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# Problems associated with selecting the most sensitive species for toxicity testing

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## Abstract

When the field of aquatic toxicity testing began its first major expansion about 40 years ago, it was uncommon to use more than one test species (usually a fish). Later, it became customary to use individual microorganisms (usually algae) and macroinvertebrates as well. Most attention was then given to the response of the most sensitive species *in that test series* when calculating the 'biologically safe' concentration acceptable for use in natural systems. However, in recent years, there has been an attempt to equate the most sensitive species in a laboratory test series to the most sensitive species in natural systems. Since laboratory test species represent only a tiny part of natural systems and since response variability is well established, that can be a dangerous assumption. The purpose of this discussion is to re-examine the scientific support for this practice.

## Introduction

The single species toxicity test has provided the great majority of data used in evaluating the hazard of waste materials and is an unsurpassed tool for studies of relative sensitivity of organisms, relative toxicity of chemicals or effluents, or effects on population level responses such as growth or reproduction. However, these tests are also widely used to derive limits of exposure to protect entire ecosystems, and this use of single species tests is more problematic. In all but the most sophisticated and expensive applications, the use of single species toxicity tests to protect ecosystems involves the search and testing of 'the most sensitive species.' If one finds this most sensitive species and sets standards for presence of toxic materials and other stressors in natural systems accordingly, presumably all other species and all other activities at higher levels of biological organization will be protected (e.g., Weis, 1985 commenting on an article by Kimball & Levin, 1985). However, there are a number of troublesome assumptions inherent in the most sensitive single species approach, although they are not always explicitly stated. This paper re-examines these assumptions and the scientific support for the 'most sensitive single species' approach.

## Assumption 1

*The responses of the species tested will have a correspondence with the responses of the most sensitive species in the much larger array of organisms that would be exposed in a natural system*

In a recent field investigation carried out primarily

by the Academy of Natural Sciences of Philadelphia with some assistance from Florida State University and Virginia Polytechnic Institute and State University, almost 1 000 species were identified from the Flint River and Lake Blackshear in Georgia. This number of taxa is certainly a minimum since the study involved $<10\%$ of the total drainage area of the main stem of the Flint River and covered a time span of $<2$ years, therefore not encompassing many successional processes. An estimate many times as high would not be unreasonable when incorporating these factors. However, even this minimum estimate of taxonomic richness in a natural system stands in interesting contrast to the number of organisms that can be held in the laboratory and used in aquatic toxicity tests. The very commonly used aquatic test organisms for single species toxicity tests probably do not exceed 20 for the contiguous United States. Add those less commonly used to the list, with the requirement that they be used by at least five investigators with reasonable regularity, and the list would probably not exceed 50 species. Given these numbers, the probability of actually testing the most sensitive species, not the most sensitive of the list of species commonly used in laboratory tests but, rather, the most sensitive species for the entire array of organisms in aquatic ecosystems in the contiguous United States, seems rather remote.

If what we mean by most sensitive species is that species that is most sensitive out of the limited array of commonly used test species (assuming for the moment that there is one species that is so for all chemicals in all test conditions, see Assumption 2), expectations of what test data mean must change considerably. Sound judgments in management and regulatory decisions will only be possible if one can extrapolate or predict, based on the results from the tested species, the responses of a much larger array of species and behaviors at different levels of biological organization.

Differences in sensitivity to a single chemical of two or three orders of magnitude are quite common among the most tested organisms. It is not unreasonable to expect similar or larger variability in the sensitivity of other untested organisms. Accounting for this variability has made it increasingly common to test an array of species in critical applications in order to observe the range of sensitivities, thus increasing costs considerably. But, given a large enough sampling of single species, it may be possible to make scientific inferences about the distribution of sensitivities of organisms in general and predictions about the proportion of species likely to be adversely affected by a discharge under conditions like those used in testing (e.g., Stephan *et al.*, 1985; Niederlehner *et al.*, 1986). However, this approach is undermined by the possibility that test species are not a random sample (e.g., Seegert *et al.*, 1985). They must be chosen, at least in part, for their convenience and adaptability, and this may bias selection toward hardier organisms. A possible counterbalancing bias may stem from the historical search for the most sensitive species. Work on more resistant species may have been abandoned as unproductive. A more daunting problem is that this approach may not necessarily protect behaviors or properties at levels of organization higher than the single species (see Assumption 3).

If one could find a single species that was consistently more sensitive than others tested, and thus avoid an array of tests, this species would most probably be at the extreme end of any tolerance distribution. It necessarily would be much more sensitive than the majority of species. Consequently, if data from this species were consistently used to make management decisions for systems in which it did not occur, there could be substantial overprotection.

To summarize, the ultimate difficulty with testing the most sensitive species is that we cannot do it because we do not know which species of the thousands possible is most sensitive, or, in all likelihood, how to test it. We can test an array of species, at substantially increased cost, and observe a range of sensitivities of tested organisms, then extrapolate to untested organisms, conditions, and levels of behavior. The distinction between the most sensitive species and the most sensitive tested species is important because extrapolation becomes necessary and must be scientifically justified.

**Assumption 2**

*A species shown to be most sensitive to the tested array of toxic substances will invariably be so for a much larger array of toxic substances and conditions*

This assumption is closely related to the first assumption. Even if we knew with certainty the most sensitive species for one chemical and set of circumstances, that information would not be transferable to another chemical or condition. Divergence would increase with the dissimilarity of the chemicals and circumstances. Relative sensitivity to chemical X for two or more species is unlikely to be exactly the same as the relative sensitivity to chemical Y. Therefore, the degree of sensitivity of a species designated most sensitive will probably vary enormously from test to test.

The sea lamprey in the Great Lakes is not usually thought of as a particularly sensitive species. Yet, after a search that involved testing literally thousands of different chemicals, one particular chemical was found (TFM) to which the lamprey was significantly more sensitive than a variety of 'desirable' and 'sensitive' species (e.g., rainbow trout). The hypothesis of the US Fish and Wildlife Service, which ultimately proved to be correct, was that a chemical existed to which the lamprey was significantly more sensitive than the other species inhabiting the same ecosystem. The difference in sensitivity was small, but it was real. Of course, not all of the thousands of species associated with the lamprey have been studied extensively, so some may be even more sensitive to TFM than the lamprey.

Other examples of reorderings in sensitivity can be found in compilations of conventional single species test data. Fathead minnows are two orders of magnitude more sensitive than daphnids when chronically exposed to nitroglycerine (Bentley *et al.*, 1976). But when the toxicant is the insecticide diflubenzuron, the daphnid is at least three orders of magnitude more sensitive than the fathead minnow (Hansen & Garton, 1982). These reorderings do not pose an insurmountable problem if one is using an array of evidence, but might result in significant errors in judgment if a single species designated as most sensitive is the sole source of information.

The degree of sensitivity demonstrated by the most sensitive species may also vary greatly depending on test conditions. In addition to variation with water characteristics (e.g., hardness, temperature, pH, etc.), sensitivity may be greater in a natural environment or a laboratory test situation with high environmental realism: ultraviolet light, harvesting pressure, competition for food, or disease. Conversely, a species that is extremely sensitive in the laboratory may be so only because its environmental requirements are not being fully met and stress of test conditions is interacting with toxicant stress. In a standardized test, where the goal is to produce equivalent data for comparisons of relative toxicity, these factors are less confounding than in applications to the prediction of safe concentrations for ecosystems.

So, as in Assumption 1, one cannot be assured of testing the most sensitive species because it is impossible to know with certainty which species that is for any given combination of chemicals and conditions. One can test an array and extrapolate with error.

**Assumption 3**

*There will be no significant responses at any level of biological organization that are more sensitive than the responses chosen and observed in the most sensitive single species toxicity test*

This is, perhaps, the most interesting assumption for the ecologist. Many researchers express concern that, while single species tests may adequately demonstrate effects on population growth or relative toxicity, they cannot demonstrate effects on important properties of ecosystems not found in the single species test (e.g., Odum, 1984; O'Neill & Waide, 1981; Kimball & Levin, 1985; Cairns, 1983). In addition, studies using more complex test systems to study effects of toxicants have demonstrated that there are emergent responses that can be more sensitive than the most commonly used end points in single species toxicity tests in some instances. For example, effects of PCBs on algae were apparent at concentrations two orders of magnitude lower when competition was examined rather than the growth of individual species (Mosser *et al.*, 1972). Cadmium exposures af-

fected the predator-prey behavior of largemouth bass and fathead minnow at concentrations half of the reproductive MATC for fathead minnow (Sullivan *et al.*, 1978). In pond studies with pentachlorophenol (Crossland & Wolff, 1985) and methyl parathion (Crossland, 1984), unanticipated algal kills depleted oxygen, subsequently killing trout. As these studies demonstrate, the assumption that there are no behaviors at higher levels of organization that are more sensitive than the most commonly measured responses of single species is demonstrably false. Whether this will necessarily remain so is open to debate.

The argument that emergent behaviors will inevitably be less sensitive to toxic stress than population responses is most frequently stated as the contention that any ecosystem response must originate in the response of its parts; therefore, by judiciously choosing the most sensitive and key end point or response, a single species test will protect the environment. This reductionist argument certainly has theoretical value. But it does not provide practical guidance, because, once again, it is not possible to know a priori which response is the key or most sensitive and must be measured in order to provide protection. The problem becomes a restatement of Assumption 1 and 2 on an even grander and more ambitious scale. Not only must we choose the most sensitive species for a unique combination of chemical and condition, but we must also choose the most important and sensitive end point to monitor out of myriad processes occurring in the natural system. Commonly used end points will not always be sufficient. For example, in their comprehensive study of copper contamination of a stream, Geckler *et al.* (1976) found that single species toxicity tests could generally predict mortality and reductions in reproductive success in fish populations, but a more significant factor in the observed field effects was avoidance. Clearly, a scientifically justifiable choice of end points may require extensive background research, or, if one is forced to use a large array of end points in most sensitive species toxicity tests, it will increase the cost substantially.

A more serious problem is that of how to translate the magnitude and significance of a change in a single species parameter into a concern for ecosystem health. Although inhibitions in collagen metabolism may presage fish growth impairments (e.g., Mayer *et al.*, 1977), which, in turn, may presage reductions in catchable fish and a degradation of an ecosystem resource, how much of a change in collagen synthesis should be cause for regulatory concern? Translations of magnitude and significance of effects are essential in order to judge the point at which regulatory mechanisms should be called into effect. These translations require explicit models and documentation of their performance, and this model formulation and documentation requires a considerable amount of scientific effort. Instead, we frequently rely on application factors and have little or no information about how well our predictive models perform.

Some problems of translation can be avoided by the use of micro- or mesocosm toxicity tests measuring the same characteristics that are the object of protective management. For example, if our management goal is to preserve species richness and no effects on species richness are observed in microcosm toxicity tests, this information can be applied with minimal translation. Taxonomic diversity and richness in response to toxic stress have been used as test end points (e.g., Niederlehner *et al.*, 1985; Tagatz, 1986). Productivity has been a focus of microcosm toxicity tests (e.g., Hedtke, 1984; Van Voris *et al.*, 1985). Community metabolism has also been monitored (Giddings & Franco, 1985; Sheehan *et al.*, 1986). However, there is concern that the results from these test systems are not generalizable and that advantages of micro- and mesocosm tests may be lost as attempts are made to standardize these tests for inclusion in hazard assessment protocols (e.g., Giesy, 1985).

So, although it is possible (but hotly disputed) that a single species test judiciously chosen for its sensitivity and the importance of its response might adequately protect the environment, it may also be the most difficult and indirect way to approach the problem of hazard management. The most sensitive species approach would properly require extensive background research to choose the appropriate end point and to construct models for translation of effects observed into ecosystem terms. By using micro- or mesocosm tests measuring the same characteris-

tics likely to be of concern in the environment, i.e., changes in diversity, metabolism, nutrient flow, one can avoid some of the problems of anticipating the single most sensitive link in a very large chain and translating the magnitude of that response into ecosystem terms, although translations over scale remain.

**Assumption 4**

*The savings resulting from using the most sensitive single species approach will outweigh any costs engendered by making inappropriate management decisions*

Management requires techniques that will enable the cost effective prediction with a known degree of accuracy of the concentration of a waste that will not harm an ecosystem. The degree of accuracy with which we can make predictions of hazard may be quite important because this accuracy determines the success with which we can avoid the costs of underprotection, i.e., damage to environmental resources, and those of overprotection, i.e., construction of treatment facilities that provide no biological benefit. Validation studies (Cairns, 1986a) are the most obvious way to determine if our predictions are meeting our expectations. They can identify the margins of error of our predictive tools and then a judgment can be made about the sufficiency of these tools in light of the costs of errors.

It is often argued that the most sensitive species approach has proven adequate and therefore should not be changed. Because of the wealth of experience with single species toxicity tests, one expects that this contention should be easy to document. But the amount of data documenting how well predictions from single species tests match environmental effects is limited. In their landmark study, Geckler *et al.* (1976) found that single species tests provided valuable indications of potential for mortality and reproductive impairments, but laboratory tests could not predict several significant events in field exposures: fish avoided exposure; variations in water quality changed toxicity considerably; predicted safe concentrations for some species were underestimated by a factor of two. More recently, Hansen & Garton (1982) found that an array of single species tests anticipated the concentrations of diflubenzuron affecting artificial stream communities within an order of magnitude. But these tests were unable to predict the nature of changes in the stream communities. Expected effects on benthos were not observed and algal community response was not anticipated. Mount *et al.* (1984) have shown significant correlations between daphnid reproductive success in ambient toxicity tests and benthic taxonomic richness in the field. But there was poor correspondence between daphnid and fish species responses. In comparisons of the magnitude of response in laboratory and field, differences ranged from 11 to 81%. Carlson *et al.* (1986) found that national water quality criteria derived from single species test data could be overprotective by one order of magnitude. It should be noted that few validation studies rely on a single most sensitive species toxicity test to make predictions of hazard. Instead, they use an array of data, a better and more expensive basis for prediction. Based on these and similar studies, it seems that an array of single species tests may indicate the general range of concentrations at which effects are likely, perhaps within an order of magnitude. However, the nature and magnitude of the response of more complex systems is unpredictable, thus making determinations of the acceptability of effects in the environment difficult. This level of predictive accuracy may or may not be judged sufficient for management purposes.

A corrollary argument has been that other types of tests, e.g., micro- and mesocosm toxicity tests, are indecisive and prohibitively expensive and, therefore, are not practical alternatives to the sensitive single species approach (e.g., Mount, 1985). Reports indicate that many more complex tests are no more expensive than traditional tests, with costs ranging from $6,000 to $29,000 for microcosm tests and $7,500 to $29,000 for conventional tests (e.g., Van Voris *et al.*, 1985; Perez & Morrison, 1985; Niederlehner *et al.*, 1986; Taub *et al.*, 1986; Sheehan *et al.*, 1986). Micro- or mesocosm tests may be more efficient and less expensive ways of determining effects on some characteristics because they simultaneously expose many organisms (e.g., Niederlehner *et al.*,

1986). Decisiveness, or the ability to specify a safe concentration, also seems to be no more troublesome, depending, as always, on statistical determinations of difference or dose-response curves and subjective decisions about what is and is not an acceptable consequence of toxic stress (e.g., Cairns, 1986b; Giddings, 1986; Niederlehner *et al.*, 1986). As previously discussed, microcosm tests may have an advantage in decisiveness because they directly observe responses that are articulated concerns of protective management. Validation of predictions from micro- and mesocosm tests is proceeding. Giddings & Franco (1985) found good correspondence between effects in laboratory littoral microcosms and experimental ponds treated with coal-derived crude oil. Acceptable levels in both systems corresponded to a dosing rate of ca. 1 uL/L/day. Portier (1985) found high correlations between measures of microbial community structure and function and toxicant half-life in laboratory microcosms and in the field. Niederlehner *et al.* (1986) found an estimate of a safe concentration of cadmium predicted from microbial community toxicity tests fell within a rational range, i.e., between concentrations reported for healthy and damaged natural systems. Livingston *et al.* (1985) calibrated laboratory estuarine microcosms with field responses and concluded that extrapolation from one system to another is possible within the range of uncertainty that is characteristic of natural systems. Despite the short time they have been in use, micro- and mesocosm toxicity tests are establishing their value in some determinations of hazard. There are legitimate concerns about the most appropriate way to incorporate this information into hazard assessment schemes, but, as work continues, these questions should be resolved.

The efficiency of our tools for hazard assessment is important to the success of the process. However, we have come to recognize that no test can stand alone in evaluations of hazard. If we rely on a most sensitive species toxicity test, testing costs would be minimal, but margins of error for predictions would be too wide, often engendering costs associated with under- or overprotection. Arrays of data improve our predictions and micro- and mesocosm test systems can be included without undue expense or loss of decisiveness. Uncertainty about the nature and magnitude of the response of natural systems may be reduced by use of such tests, thus improving the predictive capabilities of our assessments.

## Summary

Properly used single species toxicity tests are now and will continue to be the backbone of our hazard evaluation efforts. However, we are disturbed when people use simple laboratory tests of short duration to infer a degree of protection to the environment that is not yet warranted by the evidence at hand. If we are to make sound regulatory and management decisions on environmental protection, it is important that we know the responses of complex systems to a variety of concentrations of chemicals and other stresses along a gradient. This is a scientific task. Having established this gradient, we will then make a societal decision on the desired level of protections (i.e., end points or characteristics that we wish to be entirely unaffected). Finally, we may add some safety factors to allow for uncertainties about the operation of the waste treatment system, the fate and transformation of the chemical in natural systems, and inaccuracies in estimating the response of complex systems from laboratory tests, in much the same way safety factors are used for elevators, bridges, and automobiles. We may even wish to install a biological monitoring system accompanied by selected chemical and physical monitoring to verify that our assumption of protection for selected characteristics is sound. The most sensitive species approach attempts to bypass or ignore this complex array of decisions and information and, although it initially has great appeal, on examination, it may be one of the most expensive approaches possible even though the costs are not incurred in carrying out the test itself. Arrays of data improve our predictions. And, although multispecies toxicity tests, including micro- and mesocosms, are not now commonly used, they provide information that may clarify the nature, magnitude, and significance of toxicant effects. Prudently used and interpreted as part of an array of data, this additional evidence should further enhance our ability to protect natural systems from the wastes and products of an advanced tech-

nological society. In sum, to retreat to the single most sensitive species toxicity test is to ignore all the knowledge of the complexity of ecosystems that has been painfully accumulated over the last 30 or more years.

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