risk", „cost-benefit".

The concept of acceptable risk implies that the complete elimination of the risk is practically impossible or not economically viable. In line with this, a rational safety limit shall be set which optimises the costs of risk prevention and the extent of the damage in the event of an emergency.

*The first step (phase) of risk assessment is to **identify hazards*** – defining a real danger to a person or the environment. Here an important role is assigned to scientific research. Tests to identify the hazard are reduced to the search for danger signals and the selection of such a signal on the existing background.

Methods of approval, selection (*e.g.* various medicines), modelling the behavior of different substances in the environment, monitoring and diagnosis (symptom assessment, effects of exposure) are important for identifying hazards. Note that all evaluation, diagnostic and forecasting problems should be attributed to the monitoring system.

Diagnosis begins with the observation of deviations – for these deviations it is necessary to correctly identify the „disease". Almost all the data obtained through monitoring require evaluations, especially diagnostic ones.

When a hazard is identified, the first question is about what exactly constitutes a hazard and when calculating a risk, what is its extent. It is necessary to determine the likelihood of occurrence of this danger and the likelihood of adverse effects. Perspective, intuition and extrapolation can be used to determine the calculation of risk (Calow, 1998).

At the stage of the review of the risk assessment procedure, the analysis is carried out at a qualitative level.

The second stage – ***the exposure assessment*** – is an assessment of how and the environment in which, at what quantitative level, at what time and for what period of exposure the actual and expected exposure occurs; it is also an estimate of the doses received, if available, as well as an estimate of the number of people who are exposed to such exposure and for whom it appears to be probable.

The size of the exposed population is one of the most important factors in deciding on the priority of protective measures that arise when using the results of the risk assessment for the purpose of 'risk management'.

Ideally, the exposure assessment is based on the actual pollution monitoring data of different environmental components (ambient air, indoor air, soil, surface water, drinking water, food).

However, this approach is often impossible due to high costs. In addition, it is not always possible to assess the relationship between pollution and its source, and it is insufficient to predict future exposure. Therefore, in many cases, different mathematical models are used to assess the dispersion and dispersion of atmospheric emissions, their sedimentation on the soil, the diffusion and dilution of pollutants in groundwater and / or open water bodies.

The third stage – ***the evaluation of the dose-effect relationship*** – is the search for quantitative models that associate the received dose of a substance with the prevalence of an adverse effect (for health), that is, with the probability of its development. Similar patterns, as a rule, are revealed in toxicological experiments. However, their extrapolation from a group of animals to a human population is associated with too many uncertainties.

As the risk assessment procedure is complex and suffers greatly from the known uncertainty, the United States Environmental Protection Agency (U.S.EPA) developed and approved a plan for such a reseach standardization activity (Measurement Methods Standardization Strategy Document; Science Models and Research Tools (SMaRT) Search). This contains a description of the sequence of problem solving, taking into account the uncertainties and assumptions in order to obtain, to some extent, unified, approximate information about the likelihood of adverse effects on the environment.

According to this plan, the environmental risk assessment includes the following steps:

1. Formulating the problem and drawing up a plan for analyzing the situation.
2. Analysis of the environmental situation.
3. Data processing, formation of findings and presentation of materials to the customer.

As a rule, the environmental risk assessment is carried out in the form of an ordered research, performed in order to obtain information of a promising or retrospective nature and necessary for the client (legislation, management structures, etc.) to make administrative decisions.

Therefore, unlike scientific ecotoxicological studies, in which the objective rules of biocenosis reactions to the action of a stress factor are taken into account, when defining the ecotoxic risk, the environmental characteristics to be studied and „protected" may be the characteristics of a biosystem that has a value in public opinion as being very significant (Bezel et al., 1994).

The methodology for environmental risk assessment is not fully developed. In most cases, its conclusions are qualitative, descriptive. Attempts to introduce quantitative assessment methods face serious difficulties.

This is due to the complexity of ecosystems, the complex impact of stressors on the environment (not only chemically, but also physically and biologically), insufficient knowledge of the characteristics of the ecotoxic danger of a huge amount of xenobiotics used by humans, etc. In this sense, in the opinion of the environmentalists themselves, the assessment of environmental risks is largely an art that requires a permanent development.

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