ECOTOXICOLOGY: BASIC APPROACHES

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A expansão das atividades agrícolas e industriais fez emergir necessidades de planos de manejo das atividades antropogênicas e o meio ambiente natural; a revolução ambiental procura agora soluções de gerenciamento para os seus modos estruturais e funcionais. Muitos problemas de poluição ambiental relacionam-se diretamente com as atuações políticas, oriundas das administrações governamentais. Informações a respeito dos efeitos de agentes químicos em ecossistemas naturais são altamente polêmicas e cheias de incertezas. A sociedade tem acumulado cada vez mais conhecimento, agora é o tempo das ações efetivas para se prevenir futuras degradações.

1 INTRODUCTION

The extraordinary, rapid growth of the *Homo sapiens* population, coupled with its voracious appetite for planetary dominance and resource consumption, had put every measurable biological and chemical system on earth in a state of imbalance (1). The agricultural and industrial revolutions emerged from the need to manage the human environment; the environmental revolution now seeks to manage ecosystems for their necessary services. Ecosystem services include free provision of

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biomass (food & fiber) and the assimilation of wastes. The wholesale disposal of wastes and alteration of ecosystems is making ecosystems undependable in providing expected services, such as too few fish, undesirable species (e.g., harmful algal blooms), and poor drinking water quality. All present indicators point to a continuance of current trends toward a deterioration of life capacity of the planet (2).

The world has, indeed, entered into a new information age. However, important questions remain about how to obtain information to estimate and manage environmental hazards (3).

2 WHY THE BIOASSAYS ?

Getting information about the effects of chemicals on ecosystems is highly polemic and filled with uncertainties. For instance, it was only in 1984 when the U.S. Environmental Protection Agency (EPA) amended the **Guidelines for the Analysis of Pollution**, that they officially recognized the need to use living material to evaluate the toxicity of chemicals, even though the use of biological organisms to assess toxicity had been widely used by ecotoxicology for a decade. As the EPA has stated, "In many cases all potentially toxic pollutants cannot be identified by chemical methods. In such situations, it is more feasible to examine the whole effluent toxicity and instream impacts using biological methods rather than attempt to identify all toxic pollutants, determine the effects of each pollutant individually, and then attempt to assess their collective effect" (4). They also mention that the principal advantages of using biological techniques for testing to assess water quality impacts are that: the effects of complex discharges of many known and unknown constituents can be measured only by biological analyses; the bioavailability of pollutants after discharge is best measured by toxicity testing; and pollutants for which there are inadequate chemical analytical methods or criteria can thus, be addressed (4).

Therefore, chemical information alone can be misleading because environmental quality mediates chemical toxicity (e.g., water hardness, pH, dissolved organics), chemicals may act differently individually and in mixture, chemicals may produce toxicological effects at concentrations below analytical capability, various transformations may occur making the chemical vary, but living organisms integrate the toxicological effects of this continuous but varied exposure much better than can be done with nonliving models. Despite the weaknesses of chemical information alone, the correct interpretation of bioassay results would be impossible without accompanying chemical information. So neither biological nor chemical information should be examined alone when determining toxicological response (3).

It is evident that both biological and chemical evidence are needed to assess risk to ecosystems effectively. The ability to detect a compound does not ensure that biological effects can be predicted, and the failure to detect a released chemical interacts with this ability. The integration of effects by biological material is the only reliable evidence for predicting or detecting adverse impacts (3). Results of laboratory studies indicate that in the case of metals, for instance, two or more of these substances may have a joint effect that is simply additive, as has been shown with Cu and Cd. Alternatively, joint effects may be either more-than-additive as in the case of Cu and Ag, or less-than-additive as with mixtures of Cu and Hg and some combinations of Pb and Hg (5). It is apparent that the possible action of toxicants on aquatic organisms may become increasingly more complex and concomitantly less easily predicted as the number of factors available to interact increases (5).

There is, unquestionably, scientific justification for using living material to detect toxicity since no instrument devised by man will do so. Therefore, bioassays are superior to predictions made on the basis of chemical/physical measurements alone or assumptions of being harmless based on the quality of the technology of the waste treatment system. However, the confidence that may be placed in predictions based on bioassays would be vastly improved if more attention were given to validating these predictions in natural systems or surrogates thereof (3).

3 THE ASSOCIATED PROBLEMS

The ultimate goal of ecotoxicological testing is to predict ecological effects of chemicals and other stressors. Since the damage should be avoided rather than corrected after it occurs, the predictive value of such tests is crucial (3). In order to predict the behavior of a chemical in the environment, one has to consider the evaluation of fate, transport, and effects. Each of these is contingent on the ecosystem into which the chemicals are introduced; therefore none can be resolved adequately without a perspective that considers the ecosystems and its interactions with the introduced chemicals. The problem is, however, that the determination of effects relies heavily on standardized laboratory bioassays, and on extrapolation from these to prediction of effects under field conditions (6).

The problem of extrapolating among levels of biological organization has not been given the serious attention it deserves, and currently used methods have been chosen for reasons other than scientific validity (3).

Extrapolation is basically a mathematical process of estimating in values or terms a series on either side of a known value. Extrapolations are thus, quantitative, but in biology, they may be semi-quantitative or even qualitative. Extrapolation may be from laboratory animals to humans, laboratory species to environmental biota, high to low exposure situations, short term to chronic exposure, single to multiple chemical exposure, or from one chemical to another. Furthermore, extrapolation may be from one level of biological organization to another, such as when using data of a few test species studied under laboratory conditions to extrapolate to the multitude of species in the natural environment (7). The problem, however, is that as the level of organization increases, new properties are added, such as nutrient cycling and energy transfer, that are not readily apparent at the lower levels (3). Most toxicological extrapolations, of course, contain a large element of uncertainty 8).

To deal with uncertainties, it is customary to apply "safety factors". For example, when using data for estimating effects of offshore oil development, the Minerals Management Service of the U.S. Department of the Interior, applies a 10-fold "safety factor". That is, it is assumed that oil concentrations less than those 10-fold lower than the LD50 values, will not cause ecological harm. There is absolutely no scientific basis for this procedure, and it often underestimates the potential for harm (8).

The most well-developed test methods (in terms of standardization) are acute bioassays. The measured response (death) is only environmentally meaningful in the most vulgar sense (dead animals are a problem). Biologically, acute toxicity is uninteresting in the sense that rapid death and destruction, even when relatively widespread, results in short-term disruption of ecosystem services during the recovery period, unless the acute concentration approaches the expected environmental concentration over a long period. Long-term effects are more serious in their disruption of ecosystem processes, and typically occur at concentrations near chronic toxicity test end points, which are often near expected environmental concentrations of trace contaminants (3).

The inadequacy of laboratory bioassays has to some extent, been counterbalanced by the ease and economy of application of such tests and by the body of experience that has developed concerning them. Furthermore, laboratory bioassays are valuable screening tools, and the development of a standardized laboratory tests has made it possible to evaluate the effects of a large number of chemicals in relation to a substantial number of species. Thus, despite the limitations of such tests, this availability has led to a dramatic improvement in water quality and in the protection of ecosystems (6).

However, faced with the need to evaluate the proliferation of chemicals being released into the environment, including evaluating the structure and function of ecological communities, new methods have to be employed. As CAIRNS and PRATT (3) note: "ecosystems are hierarchically structured, where new properties emerged at increasing levels of biological complexity that are not simply the sum of structures and activities at lower levels". Properties of communities and ecosystems result from the simultaneous presence and functioning of many species. Therefore using the laboratory toxicity bioassays to assess the effects of pollutants on a single species does not provide an adequate basis for extrapolating to effects on populations, communities, or ecosystems, or for anticipating transformation processes of xenobiotic chemicals in the environment (6).

The alarming increase of toxic substances in the environment is a serious threat to the integrity and health of aquatic ecosystems, and with an estimated 63.000 chemicals in daily use in 1989, it is clear, though, that society cannot wait for the perfection of a totally scientifically justifiable bioassays method (3). This total of 63.000 chemicals in common use in 1989 doubled in only 2 years (9). There are about 10 million different synthetic chemicals known, of the total material in the environment, only 1/1000 is anthropogenic, but this portion includes some toxic and very persistent compounds that accumulate in living things to harmful levels (9). However, the impacts at different levels of biological organization need to be estimated, since the essence of predictive capability is the determination of effect thresholds at all levels of organization (3).

4 BEYOND THE LABORATORY...

One of the primary concerns with traditional aquatic toxicity testing is the uncertainty in extrapolating experimental responses from the laboratory to the natural ecosystem. Laboratory environments are required to evaluate the influence of individual variables but cannot mimic all of the natural physico-chemical and biological fluctuations and interactions which occur in nature (10). Hence, for the assessment of effects on ecosystems, it is required to develop approaches that go beyond the laboratory tests. There is a need to develop more sophisticated model ecosystems and modeling methods. Furthermore, it is required to couple these with the results of experimental manipulation of whole systems to develop cross-system comparisons of the responses of the ecosystem to stress, and to lay the foundations for the beginning of a predictive ecotoxicology (6).

One major advance has been provided by experimental ecosystem studies, including *in situ* manipulations of field ecosystems, as well as experimental microcosm and mesocosm techniques. These have allowed direct observation of ecological responses, across a hierarchy of ecological organizational levels, for a given stress and defined ecosystem.

These approaches facilitate a clearer understanding of the response of ecosystems to stress (6).

During the last several decades, steady progress has been made in the gathering of data on the responses of ecosystems to stress. There are many possible ways to characterize a response to stress, and accordingly, a number of ecological properties have been monitored in the face of anthropogenic activities that may directly or indirectly affect natural ecosystems. These properties range from species to ecosystem levels, where many recent efforts have grappled explicitly with understanding the roles of different species within the context of the ecosystem (4).

Many of these studies made use of the relatively recent microcosm and mesocosm methods, which are model ecosystems subject to investigator control of conditions at a boundary between the model ecosystem and the "real world". They have been applied to studies of a variety of problems involving the fate and distribution of synthetic and natural chemicals and other stressants, and the consequent effects of these stressants on ecological processes (11). In a typical mesocosm or microcosm study, the test chemical is applied to the test systems in a manner intended to simulate realistic transport pathways and create realistic exposure patterns. This is unlike the exposure regimes in most laboratory studies, and is one reason mesocosm and microcosm studies provide such useful information for ecological risk assessment (12, 13, 14, 15)

Microcosms are practical compromises between laboratory bench tests and field studies. By microcosm it is meant an experimental unit containing multiple species, usually of a size suitable for testing in laboratory settings, designed to contain the interacting biotic and abiotic components that characterize a natural ecosystem (16). They afford a measure of safety for investigator and environment, can be operated to provide mass balance of introduced pollutants, and therefore have been judged to be particularly good for fate and transport studies of toxic chemicals. Microcosms have played a key role in the development of the use of physiochemical data to predict bioaccumulation and of comprehensive indications of chemical fate. Although there remain methodological problems, laboratory model ecosystems have been used to detect effects of toxicants on species mortality, growth, and behavior and on ecological processes such as respiration, decomposition, nutrient cycling, and species interactions (11). Besides applications in evaluating the potential impact of chemicals, microcosms may be useful in evaluating the potential environmental impact of genetically engineered microorganisms (16).

Since recent advances in the commercialization of genetic engineering technology have resulted in the creation of many new and exotic microorganisms, biodegradation is increasingly being considered as a less expensive alternative to physical and chemical means of decomposing organic pollutants. For instance, the use of filamentous cyanobacteria for biodegradation of organic pollutants has opened up possibilities of genetically engineering cyanobacteria to enhance their degradation of organic pollutants (17). But the potential negative environmental effects resulting from such releases have become a source of concern for both regulators and the general public (18).

Attempts to predict the hazard of a potential pollutant before the fact have, of necessity, relied on single species bioassays methods. Multi-species microcosm toxicity tests may be useful additions to hazard evaluation techniques, particularly when the purpose of the test is a prediction of environmental effects before the fact. These tests permit direct observation of the effects of toxicants on characteristics of complex communities, such as diversity, that are important objects of protection. And, by testing many species simultaneously, one can efficiently establish the range of biological sensitivity to a toxicant (19, 20). The use of different species can also identify the nature and extent of possible variations in toxic response, because chemicals may cause different effects in different organisms at different concentrations (7). However, in natural systems, comparisons of those predictions to observed effects in natural systems can provide information about relative accuracy of the test and can also provide guidance for improving the test method (19).

Predictions of biological response to a complex effluent were made from dose-response curves in laboratory microcosm tests and compared to observed effects in the receiving system. The microcosm test actually predicted the magnitude of decreases in species richness in protozoa and macroinvertebrate communities in the receiving system at the first downstream site. Predictions of environmental effects for stations farther downstream were generally less accurate and too high, perhaps due to lack of persistence in the toxicity of the effluent (19).

In the context of environmental assessment of pesticides, microcosms are used to integrate, corroborate and extend the information derived from conventional laboratory toxicity tests and environmental fate studies (13). To assess aquatic risks associated with the use of a pesticide, one needs to have an array of data including, but not limited to agricultural usage, environmental fate of the material in soil and water, and direct and indirect effects of the product on aquatic organisms. When laboratory data suggests there is a potential for environmental effects, a microcosm study can help resolve the uncertainty (20). Such a study was done on the effects of Chlorpyrifos on an aquatic microcosm, which provided information on an ecosystem comprised of over 100 zooplankton and macroinvertebrate taxa, and their interactions with their environment, habitat and the predacious bluegill sunfish. The algae and macrophyte communities were generally unaffected, however, in the case of an herbicide, this same experiment could have provided an additional level in the food chain, with the identification of probably 50 to 100 taxa of phytoplankton, periphyton, and macrophytes. Moreover, this study provided valuable information on the environmental fate of Chlorpyrifos in an aquatic system (21).

By measuring effects across a broad but realistic range of exposure regimes, microcosms can provide risk managers with a gradient of effects on aquatic ecosystems. This gradient of effects, when used in conjunction with probabilistic environmental fate modeling, allows the assessment of potential aquatic risks associated with different scenarios (21).

Another example of a type of microcosm study, is direct toxicity testing of organisms *in situ*. Enclosing test organisms in mesh-walled chambers provides semi-natural continuous exposure to water and sediment, without sampling induced alterations. Fluctuating physiochemical concentrations are maintained, many of which may alter the toxicity responses of the test species. *In situ* testing at multiple test sites with fish, zooplankton, and benthic macroinvertebrates has shown this approach to be effective, frequently producing results which conflict with laboratory results (10).

Mesocosms differ from microcosms by being located outside of laboratories, so that they are often difficult to distinguish from field studies. Their advantage is in the high degree of realism, direct extrapolability of data, long duration, and scale and scope of processes examined. They are, however, vulnerable to catastrophic impacts of natural phenomena, costly in terms of maintenance of the boundary, and presently lack a broad experiential base regarding synthetic organic chemicals. For certain questions, mesocosms are the only practical methods for environmental safety (11).

Studies of fate of chemicals moving to groundwater and the effects of air pollution and other chemicals on plant growth, animal populations, and ecosystem processes are among the accomplishments of mesocosm research. Mesocosms have also supported mathematical modeling, pollutant regulation, and specific safety questions (11).

Mathematical modeling of environmental fate and effects is closely linked to model ecosystem design and application. Mesocosms may be particularly appropriate in testing mathematical models, whereas microcosms may be useful in developing a better understanding of processes included in the models. The models in turn influence model ecosystem structure and operation (11, 22).

Microcosm studies have produced large amounts of data on the fate and effects of chemicals tests (databases comparable in size and scope to those generated by mesocosm studies). Where direct comparisons between the two types of systems have been possible, the results and conclusions have agreed well. Test systems of both sizes have provided valuable information for ecological risk assessment (12, 14).

There are several field methodologies being developed for the assessment of the effects of several contaminants on aquatic organisms. Many of these assessments are based on the metabolic activity closely associated with photosynthesis, such as ATP information, the assimilation of radioactive carbon, oxygen evolution, and enzyme production (23).

The short-term ^{14}C assimilation algal toxicity test method (photosynthesis inhibition test) was evaluated by KUSK and NYHOLM (24) with natural phytoplankton and cultures of the marine diatom, *Skeletonema costatum*. The ^{14}C assimilation algal toxicity test with natural phytoplankton was found to be sensitive to toxicants and well-suited for testing of the toxicity of both chemicals and complex test materials, such as effluent samples. The sensitivity of natural phytoplankton, however, varied considerably, both between sampling locations and with time. So tests with natural phytoplankton are probably not suitable for use in effluent control schemes or for similar regulatory purposes because their sensitivity is too variable. But such tests are considered realistic indications of the actual acute toxic effects on the phytoplankton community of the receiving water body. If the objective is to monitor variations in effluent toxicity, to control set limits of toxicity, or to initially evaluate the toxicity of a chemical, it is preferable to use a sensitive culture microalgae as the test organism, because such tests have been demonstrated to show good reproducibility. Nevertheless, tests with natural phytoplankton constitutes an inexpensive tool for occasional toxicity screening, where maintenance of stock cultures are not necessary. In addition, tests with natural phytoplankton of a particular after body are considered to possess a high degree of ecological realism (24).

Another method of *in situ* field testing is evaluating the protein or enzyme synthesis of the test organisms. One that seems to be very promising is the evaluation of the so-called "stress" protein. The exposure of organisms to stressing agents results in a rapid increase of the synthesis and accumulation of special proteins with a molecular weight, called "stress" proteins, and a correlated decrease of proteins normally produced by the organism. The main advantages of this technique are that the cellular processes tend to be more sensitive and less variable, and, also, it allows for possible detection before the impact that is causing the stress manifests itself at the organism level. Studies show that protein synthesis increases shortly after the organisms exposure to the stress. The synthesis and accumulation of the protein continues to increase as long as the stressant acts, in a desperate attempt to adapt to the cells. However, near the maximum stress level, the protein synthesis stops and the organism dies shortly after. The results of the laboratory were compared with data from organisms tested *in situ* with different concentrations of treated domestic sewage, and the results suggest the possibilities and limits of using these "stress" proteins as bioindicators for environmental quality (25).

Closely related to the above method, is the evaluation of the enzyme acetylcholinesterase (AChE) for the detection of organophosphorous insecticides and carbamates. These pesticides inhibit the synthesis of AChE (26). Studies have been done correlating changes in physical attributes of the activity of turbidity, total solids, dissolved oxygen, phosphorous, and nitrate with that of AChE activity of *Moina macrocopa*. The study suggests this activity as a valuable indicator for determining the level of impact (27).

Another study evaluated the extent pollutants effect onthe benthic biota, since organic and inorganic compounds tend to be deposited in the sediments. The toxicity of the contaminated sediment was evaluated by examining the change in excretion of ammonium by the test organism. The change in physiology of the organism exposed to the contaminated sediment indicates that the organism is in a stress condition, which, if continued, may result in the death of the organism (28).

A reliable indicator of the toxic effects of pollutants is the use of staining results associated with individual enzymatic activities to assess *in vivo* metabolic activity of cells. The fluorescein diacetate method is based on the measurement of cell activity using a fluorometric stain, because the fluorescence depends on the physical and metabolic state of the cell (23). The test has several advantages over the other procedures of toxicity testing described, by means of radioisotopes and enzymes, which have the disadvantage of utilizing methods of great complexity. The fluorescence test, in addition, permits visual examination of the impact of the contaminants on individual cells or organisms under the microscope, which is not possible with the other techniques. Also, the microcomputer-based algal fluorescence test was found to be rapid, sensitive, and relatively inexpensive. Recent advances in microcomputer technology makes the procedure more accurate, gives it greater detail, and allows for faster analysis of acquired data (29).

The importance of developing a hierarchy of test systems is evident, ranging from laboratory tests through microcosm to controlled field experimentation. Also, there is a need to develop a theoretical framework based on comparisons, across systems and across stresses. Tools such as mathematical modeling can help to make the connections between levels of organization, and statistical analyses can help to detect and organize patterns (6).

5 WHAT FOR ?

Ecotoxicology test data have a variety of applications, including corporate industrial decisions on product development, manufacture, and commercialization, registration of products to satisfy regulatory requirements, permitting for the discharge of municipal and industrial wastes, environmental hazard evaluations, and prosecution and defense of chemical-related activities in environmental mitigation (30).

6 REGULATORY ISSUES...

Relatively few countries have requirements for ecotoxicological data, even though concern for the environment is being strongly expressed.

A significant event in the public perception of chemical risk is the rapid growth of environmental protection movements which consider chemicals as one of the major threats to mankind. Environmental concerns extend beyond the chemical pollution of air, land and water, to cover nuclear power plants, noise in the urban environment and industrial installations, particularly of the chemical industry. Pesticides receive particular attention, followed by environmentally persistent chemicals, such as organochlorine compounds and environment threatening chemicals, such as chlorofluorocarbons. The public concern for human and environmental health has been particularly shaped by pollution incidents in Italy (Seveso, dioxin), Japan (Minamata Bay, mercury), England (London, smog), USA and Taiwan (Polybrominated biphenyl's), Brazil (Goiás, radiocesium-137), and chemical warehouse fires in Switzerland, waterways. These incidents have been among the driving forces for imposing controls on potentially toxic chemicals (7).

In conclusion, it is crucial that scientists and administrations in countries who have responsibility for chemical risk assessment and establishment of national legal exposure limits understand the scientific complexities and uncertainties involved in the methods. The pace of development of the scientific basis for risk assessment indicates that an open-minded and practical approach is essential (7). Science that can ensure sound and planned steering of the harmonious balance and relationship between man and nature, a relationship and balance, which is absolutely needed, if it is wanted to survive on this planet (31).

Multidisciplinary Science can go a long way toward reducing the unknown, however, uncertainty will always remain on any evaluation. Therefore the problem of decision making under uncertainties, and the complementation of risk assessment with risk management, is the main challenge in ecotoxicology.

Abstract

The agricultural and industrial revolutions emerged from the need to manage the human environment; the environmental revolution now seeks to manage ecosystems for their necessary services. Many pollution problems have in fact derived directly from the practice of politics and from the actions of governmental administration. Getting information about the effects of chemicals on ecosystems is highly polemic and filled with uncertainties. Society has been gathering much information and now it is time to act to prevent further degradation.

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