

## **Ecotoxicology : An Opportunity for the Experimental Sciences**

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**Abstract :** The primary purpose of ecotoxicology is to provide a means of predicting the probability of harm from the use of chemicals or other environmental stressors (*e.g.*, heat or suspended solids) upon complex natural systems. Although this statement may appear platitudinous, an examination of toxicological literature involving non-human species shows an inordinate preoccupation with studies of dose-response curves at the single species level of biological organization and relatively few studies dealing with properties at higher levels of biological organization such as natural communities and ecosystems. Detractors of the attempts to incorporate ecological principles into the field of environmental toxicology to a degree sufficient to justify the term “ecotoxicology” often categorize such attempts as impractical. However, the field of ecotoxicology will not reach maturity until it involves toxicity tests at different levels of biological organization using end points or parameters characteristic of these levels and the results of laboratory tests are validated in a scientifically justifiable way in the natural systems that the tests are presumably designed to protect. This manuscript explores the present situation with regard to the major components of the hazard evaluation process in ecotoxicology– (1) screening or range-finding tests, (2) predictive toxicity tests, (3) validating or confirming tests, and (4) monitoring. A clear tendency in the literature in recent years has been to incorporate a higher degree of environmental realism into laboratory ecotoxicological tests and to be concerned in principle, if rarely in practice, about the validation or confirmation of the predictions made on these tests. However, it is abundantly clear that of the four components just mentioned the first two have received inordinate attention (particularly at the single species level) while the latter two have received relatively little attention. In order for the field of ecotoxicology to develop properly, a balance must be reached in both research activities and practical application of all four components of the ecotoxicological hazard evaluation process.

**Keywords :** Ecotoxicology, Bioassay, Pollution assessment, Multispecies toxicity, testing, Hazardous chemicals

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### **Introduction**

The continent of Australia provides a dramatic illustration of the rapidity with which ecotoxicological problems developed. Over 200 years ago, human activities were primarily hunting and gathering of foodstuffs

with a relatively primitive technology. Industrial technology has only been notable for approximately 100 years, and only in the period since World War II has it been of pressing ecological concern. Globally, the initially isolated patches of technological stress have grown larger and the stressors more global to the point where, in some cases, such as atmospheric CO<sub>2</sub>, depletion of the ozone layer, and acid rain, they are a matter of international concern. We are now witnessing a global loss of biodiversity that may be unparalleled even in geological time. To further confound the difficulty, again beautifully illustrated in the case of Australia, species may become extinct before they have even been inventoried. Of course, this biotic impoverishment is primarily due to habitat destruction through deforestation, draining and filling of wetlands, desertification, and the like. In short, even if all the materials that caused toxicological effects suddenly vanished from the face of Earth, problems due to destruction of habitat would still have to be faced. Thus, the challenge to ecotoxicologists is to carry out, during a period of unprecedented ecological change, the four major activities of hazard evaluation: (1) screening or range-finding toxicity tests, (2) predictive toxicity tests, (3) validating or confirming tests, and (4) monitoring. Furthermore, methods must be developed to protect species yet to be inventoried and, therefore, of unknown toxicological response thresholds.

The amount of work needed for a maturation of the field of ecotoxicology is indeed formidable. However, the truly astonishing progress made over the last 50 years in environmental toxicology gives reason for cautious optimism. In addition, the number of research investigators and practitioners today is far greater than 50 years ago, and the amount of funding and other resources available are proportionally larger. There are two basic problems, however. (1) The subdiscipline of ecology is also a relatively new one and is itself just entering the predictive stage. In fact, in that regard, toxicology is well ahead of the field of ecology because toxicity began with the predictive stage. (2) Ecotoxicologists have concentrated too much on screening and predictive toxicity tests at the single species level in test systems low in environmental realism and have only recently put relatively modest resources into validation and monitoring. Some of the problems of ecotoxicology will not be resolved until ecologists develop a more substantive predictive capability, which does not appear to be likely

in the near future. In the meantime, however, ecotoxicologists can make better use of the limited array of end points ecologists have provided for ascertaining changes in community and ecosystem structure and function. I hasten to add that the word *provided* does not yet mean widespread professional endorsement of any of the methods for pollution assessment.

I am making the assumption that the basis for ecotoxicology is the hazard evaluation process outlined in the first of the so-called “Pellston Series” books (Cairns *et al.*, 1978) and subsequently developed further in the other volumes (Dickson *et al.*, 1979, 1982; Maki *et al.*, 1980; Bergman *et al.*, 1985). This hazard evaluation process encompasses the four steps already mentioned. What the Pellston Series did not do is explore in detail the levels of biological organization appropriate to this process nor was much attention given to the ecological aspects of hazard evaluation. The National Research Council (1981) book does give attention to the levels of biological organization and the ecological aspects but does not make the connections with the field of ecotoxicology in quite the same way I intend to do in this discussion. If the pivotal assumption in judging the merit of an ecotoxicological test is its ability to predict events accurately in complex natural systems, then the field of ecotoxicology must determine with statistical reliability how well the four components mentioned fit together and how accurately events can be predicted in one level of biological organization from events in another. Carried to the ultimate predictability, extrapolation could be done reliably from laboratory to field or vice versa. Furthermore, an assertion with confidence could be made that, given a certain series of conditions, a specific ecological outcome is highly probable.

Finally, although restoration of damaged ecosystems and ecotoxicology have not been closely associated, they should be. A failure in the field of ecotoxicology will require the services of those knowledgeable in the field of restoration ecology. The latter field must also develop a predictive capability using many of the parameters ecotoxicologists should be using (Hoffman *et al.*, 2003). Some ethical issues (Cairns 2002a, 2003), as well as the biological monitoring issues (Cairns 2002b), must be considered in the practice of ecotoxicology. A discussion of desirable changes in parts of the hazard evaluation process follows.

## **Screening And Range-Finding Toxicity Tests**

Screening or range-finding tests should provide two useful types of information. (1) Such tests should determine whether the environmental concentration of the chemical is well below the concentration causing adverse biological effects, above that concentration, or in such close proximity that more definitive tests are needed. (2) If further tests are needed, the critical range of concentrations should be identified so that the most useful predictive information can be obtained.

At one time, the “most sensitive species” was chosen on the belief that any concentration that did not affect that species would thereby protect all other species as well as community and ecosystem structure and function. Although this position had its defenders (*e.g.*, Weiss, 1985), ample evidence shows that this sensitivity to one chemical is not likely to mean the same relative sensitivity to other chemicals. More important, since only a relatively few species have been tested (since many of Earth’s species are not yet even inventoried or named), the likelihood is minimal of selecting the most sensitive species in a series of laboratory tests due to the limited number of species used for this purpose (Cairns, 1986a). If the goal is to protect a particular species of commercial, ecological, or recreational importance, it is, of course, prudent to determine the effects of the presumed toxicant on the growth of the species, its reproductive success, its survival, and the like. The value of single species toxicity tests for this purpose has never been doubted, especially given the history of the use of single species tests to protect human health and well being (*e.g.*, Schettler *et al.*, 1999).

The value of screening, single species tests (*i.e.*, short-term, low in environmental realism) using lethality as an end point for establishing critical concentrations to be tests for sublethal effects over a long period of time (*i.e.*, chronic tests) is better than having no evidence at all, but this approach certainly lacks precision. Stated differently, no “k factor” is available for use in multiplying a screening test concentration to determine which concentrations will furnish the best predictive information for the next series. An alternative possibility is the use of multispecies toxicity tests involving microorganisms and invertebrates for screening or range-finding purposes. The advantage of using an array of species is that evidence can be obtained

on both the concentrations likely to harm a system and also on the range of sensitivities for the number of organisms. If functional tests are being used, evidence can be gathered on the threshold concentration at which functional attributes are disrupted, which might not occur with the loss of a few species. Using an artificial substrate colonized in natural systems as the basis for the multispecies tests in the laboratory means that it is highly unlikely that the same array of species will be tested at different time intervals or even that precisely the same array will be on two different artificial substrates. Nevertheless, if the colonization process is independent of sensitivity to the particular toxicant being tested, it is quite probable that the total response range will not vary strikingly. In fact, my laboratory has recorded constancy in this regard not much different from single species tests in which some populations may be physiologically distinct from others.

### **Predictive Toxicity Tests**

Most investigations on the toxicological responses of non-human organisms, particularly those of aquatic ecosystems, continue to be carried out with single species laboratory systems low in environmental realism because the four following assumptions are thought to have considerable merit :

- (1) by selecting the most sensitive species and determining the concentration below which no adverse effects are noted, all species could be protected as well as communities and ecosystems at concentrations below this threshold.
- (2) a representative species could be selected that is neither ultrasensitive nor ultratolerant to pollutants and this species represents the majority of species. By finding the no-adverse-effects threshold for the representative species, a safety factor or application factor could be added or that concentration could be multiplied by a safety factor or application factor that would protect the more sensitive species, life history stages, modification of the toxicological response by environmental conditions, etc.

- (3) enough is known about both the environmental and physiological factors that modify the toxicological response to develop an application factor that would take care of all deficiencies due to duration of tests, lack of environmental realism, limitations of test species, etc.
- (4) these assumptions need not be confirmed or validated because they appear to be working. This “appearance of working” is based almost entirely on absence of contrary evidence, anecdotal evidence, and lack of rigorous examination of natural systems and not on scientifically justifiable evidence. This position has been strengthened because ecologists have not agreed as a profession on what types of measurements should be made to determine the health or condition of natural communities and ecosystems.

### **Alternative to Biological Monitoring**

Before examining these assumptions, some attention should be given to non-biological alternatives for assessing and preventing pollution. Long before toxicity testing came into vogue in the late 1940s, methods focused on measuring the chemical concentration alone. Assumptions were then made about the toxicological effects, often in the absence of rigorous evidence related to these assumptions. The three primary reasons explain the failure of chemical measurements alone as the prime criteria for estimating toxic effects: (1) chemicals may have toxic effects at concentrations below analytical capability, (2) environmental quality mediates the toxic response, and (3) compounds may act together synergistically, antagonistically, or in an additive way. Gradually, realization surfaced that no instrument devised by man can measure toxicity, only living material can be used for this purpose. Of course, having the biological response alone is not useful either because the integration of two lines of evidence—the environmental concentration of a chemical and the toxicological response at these concentrations—provides the basis for a systematic and orderly process of hazard evaluation. This integration of different evidence, of course, forced reconsideration of what kind of toxicological information should be coupled with the environmental

concentration, fate, transformation, partitioning, and persistence of the chemicals for which a toxicological response was being determined. A second major thrust was essentially an attempt to bypass biological information entirely using a technology-based strategy. The basic assumption of this approach (which in the United States has been referred to as the best applicability technology [BAT] or the best practicable technology [BPT]) is that, if either the best available or the best practical technology is used in every waste discharge situation, the environment is protected. This approach has a number of deficiencies (Cairns, 1983*a*) and was ultimately rejected by engineers who once espoused it. The technology-based standards ignored the size of the receiving system, its sensitivity or robustness, the number of other dischargers into the system, the vast amount of pollution originating from nonpoint sources, and the fact that environmental quality mediates the toxic response. Probably the main reasons for rejection were not these theoretical deficiencies but rather the fact that the technology of waste treatment could change very rapidly and industries would be in a constant turmoil installing new equipment that might not be operated as it should by engineers and chemists without a long history of experience with the equipment. In addition, the environmental benefits of any improvements would remain undefined. In short, if the environment is protected with present technology, why bother with new; if it is not, should the environment be damaged with the present technology until superior technology is developed? As George Bernard Shaw has been reputed to say about life, "It may not be splendid but it is certainly preferable to the alternative." One could certainly say the same thing about toxicity testing and alternative methods of estimating environmental response to toxicants.

### **The Most Sensitive and Representative Species Myths**

Probably the most persuasive single database to refute these assumptions about most sensitive and representative species is that of Mayer and Ellersieck (1986). The first of their interesting conclusions states "No one species, family, or class was the most sensitive to all chemicals all of the time." Two other statements deserve considerable attention: (a) "the utility of predictive techniques should be realized, but no single approach correctly predicts acute toxicity under all situations" and (b) "toxicity studies

are too often conducted to develop data, without subsequent comprehensive interpretation.” Others (Cairns and Niederlehner, 1987) have discussed the deficiencies of the most sensitive species approach. However, Mayer and Ellersieck (1986) have shown that certain groups of organisms are more sensitive than others a certain percentage of the time and that, if one were making wagers on the probable sensitivity for a large range of chemicals and test conditions, one could win the wager a satisfactory number of times by betting on their sensitivity. However, using a single line of evidence is dangerous, particularly without rigorous analysis of the significance of the data. The Executive Summary of the National Research Council (1981) book *Testing for Effects of Chemicals on Ecosystems* states the situation well: “Single-species tests, if appropriately conducted, have a place in evaluating a number of phenomena affecting an ecosystem. However, they would be of greatest value if used in combination with tests that can provide data on population interactions and ecosystem processes.” In short, single species tests have performed very well and will continue to do so in the future. This success should not prevent examination of ways in which predictive capability can be improved.

Single species tests should be used prudently to determine how well extrapolation can occur from one species to other species. Kenaga (1978) approached this problem by correlating the LC50s of one species for all chemicals tested with those of two other species. He found that correlation within the three fish species he used was good ( $r = 0.85-0.99$ ), but the correlation with *Daphnia* and rainbow trout was poor ( $r = 0.56$ ) with 95% confidence limits of 2.2 to 3.3 orders of magnitude. Doherty (1983) used essentially the same approach. The correlations were good in Doherty’s study ( $r = 0.89-0.97$ ), providing evidence that the LC50 for one species can be estimated from the LC50 of another species using suitable regression equations. Mayer and Ellersieck (1986) also found that toxicity values for *Daphnia magna* did not correlate well with those of other species. In short, even at the single species level, the results of extrapolation are mixed with quite good results in some cases and rather poor results in others. Kenaga (1978) and Doherty (1983) each used four species of freshwater organisms (*D. magna* or *D. pulex*, rainbow trout, fathead minnows, and bluegill sunfish) in their correlations. Mayer and Ellersieck (1986) also examined



the relative sensitivity of 34 families of aquatic organisms tested with 203 chemicals and found that it varied greatly. They note that some of the families were represented by only one species and others by many and that a more complete representation might easily change the ranking. In short, the interesting evidence provided by Mayer and Ellersieck suggests that even a relatively large database is inadequate for reliable extrapolations from one family to another. Mayer and Ellersieck (1986) essentially restricted their examination of the problem to single species, extrapolations from one species to another, or ranking of species. They tried this examination at the individual level and by using larger and more comprehensive taxonomic groupings such as families. Attempts to extrapolate from single species to communities are relatively rare. Niederlehner *et al.* (1986) did compare responses at three different levels of biological organization and found surprisingly good agreement. Expanding the database either by using different aggregations of organisms for comparison or more chemicals probably does not produce the same results, as the analysis of Mayer and Ellersieck (1986) for single species shows.

Mayer and Ellersieck (1986) have also analyzed the ability to extrapolate from one set of environmental conditions to another with somewhat similar results to those obtained from one species to another. In short, in some instances the results were quite good, in others substantively less than desirable.

The most fundamental question about developing an ecotoxicological capability has yet to be answered in even a preliminary way. The question concerns the success of extrapolating from laboratory systems low in environmental realism to the highly complex, sometimes extraordinarily variable, natural systems. Even though Mayer and Ellersieck (1986) provide evidence on correlations from one laboratory test to another, the important correlations are between laboratory systems and natural systems. The crucial question concerns how the accuracy of extrapolation from artificial systems to natural systems and from one level of biological organization to another for each of these.

## **Validation**

Practically all the theoretical and applied investigations in the area of environmental toxicology have involved laboratory tests at the single species level low in environmental realism. If these tests are truly ecotoxicological, they should be suitable for extrapolating to responses at higher levels of biological complexity, such as communities and ecosystems under natural conditions. Scientifically justifiable evidence that this process can be accomplished is extraordinarily slim considering the seriousness of the problem. Elsewhere (Cairns, 1986*b*, 1988*a*, 1988*b*), I have discussed the theoretical aspects of this problem. Hierarchical theory (*e.g.*, Webster, 1978) indicates that when one is studying a particular level of a hierarchical organization such as an ecosystem, one should use the characteristics that are unique and critical to that level of organization and not those of other levels. Useful information possibly may be obtained at the next lower level of organization (for ecosystem level, this level would be community); however, if lower levels are then used (*e.g.*, population), the number of possible interactions relative to the original level being studied are so complex that studying them in a systematic and orderly way becomes virtually impossible. Webster (1978) also indicates that, for the same reasons, extrapolation from lower to higher levels of biological organization is extraordinarily difficult. An added difficulty is that new properties are added as one progresses from lower to higher levels of biological organization that are simply not apparent at the lower levels. For example, one can hardly study nutrient spiraling through an aquatic ecosystem by working with single species.

The problem of validation is particularly crucial—confidence in a predictive model or method relies on confirming the accuracy of the prediction. Any failure to confirm is probably the result of two factors: (1) environmental toxicologists and ecologists rarely associate with each other and (2) ecologists have not endorsed (as a profession) the criteria, standards, and methods suitable for determining ecosystem condition or health. The well known Welsh ecologist John Harper (1982) feels that ecology has tended to be highly descriptive in nature and has thus far made little progress toward reaching maturity as a rigorous experimental and predictive science. He feels that so long as ecological work remains basically descriptive in

nature and has thus far made little progress toward reaching maturity as a rigorous experimental and predictive science. As long as ecological work remains basically descriptive, these weaknesses will not be evident because validation of predictive models, etc. is either not done or is not done as it should be (Harper, 1982). The first volume of the Pellston Series on hazard assessment (Cairns *et al.*, 1978) and the book published by the National Research Council (1981) both identify validation as an important component of environmental risk analysis or hazard assessment. Some toxicologists have confused calibration of the test methodology with validation of a predictive model. This situation has probably occurred because the predictions that entire ecosystems are protected are not explicitly stated. If the test species used is not indigenous to the ecosystem receiving the stress or hazardous chemical, clearly the extrapolation is being made from the test species to some other biological unit. If this biological unit were merely another species (other than a rare and endangered species), the toxicity tests could probably be carried out with that species rather than a surrogate species. Regardless of the nature of the prediction, some validation of a prediction developed on laboratory generated evidence should be made in natural systems or field enclosures of natural systems not only to validate or confirm the accuracy of the prediction but to provide an error control loop so that the predictive models can be corrected when their accuracy is less than desirable. If ecotoxicology is to live up to the responsibilities implied by its name, some significant ecological component must be present in the toxicity testing protocol. The most obvious way to rectify this deficiency is through the validation process, i.e., by incorporating some ecological parameters into the toxicological testing procedures. I have not seen any remarkable activity among ecologists to assist in this process, although I would be delighted to be proven wrong. Nevertheless, until ecologists assist in this effort, environmental toxicologists must assume this responsibility.

The development of multispecies toxicity tests has provided a good basis for making the first step in developing comprehensive validation procedures. The end points utilized in many of these laboratory multispecies toxicity tests (such as colonization, respiration, detritus processing and the like) also occur in natural systems and involve higher levels of biological

organization than single species. The same end points or characteristics to be measured could conveniently be used in both laboratory and field. Earlier (Cairns, 1983b), I developed a case for testing simultaneously at different levels of biological organization. Doing so would provide a basis for not only determining the accuracy of predictions based on laboratory-generated evidence at each level but also simultaneously determining the accuracy at different levels of biological organization while using different end points. Toxicologists capable of making these measurements in the laboratory can certainly learn how to do so in the field. These tests will be absolutely crucial in responding to Macek's (1982) description of the state of environmental toxicity: "there has been an incredible increase in data but virtually no increase in knowledge." This situation is improving, but not with the rapidity that anthropogenic environmental stresses are developing.

### **Biological Monitoring**

Biological monitoring (Cairns *et al.*, 1977, 1979, 1982; Wolfe, 1980; Stauffer and Hocutt, 1980; Pascoe and Edwards, 1984; Gruber and Diamond, 1988; Holl and Cairns, 2003; Breckenridge *et al.*, 2003) should, of course, always be accompanied by a chemical/physical monitoring, or a determination of the biological response will not be possible. Biological monitoring can serve two primary uses: (1) serving as early warning systems (*e.g.*, Cairns and van der Schalie, 1980) and (2) ensuring that previously established quality control conditions are being met (Helawell, 1978).

### **Early Warning Systems**

The problem of validating the signals generated by early warning systems is essential but has not received the attention it deserves. Basically, the number of false positives (an early warning signal that indicates harm when none has occurred) or false negatives (the early warning system that indicates acceptable conditions when they are not) must be reduced. The only way to accomplish this reduction is to validate both positive and negative signals from an early warning system in the natural system it is designed to protect. Monitoring systems are designed to provide evidence on a frequent or continuous basis that system conditions are as expected. Since the early warning system will be less effective if the time required to

generate information is too lengthy, time for information generation must be optimized. Again, the validation process is essential to proper management plans involving early warning systems.

### **Ensuring Quality Control Conditions Are Being Met**

The assumption in the use of monitoring systems for this purpose is that one can select a few key characteristics of the system identify malfunction in the entire system, preferably as rapidly as possible. Ideally, monitoring systems should provide evidence of malfunction before the malfunction occurs or, if that is impossible, before irretrievable damage is done. This assumption is the basis for designing monitoring systems for intensive care patients in hospitals, for monitoring condition of astronauts in space flight, and for determining human health in other crucial circumstances. Usually, early warning biological monitoring systems are installed before the material reaches the natural system, whereas the quality assurance monitoring units are placed in the natural systems themselves. Since all measurements available cannot be made, certain ones must necessarily be selected for a variety of reasons, including practicality, the capability of being measured by a large number of people, and, of course, most important of all, a high correspondence to characteristics crucial to the health and well being of the natural system.

### **Conclusion**

As Macek (1982) notes, the amount of data generated by environmental toxicologists is overwhelming. He is also correct in stating that knowledge has not greatly increased. Expanding the knowledge base is unlikely to happen by developing more single species tests, finding more sensitive species, or trying to develop application factors to cover the deficiencies of existing methodology. For knowledge about ecotoxicology to increase, the accuracy of the responses in present laboratory toxicological test systems must be quickly determined, along with how the events in natural systems correspond at not only the single species level of biological organization but higher levels as well. If the field of ecotoxicology is to be truly that—a toxicity recognition system for ecosystems—the effect of toxic substances upon ecosystems must be predicted using parameters, end points,

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or attributes at that level of biological organization. Extrapolations from single species response to ecosystem response are possible in practical terms, but better, more accurate alternatives are possible. Even if multispecies toxicity tests do not provide a better base for making such extrapolations, this possibility must be investigated. If the laboratory methods for ecotoxicology at various levels of biological organization (*e.g.*, single or multispecies toxicity tests) are inaccurate, then developing monitoring procedures for natural systems is even more crucial. Under these circumstances, ecologists must develop the predictive capability espoused by Harper (1982). The degree to which ecotoxicology remains primarily a laboratory-based profession, becomes primarily a field-based profession, or becomes a highly integrated combination of the two will depend on how satisfactorily predictions made with laboratory-generated evidence can be validated in natural systems.

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