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# Ecotoxicology, Ecological Risk Assessment and Multiple Stressors

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# FROM MOLECULE TO ECOSYSTEMS: ECOTOXICOLOGICAL APPROACHES AND PERSPECTIVES

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## ABSTRACT

Ecotoxicology belongs to one of the new ecological branches, which emerged as a consequence of the adverse effects of pollution on various ecosystems. These ecosystems are complex and it is difficult to fully understand all their details. Therefore, the description of ecosystems and their processes inevitably has a certain degree of uncertainty, due to their enormous complexity.

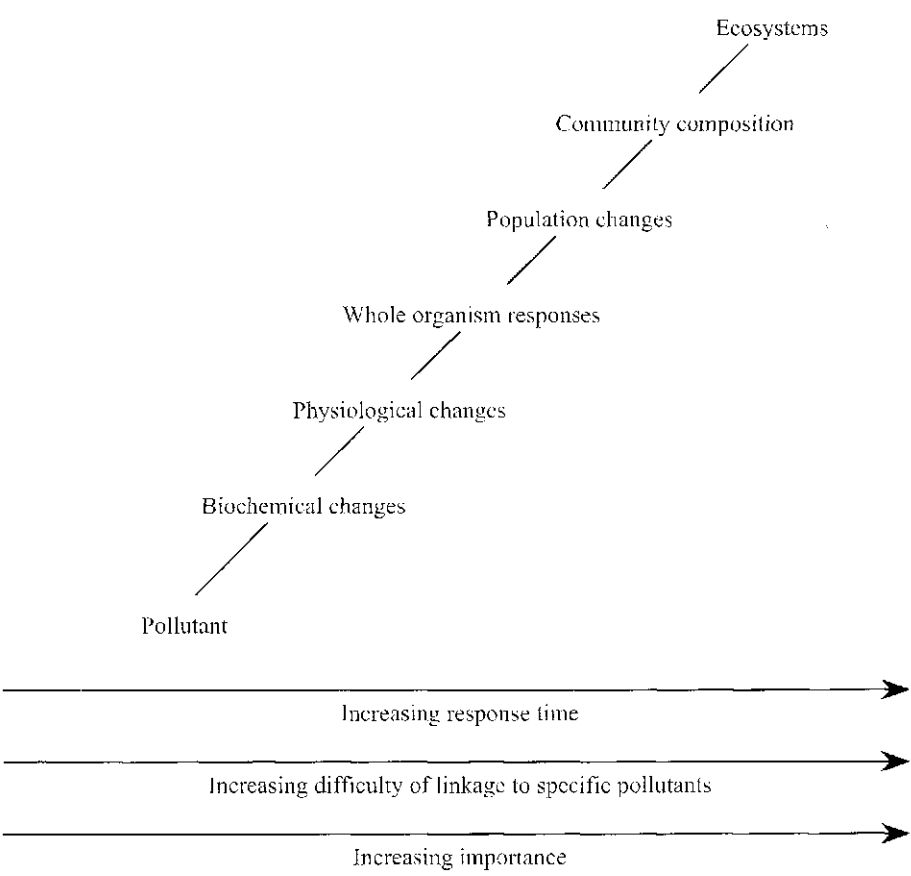
Nowadays, the ecosystem as a whole, starts to be considered as a living, evolving and dynamic entity, and not simply a conglomeration of physical and biotic components. In fact, appropriate examples drawn from various species, populations, communities and ecosystems emphasise and explain the role of ecological factors and phenomena. Thus, at the level of organisms the effects and the way they adapt for example to temperature, moisture, light, photoperiod, ionising radiation, salinity, pH and toxicants, must be taken into account. At the population level, parameters such as growth, reproduction, mortality, spatial pattern, dispersal, migration and communication are important. At the community level, additional attributes such as diversity, competition, parasitism, predation, etc. are of great significance. At the ecosystem level, the concepts of trophic levels and webs, nutrient cycles, maturity, succession, niche, stability, homeostasis, etc., must also be taken into consideration.

## 1. INTRODUCTION

Ecotoxicology belongs to one of the new sciences which emerged as a consequence of the adverse effects of pollution on complex natural systems. The term "ecotoxicology" was introduced by Truhaut in 1969 and was derived from the words "ecology" and "toxicology" (Walker et al., 1996). The introduction of this term reflected a growing concern about the effects of environmental pollutants upon species other than man (Ramade., 1977; 1992). It has been acknowledged in this new scientific discipline, that

natural systems are so complex that it is impossible to reach an understanding of all the details of these systems. Due to this enormous complexity, the description of natural systems and their processes have a certain degree of uncertainty. Wolfram talked about irreducible systems, to which most biological systems belong, but required a synthesis of many laboratory experiments and/or observations in situ (Wolfram., a; b, 1984).

Ecotoxicology can be simplified to the understanding of the following three functions. First, there is the interaction of the introduced toxicant, xenobiotic, with the environment. This interaction controls the amount of toxicant or the dose available to the biota. Second, the xenobiotic interacts with its site of action. The site of action is the particular protein or other biological molecules that interacts with the toxicant. Third, the interaction of the xenobiotic with a site of action at the molecular level produces effects at higher levels of biological organization. Figure 1 shows schematically the relationship of linkage between responses at different organization levels (Walker et al., 1996).



**Fig. 1.** Schematic relationship of linkage between responses at different organisation levels (Walker et al., 1996)

It would be possible to accurately predict the effects of pollutants in the environment, if we can write the appropriate functions that describe the transfer of an effect from its interaction with a specific receptor molecule to the effects seen at the community or ecosystem levels. However, we are far from a suitable understanding of these functions and, unfortunately, we do not clearly understand how the impacts seen at the population and community levels are propagated from molecular interactions (Landis and Van, 1995). Nevertheless, techniques have been derived to evaluate effects at each step from the introduction of a xenobiotic to the biosphere, to the final series of effects. These techniques are not uniform for each class of toxicant, and mixtures are even more difficult to evaluate.

Given this background however, it is possible to outline the basic aspects of biological interaction with a xenobiotic, which are molecular interactions and bioaccumulation, ecological effects on species, population, community and ecosystem, and risk assessment ( Jorgensen et al., 1995; Jorgensen., 1997).

## **2. BIOACCUMULATION**

Xenobiotics interact with the organism at the molecular level and the receptor molecule, or site of action, may be the nucleic acids, specific proteins within nerve synapses or even present within the cellular membrane. An important process through which toxicants can affect living organisms is bioaccumulation. Bioaccumulation means an increase in the concentration of a compound in a biological organism over time, compared to the compound's concentration in the environment. Toxicants accumulate in living organisms any time they are taken up and stored faster than they are metabolized or excreted. Understanding of the dynamic process of bioaccumulation is very important in protecting organisms from the adverse effects of pollutants exposure.

### **2.1. Bioaccumulation Process**

Bioaccumulation is a normal and essential process for the growth and nurturing of organisms. For example, all animals bioaccumulate many vital nutrients, such as vitamins, trace minerals, and essential fats and amino acids. What concerns toxicologists is the bioaccumulation of substances to levels in the organism that can cause harm. Because bioaccumulation is the net result of the interaction of uptake, storage and elimination of a toxicant, these parts of the process will be analysed further.

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### 2.1.1. Uptake

Uptake is a complex process which is still not fully understood. Scientists have learned that toxicants tend to move, or diffuse, passively from a place of high concentration to one of low concentration. The force or pressure for diffusion is called chemical potential, and it works to move a toxicant from outside to inside an organism.

A number of factors may increase the chemical potential of certain substances. For example, some lipophilic or hydrophobic compounds tend to move out of water and enter the cells of an organism, where there are lipophilic microenvironments.

### 2.1.2. Storage

The same factors affecting the uptake of a xenobiotic continue to operate inside the organisms, hindering its return to the outer environment. Some substances are attracted to certain sites, and by binding to proteins or dissolving in fats, they are temporarily stored. If uptake is slow, or if the xenobiotic is not very tightly bound in the cell, the organism can eventually eliminate it. One factor important in storage is water solubility. Usually, compounds that are highly water soluble have a low potential to bioaccumulate and do not enter easily the cells of an organism. Once inside, they are removed unless the cells have a specific mechanism for retaining them.

Heavy metals like mercury are an exception, because they bind tightly to specific sites within the body. When binding occurs, even highly water-soluble chemicals can accumulate. This is illustrated by cobalt, which binds very tightly and specifically to sites in the liver and is accumulated there despite its water solubility. Similar accumulation processes occur for copper, cadmium, and lead.

Many lipophilic compounds pass easily into organism's cells through the fatty layer of cell membranes. Once inside the organism, these substances may move through numerous membranes until they are stored in fatty tissues and begin to accumulate. The storage of toxicants in fat reserves serves to detoxify the compounds, or at least removes it from harm ways. However, when fat reserves are called upon to provide energy for an organism, the materials stored in the fat may be remobilized within the organism and may again be potentially toxic. If appreciable amounts of a toxin are stored in fat and fat reserves are quickly used, significant toxic effects may be seen from the remobilization of the toxicant.

### 2.3. Elimination

Another factor influencing bioaccumulation is whether an organism metabolises and/or excretes a xenobiotic. This ability varies among organisms and species and also depends on characteristics of the xenobiotic itself. Lipophilic compounds tend to be more slowly eliminated by the organism and thus have a greater potential to accumulate. Many metabolic reactions change a toxicant into more water soluble metabolites that are easily excreted. However, there are exceptions such as, for example, natural pyrethrins are highly fat-soluble pesticides, but they are easily degraded and do not accumulate. Factors affecting metabolism often determine whether a chemical achieves its bioaccumulation potential in a given organism.

### 2.4. Dynamic Equilibrium

When a chemical enters the cells of an organism, it is distributed and then excreted, stored or metabolized. Excretion, storage, and metabolism decrease the concentration of the chemical inside the organism, increasing the potential of the chemical in the outer environment to move into the organism. During constant environmental exposure to a chemical, the amount of a chemical accumulated inside the organism and the amount left, reach a state of dynamic equilibrium.

An environmental chemical will at first move into an organism more readily than it is stored, degraded, and excreted. With constant exposure, its concentration inside the organism gradually increases. Eventually, the concentration of the chemical inside the organism will reach equilibrium with the concentration of the chemical outside the organism, and the amount of chemical entering the organism will be the same as the amount leaving. Although the amount inside the organism remains constant, the chemical continues to be taken up, stored, degraded, and excreted.

If the environmental concentration of the chemical increases, the amount inside the organism will increase until it reaches a new equilibrium. Exposure to large amounts of a chemical for a long period of time, however, may overwhelm the equilibrium potentially causing harmful effects. Likewise, if the concentration in the environment decreases, the amount inside the organism will also decline. When the organism moves to a clean environment, so that exposure ceases, then the chemical eventually will be eliminated.

## 3. FACTORS AFFECTING BIOACCUMULATION

Some toxicants bind to specific sites in the organism and prolong their stay; whereas others move freely in and out. The time between uptake and

eventual elimination of a substance directly affects bioaccumulation. Compounds that are immediately eliminated, for example, do not bioaccumulate.

Similarly, the duration of exposure is also a factor in bioaccumulation. Most exposures to pollutants in the environment vary continually in concentration and duration, sometimes including periods of no exposure. In these cases, equilibrium is never achieved and the accumulation is less than expected.

Bioaccumulation varies between individual organisms as well as between species. Large, fat and long-lived individuals or species with low rates of metabolism or excretion of a xenobiotic will bioaccumulate more than small, thin and short-lived organisms. Thus, an old lake trout may bioaccumulate much more than a young bluegill in the same lake.

### **3.1. Ecological Effects**

Xenobiotics released into the environment may have a variety of adverse ecological effects. Ranging from fish and wildlife kills to forest decline, ecological effects can be long-term or short-lived changes in the normal functioning of an ecosystem, resulting in economic, social, and aesthetic losses.

The physical environment along with the organisms (biota) inhabiting that space make up an ecosystem. Some typical examples of ecosystems include, for example, a farm pond, a mountain meadow, or a rain forest. An ecosystem follows a certain sequence of processes and events through the days, seasons and years. The processes include not only the birth, growth, reproduction and death of biota in that particular ecosystem, but also the interactions between species and physical characteristics of the non biotic environment. From these processes the ecosystem gains a recognizable structure and function, and matter and energy are cycled and flow through the system. Over time, better adapted species come to dominate; entirely new species may change in a new or altered ecosystem.

### **3.2. Organisation Of Ecosystems**

The basic level of ecological organization is the individual organism, a single animal, plant, insect or bird. The definition of ecology is based on the interactions of organisms with their environment. In the case of an individual, it would entail the relationships between that individual and numerous physical (rain, sun, wind, temperature, nutrients, etc.) and biological (other plants, insects, diseases, animals, etc.) factors.



The next level of organization is the population. Population is a collection of individuals of the same species within an area or region. We can see populations of humans, birch trees, or sunfish in a pond. Population ecology is concerned with the interaction of the individuals with each other and with the environment.

The next, more complex, level of organization is the community. Communities are made up of different populations of interacting plants, animals and microorganisms, also within some defined geographic area. Different populations within a community interact more among themselves than with populations of the same species in other communities, therefore, there are often genetic differences between members of two different communities. The populations in a community have evolved together, so that members of that community provide resources (nutrition, shelter) for each other.

The next level of organization is the ecosystem. An ecosystem consists of different communities of organisms associated within a physically defined space. For example, a forest ecosystem consists of animal and plant communities in the soil, forest floor, and forest canopy, along the stream bank and bottom, and in the stream. A stream bottom community, for example, will have various fungi and bacteria living on dead leaves and animal wastes, protozoa and microscopic invertebrates feeding on these microbes, and larger invertebrates (worms, crayfish) and vertebrates (turtles, catfish). Each community functions separately, but is also linked to the others by the forest, rainfall and other interactions. For example, the stream community is heavily dependent upon leaves produced in the surrounding trees falling into the stream, feeding the microbes and other invertebrates. For another example, the rainfall and groundwater flow in a surrounding forest community greatly affects the amount and quality of water entering the stream or lake system.

Terrestrial ecosystems can be grouped into units of similar nature, termed biomes (such as a "deciduous forest," "grassland," "coniferous forest," etc.), or into a geographic unit, termed landscapes, containing several different types of ecosystems. Aquatic ecosystems are commonly categorized on the basis of whether the water is moving (streams, river basins) or still (ponds, lakes, large lakes) and whether the water is fresh, salty (seas and oceans), or brackish (estuaries). Landscapes and biomes (and large lakes, river basins, and oceans) are subject to global threats of pollution (acid deposition, stratospheric ozone depletion, atmospheric pollution, greenhouse effect) and human activities (soil erosion, deforestation).

### 3.3. Effects On Species

Most information on ecological effects has been obtained from studies on single species of biota. These tests have been performed in laboratories

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under controlled conditions and exposures, usually with organisms reared in the laboratory representing inhabitants of natural systems. Most tests are short-term, single exposures (acute toxicity assays), but long-term (chronic) exposures are used as well. Although such tests reveal which chemicals are relatively more toxic, and which species are relatively more vulnerable to their effects, these tests do not disclose much about either the important interactions noted above or the role of the range of natural conditions faced by organisms in the environment.

Generally, the effects observed in these toxicity tests include reduced rates of survival or increased death rates; reduced growth and altered development; reduced reproductive capabilities, including birth defects; changes in body systems, including behaviour; and genetic changes. Any of these effects can influence the ability of species to adapt and respond to other environmental stresses and community interactions.

Population numbers or densities have been widely used for plant, animal, and microbial populations in spite of the problems in mark recapture and other sampling strategies. Since younger life stages are considered to be more sensitive to a variety of pollutants, shifts in age structure to an older population may indicate stress. In addition, cycles in age structure and population size occur due to the inherent properties of the age structure of the population and predator-prey interactions.

Ecotoxicological studies performed on species in the laboratory provide the basis for much of the current regulation of pollutants and have allowed major improvements in environmental quality. However, these tests yield only a few clues to effects on more complex systems. Long-term studies and monitoring of ecological effects of new and existing xenobiotics released into the environment (including multiple stressors) are needed in order to create understanding of potential adverse ecological effects and their consequences.

### **3.4. Effect On Communities**

During the last years, scientists are most concerned about the effects of pollutants on communities. Short-term and temporary effects are much more easily measured than long-term effects of pollutants on ecosystem communities. Understanding the impact of effects requires knowledge of the time course and variability of these short-term changes.

Pollutants may adversely affect communities by disrupting their normal structure and delicate interdependencies. The structure of a community includes its physical system, usually created by the plant life and geological processes, as well as the relationships between its populations of biota. For example, a pollutant may eliminate a species essential to the functioning of

the community; it may promote the dominance of undesirable species (e.g., trash fish), or it may simply decrease the numbers and variety of species present in the community. It may also disrupt the dynamics of the community by breaking existing dietary linkages between species. Most of these adverse effects in communities can be measured as changes in productivity in the ecosystem.

The structure of biological communities has always been an indicator of ecosystem health. Numerous biological indices have been developed to judge the health of ecosystems by measuring aspects of the invertebrate, fish, or plant communities. One of the most widely used indexes of community structure is species diversity. Many measures for diversity are used, from such simple forms as species number to measures based on information theory. A decrease in species diversity is usually taken as an indication of adverse impact upon a particular ecosystem. Diversity indexes, however, ignore the dynamic nature of the system and the effects of island biogeography on the natural state. The notion of static and dynamic stability in ecosystems is related to diversity. Traditional dogma stated that diverse ecosystems were more stable and therefore healthier than less rich ecosystems. May's work in the early 1970s did much to question these almost unquestionable assumptions about properties of ecosystems (May, 1973). We certainly do not doubt the importance of biological diversity, but diversity itself may not indicate the longevity and size of the habitat rather than the inherent complexity of the ecosystem. Rarely are basic principles, such as island biogeography, incorporated into comparisons of species diversity when assessments of community health are made. Diversity should be examined carefully as to its worth in determining xenobiotic impacts upon biological communities.

Another important facet of biological communities is the number and intensity of interactions between species. These interactions make the community greater than simply the sum of its parts. The community is stronger than its populations, and the ecosystem is more stable than its communities. A seriously altered interaction may adversely affect all the species dependent on it. Even so, some ecosystem properties or functions (such as nutrient dynamics) can be altered by pollutants without apparent effects on populations or communities. Thus, an important part of research in ecological effects is concerned with the relative sensitivity of ecosystems, communities, and populations to chemicals and to physical stresses.

Effects of chemicals on communities can be measured in laboratory model ecosystem (microcosm) studies, in intermediate sized systems (mesocosms) and in full field trials. Thus, data gathered about effects of toxicants on processes and species can be evaluated in various complex situations that reflect the real world.

### 3.5. Effects On Ecosystems

Ecotoxicology focuses on the effects of toxic substances not only at the organism and population level, but also increasingly at the ecosystem level. During the last decade, generally there has been an increasing effort to understand ecosystems at the system level (Hall., 1995; Jorgensen., 1992; 1997). Through the research in this field during the last years it has been possible to reach to an understanding of the hierarchical organization of ecosystems, the importance of the network that binds the ecosystem components together, and the cycling of mass, energy and information.

While many natural forces, such as drought, fire, flood, frost or species migration, can affect it, an ecosystem will usually continue to function in a recognizable way. For instance, a pond ecosystem may go through flood or drought but continues to be a pond. This natural resilience of ecosystems enables them to resist change and recover quickly from disruption. On the other hand, toxic pollutants and other non-natural phenomena can overwhelm the natural stability of an ecosystem and result in irreversible changes and serious losses, as illustrated by the following examples:

- Decline of forests, due to air pollution and acid deposition;
- Loss of fish production in a stream, due to death of invertebrates from copper pollution;
- Loss of timber growth, due to nutrient losses caused by mercury poisoning of microbes and soil insects;
- Decline and shift in age of eagle and hawk (and other top predators) populations, due to the effects of DDT in their food supply on egg survival;
- Loss of numbers of species (diversity) in ship channels subjected to repeated oil spills;
- Loss of commercially valuable salmon and endangered species (bald eagle, osprey) from forest applications of DDT.

Each of these pollutant-caused losses has altered ecosystem processes and components and thus affected aesthetic and commercial value of an ecosystem. Usually, adverse ecological effects take place over long period of time or even at some distance from the point of release of a toxicant. The long-term effects and overall impacts of new and existing chemicals on ecosystems can only be partially evaluated by current laboratory testing procedures. Nevertheless, through field studies and careful monitoring of chemical use and biological outcome, it is possible to evaluate the short-term and long-term effects of pesticides and other chemicals.

Biomarkers have been developed to improve the estimation of exposure – including sublethal exposure – of populations of critical species in ecosystems (Peakall and Bart., 1983). They provide increased accuracy

estimation of impacts from chronic exposures to defined toxicants in the environment. Whatever their usefulness might be, these methods can not be used to assess the impacts of toxic substances and even chemical mixtures at system level. It must not, in this context, be forgotten that the properties of an ecosystem can not be equated to the sum of the properties of its individual components. First, many detrimental effects, e.g. impairment of reproductive performance and reduction of growth potential, may occur at concentrations well below those causing lethality. Second, even if perfectly understood, the toxicity of a chemical for a specific population is of little use in characterizing the toxicity that may be manifested in many systems. Therefore, the current approach must be replaced with estimations of the toxicity of toxicants throughout several ecosystems, and will require a strong emphasis on basic ecological research.

## **- RISK ASSESSMENT**

During last decades people have become increasingly concerned with toxicants, especially those that cause adverse effects after a long period of exposure. This is possible due to the fact that the industrial revolution has led to new and increased uses of known toxicants and the synthesis and widespread use of newly developed compounds. This tremendous increase in both the quantity and variety of chemical use has led to greater awareness of possible health effects of industrial products. One result of this attention was the establishment in EU, USA and elsewhere of environmental protection institutions and the enactment of new legislations to regulate chemicals in the environment. With the adoption of new laws, an important problem was how to evaluate the severity of the threat that each toxicant posed under the conditions of use. This evaluation is known as risk assessment, and is based on the capacity of a toxicant to cause harm (its toxicity), and the potential for humans to be exposed to that chemical in a particular situation. Moreover, it is taken into account their ecotoxicological impact and their fate in the environment. Standardized tests were also developed so consistent evaluations could be performed and the scientific basis of regulations could be more easily applied.

The definition of risk assessment made up of two components: toxicity (dose-response assessment) and exposure assessment. The former is a measure of the extent and type of negative effects associated with a particular level of exposure and the latter is a measure of the extent and duration of exposure to an individual or a population. For example, characterizing the risk of a pesticide to applicators requires knowing exactly what dose (amount) of this pesticide causes what effects (dose-response assessment) and what dose organisms are exposed to (exposure assessment). Sometimes, this distinction between an exposure assessment and a dose-

response assessment is not taken into account and conclusions are drawn without any measures of exposure having been made. For example, dioxin is often referred to as the most toxic man-made chemical known based on dose-response data and thus, is taken to mean that it poses the greatest risk to society. This is not the case because the potential for exposure is usually very small.

Risk assessments of widely used toxicants are often based on more or less complex models (Jorgensen., 1983; 1990; Suter., 1993). It is necessary to expand these risk assessments to encompass the ecological risk of: a) reductions in population size and density, b) reduction in diversity and species richness, c) effects on frequency distribution of species, and d) effects on the ecological structure of the ecosystem, particularly on a long-term basis (Jorgensen., 1998).

This expansion of the risk assessment concept to a much wider ecosystem level has not yet provoked much research. New approaches, new concepts, and creative ideas are probably needed before a breakthrough in this direction will occur. The concepts of ecosystem health and ecosystem integrity are probably the best tools developed up to now.

#### 4.1. Exposure Assessment

The exposure assessment can be accomplished using the following three basic approaches: a) analysis of the source of exposure (i.e., levels in drinking water, food or air). b) measurements of the environment (i.e., human blood and urine levels) and c) laboratory tests; for example, blood or urine of the people thought to be exposed. Analyses of air or water often provide the majority of usable information. These tests reveal the level of contamination in the air or water to which people and other organisms are exposed. However, they only reflect concentration at the time of testing and generally can not be used to quantify either the type or amount of past contamination. Some estimates of past exposures may be gained from understanding how a toxicant moves in the environment.

Some other types of environmental measurements may be helpful in estimating past exposure levels. For example, analyses of fish or lake sediments can provide measures of the amounts of persistent chemicals which are and were present in the water. Past levels of a persistent chemical can be estimated using the age and size of the fish, and information about how rapidly these organisms accumulate the chemical. Direct examination of a population may provide information as to whether or not exposure has occurred but not the extent, duration or source of the exposure.

Overall, exposure assessments can be performed most reliably for recent events and much less reliably for past exposures. The difficulties in exposure assessment often make it the weak link in trying to determine the connection

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of an environmental contaminant and adverse effects on human health. Although exposure assessment methods will undoubtedly improve, it is likely that there will be significant uncertainty in the foreseeable future.

## **4.2 Dose – Response Assessment**

Related to the dose-response assessment, a distinction must be made between acute and chronic effects. Acute effects occur within minutes, hours or days, while chronic effects appear only after weeks, months or years. The type and quantity of scientific evidence gathered is different for each type of effect and, as a result, the confidences placed in the conclusions from the studies are also different.

Acute toxicity is the easiest to deal with. Short-term studies with animals or humans provide evidence as to which effects are linked with which chemicals and the levels at which these adverse effects occur. When these types of evidence are available, it is usually possible to make a good estimate of the levels of a particular toxicant that will lead to a particular adverse effect in organisms.

Chronic toxicity is much more difficult to assess. There are a variety of specific tests for adverse effects such as reproductive damage, behavioural changes, mutagenesis, cancer, etc. Thus, the techniques available for assessment of chronic toxicity, especially carcinogenicity, provide rather weak evidence as to whether or not a particular chemical causes a particular effect in animals. However, there is great uncertainty about the amounts needed to produce small changes in cancer incidence. This uncertainty, together with the difficulties in exposure assessment, makes it difficult to draw definite conclusions about the relationship between most environmental exposures and chronic effects on organisms.

Overall, risk assessment is a complex process which depends on the quantity of scientific information that is available. It is best for assessing acute effects where effects appear soon after exposure occurs. Uncertainty becomes greater the longer the period of time between exposure and appearance of symptoms. In many circumstances, these uncertainties make it impossible to come to any firm conclusions about risk. Thus, risk assessment is a process which is often useful but cannot always provide the answers that are needed.

## **CONCLUSIONS**

A simplified ecotoxicological approach can be expressed through the understanding of mainly three functions: a) the interaction of the introduced toxicant, xenobiotic, with the environment. This interaction controls the amount of toxicant or the dose available to the biota. b) The xenobiotic

interacts with the site of action, a particular protein or another biological molecule. c) The interaction of the xenobiotic with a site of action at the molecular level produces effects at higher levels of biological organisation. Unfortunately, it is not clearly understood how the impacts seen at the population and community levels are propagated from molecular interactions. Nevertheless, it is possible to outline the current levels of biological interaction with a xenobiotic: Chemical and physicochemical characteristics, bioaccumulation/ biotransformation/ biodegradation, site of action, biochemical monitoring, physiological and behavioural, population parameters, community parameters and ecosystem effects.

Lately, a wide range of ecotoxicological models have been developed in order to provide with the overview needed to consider, at least the most important ecological components and processes that are known, not only at the organism and population level, but also at the ecosystem level. During the past years, an increasing effort started to understand ecosystems at the system level, especially its hierarchical organisation and the importance of the network that binds the ecosystem components together, and the cycling of mass and energy.

Moreover, bioindicators and biomarkers have been developed to improve the estimation of exposure – including sublethal exposure – of populations of critical species in ecosystems, since they provide increased accuracy in the estimation of impacts from chronic exposures to defined toxicants. However, they are not so useful to assess the impacts of toxic substances at ecosystem level. The problem becomes more intense when using toxicants mixtures.

In this context, it must always be considered that the properties of an ecosystem cannot be equated to the sum of the properties of its individual components. Consequently, in the current ecotoxicological approach must be included studies of the toxicity of toxicants throughout several species, populations, communities and ecosystems, which will require a stronger emphasis on basic ecological research.

Finally, related to the risk assessment of toxicants, which is often based on complex models, it is necessary to expand it, in order to cover the risk of:

- a) reductions in population size and density
- b) reduction in diversity and species richness
- c) effects on frequency distribution of species,
- d) effects on the ecological structure of the ecosystem, particularly on a long-term basis.

This expansion of the risk assessment concept to a much wider ecosystem level needs much more scientific effort. Innovative research approaches, new concepts and creative ideas are also needed, but the concept of ecosystem integrity is probably the best tools developed up to now and it must be more and more used in Ecotoxicology.

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