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Multispecies Tests: Research Needs to Assess the Effects of Chemicals on Aquatic Life

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ABSTRACT: Aquatic multispecies tests are an extremely flexible and powerful tool in aquatic ecology, especially in the study of the fates and effects of xenobiotics, but are not amenable to the type of protocol development and precision required to be part of a formal hazard assessment scheme. Such methods are completely antithetical to the most efficient use of multispecies tests, which is the ability to design a system to elucidate particular mechanisms and interactions in specific ecosystems. One goal of hazard assessment schemes is to remove the subjectivity of individual researchers from the process. Multispecies tests will not achieve this goal. Instead, aquatic multispecies tests of all sizes and configurations should be used by researchers to answer questions posed about the behavior and effects of xenobiotics in aquatic environments. Therefore, rather than designing experiments to test whether multispecies toxicity tests are accurate or replicable they should be used to elucidate mechanisms and make tests of relevant hypotheses within the overall framework of aquatic ecotoxicology. Because of the diversity of interactions among organisms and xenobiotics and the environments on which they exist, one will never be able to absolutely establish the accuracy of multispecies tests. Simply because multispecies tests are more complex does not mean that they are more realistic or have more predictive power than single-species tests. In fact, they may be more prone to artifacts. There is no evidence to suggest that multispecies toxicity tests, as a class, are more sensitive than the more traditional method of establishing criteria from final chronic values, based on single-species tests. More research on multispecies replicability is not necessary. Replicability is a statistical problem rather than an intrinsic property, and appropriate tests and designs are available to determine the relative variability, and therefore the sensitivity of laboratory-scale multispecies test, and they allow the appropriate number of stratified replications to be determined to make the test sufficiently replicable to make precise conclusions. Multispecies tests may be more efficient than conducting several single-species tests because of a greater range of sensitivities of test organisms, however, this has not been established. The most useful types of multispecies toxicity tests will be in-situ-type systems where some replication can be accomplished in a more realistic system

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or multispecies tests of mesocosm-scale. Further research is needed in the area of validation studies of in-situ systems.

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The legislative and litigative processes have placed a premium on precision in testing procedures, which has resulted in the use of acute toxicity tests. Furthermore, the strategy of protecting target species or monitoring indicator species has been implemented because of the relative ease with which single species can be tested in the laboratory and single populations can be monitored in the environment. This does not necessarily mean that single species tests have good predictability for the effects that toxic substances will have in an aquatic ecosystem. Rather the standard LC_{50} is more a comparative tool than a predictive one. For predictive purposes, one must consider all of the biotic and abiotic accessory factors that will mediate responses of organisms in a complex ecosystem, as well as the dose intensity and dose duration. However, statistical relationships between acute and chronic effects and the effects of assessory factors have been established. Kenaga [*1*] states than "an important need for evaluation of toxicity is a method of prediction of chronic effects based on as little data as are needed for accuracy and usefulness." The question to be discussed here is not whether these simple tests are accurate, whether they are adequate and sufficient, or whether they should be replaced by multispecies tests. I will address the question of whether a standardized multispecies testing protocol would provide useful additional information, relative to the increased cost and potential for subjective interpretation. A number of reviews and treatises have been published on multispecies tests, and their use in ecological and hazard assessment studies has been discussed [*2-40*]. In this article I argue that a standardized multispecies toxicity testing scheme for aquatic systems is neither required nor would it be useful as an additional test. However, laboratory-scale multispecies tests may be more efficient. In some testing schemes a number of algae, bacteria, protozoans, and crustaceans of varying sensitivity can be tested simultaneously, thus reducing the level of effort to expose a large number of these species separately.

Multispecies Test Rationale

It has been suggested that information on responses to toxic substances should be gathered on ecologically important "keystone" species which perform a function critical to maintaining ecosystem integrity [*17*]. In fact, knowledge of the species that occur in a given aquatic system conveys a great amount of information about the abiotic structure of the ecosystem as well as the biotic interactions [*18*]. An underlying assumption of management strategies to protect "target" or "keystone" species by single species testing is that

"keystone" species are the most sensitive species or that limits on allowable concentrations of toxic substances somehow preserve enough of the other biological components of the biotic community to maintain a viable environment for the target species [*17*]. Cairns et al [*17*] state that a complex community with many interlocking reciprocal cause-effect linkages cannot be preserved by protecting one or a few target species and that monitoring the effects of stressors should include more important aspects of ecosystem structure and function. The realization that ecosystems are complex, interacting systems has resulted in the argument that single species tests are too simplistic to be useful predictive tools to protect aquatic ecosystems from the potential adverse effects of xenobiotics [*20*]. Certainly this is true, however, one must address the question, Do standardized multispecies tests offer improvements such as greater predictive power, greater sensitivity, a more rapid test, a more efficient test or a more cost-effective test?

Individual ecosystems are unique but do have some common features. To have perfect predictability about the response of an ecosystem to a given intensity and duration of exposure to a toxic substance, one would literally need to know all of the information embodied in the ecosystem [*18,19*]. This is impossible so the salient question is, "Do multispecies tests allow the study of specific, important ecosystem-level interactions?"

The major reason for using single-species tests is that they are reproducible. Few aquatic toxicologists would predict the effect on a population, let alone a community or ecosystem from acute, single-species toxicity data. Single-species toxicity tests are not designed to measure important interactions or compensatory reactions and eliminate many of the important interactions between a population of organisms and its environment.

Multispecies tests have the positive attribute that they are physically real, unlike simulation models, but do they more accurately represent the effect of xenobiotics in the real world? Certainly some multispecies tests can more accurately represent the real world than single-species tests. The boundaries on some multispecies tests are so large that they accurately represent the dynamics of the larger ecosystem. However, the tests are so large that they often do not offer advantages such as replicability and reduced expense over ecosystem-level tests. The question of realism of multispecies tests is critical in evaluating their utility in hazard assessment schemes. Some authors argue that multispecies tests need not exactly mimic natural systems at all levels of organization but for the study of general ecological principles or gross toxicological effects can be thought of as analogous. An underlying assumption of all multispecies tests is that the experimental system represents either a single function or a complex set of functions expressed by larger uncontrollable field situations regardless of simplifications. Thus, all simplifications made must be evaluated to determine the validity of the results obtained.

Cairns [*41*] argued that basic ecological properties, such as nutrient spiraling, mineralization rates, energy flow, and successional processes, must be

studied in toxicity tests and went on to suggest that laboratory systems can be developed for such a purpose. The "functional integrity" school of researchers maintains that laboratory multispecies tests are useful analytical tools because they exhibit community- and ecosystem-level processes, even though the magnitude and rate of these processes may not exactly mimic any naturally occurring system, to which one would like to extrapolate. Also, from a structural point of view it can be argued that the same species need not be included in a multispecies test as in a natural system, if the functional integrity is approximated. This approach will not allow a very great amount of predictability from multispecies tests to specific aquatic ecosystems. Multispecies tests will not be more effective predictive tools than single-species tests in hazard evaluation schemes. Identifiable ecosystem-level properties are the results of interactions among individual organisms and populations of organisms, and effects of xenobiotics are the result of effects on individuals in populations with different sensitivities to the toxicant of interest. More specifically, the effects are due to interactions with biochemical receptors. This is important in interpreting the results of multispecies tests because the results obtained are as much a function of the community structure as the effect of the toxicant. Just as the structure or species assemblage and relative densities determine the responses of toxicants elicited in diverse ecotypes, these same parameters will determine the response of multispecies tests.

Furthermore, the abiotic structures, such as inorganic and organic constituents of the water and sediment, determine ecosystem-level properties as well. Also, the source and relative densities of recolonizing populations and natural rates of increase will affect the dynamics of populations exposed to xenobiotics. For instance, population densities, as well as diversity of stream benthic organisms, are greatly controlled by colonization from upstream areas, caused by drift. Thus, artificial bounding, which restricts this type of variability, will drastically influence the results and interpretations of multispecies tests with natural assemblages. When it is suspected that these events are important in controlling the structure of or regulating the function of an ecosystem, multispecies tests will have low predictability of the effect of xenobiotics in that system.

The greatest value of multispecies tests is that they enable the study of functioning ecosystems with cybernetic or negative feedback loops in place. If the structure of the microcosm is too specific or the scale of the system too small generality is lost, and if it is too general, predictability is lost. Therefore, creating a generalized multispecies test of known complexity for hazard evaluation is impossible. Two approaches could be taken: (1) a site-specific multispecies test could be used to assess localized effects on a very specific area or (2) a more generalized system with a number of components and pathways general to all ecosystems could be used. In the second approach, two levels of complexity can be designed. In the more simple system all interactions are well defined. A more complex system can be used where mechanisms are

poorly defined but overall community effects are measured. The simpler system is the gnotobiotic or completely defined system and is represented by the "Taub"-type system. The more complex system is the undescribed assemblage system such as that described by Carins et al [*42*]. The degree of replicability required in standardized toxicity testing would require the use of gnotobiotic systems, which would not allow for the use of endemic species assemblage.

Multispecies tests will increase the magnitude of intralaboratory variability and thus it will become more difficult to interpret these results. Presently, the results of single-species tests can vary by an order of magnitude or more in some species, depending on age and nutritional state. Aquatic toxicologists have gone to great lengths to standardize these tests. Anyone who has conducted a "simple" *Daphnia* bioassay knows that these tests are anything but simple. Therefore, are the results to be gained worth the added complexity? My experience has been that they are not. The increased predictability is not sufficient to warrant the increased complexity of multispecies tests.

One other limitation of multispecies toxicity tests is the sampling and enumeration of many different species. This is particularly true of the algae and protozoans. Therefore, for naturally derived, nongnotobiotic systems standardization will be extremely difficult. To address this concern, Cairns has developed an automated system to scan and identity diatom cells and a rapid visual identification system for protozoans [*43*]; however, these techniques will not overcome the problem of identification.

Single Species-Multispecies Comparisons

Because of the diversity in toxicants and potential single- and multispecies tests, it would be extremely difficult to make a critical test of the null hypotheses that there is no difference in (1) sensitivity or (2) cost efficiency between a particular multispecies test and a set of single species tests. The best mechanism for such a test would be to compare the maximum acceptable toxicant concentration (MATC), as determined by both the multispecies and the single-species test.

A number of multispecies tests have been conducted to examine the effects of toxicants; however, there are relatively few instances where toxicity information from multispecies tests can be directly compared to that from single-species tests. The greatest amount of information exists for the metals cadmium and copper (Cd and Cu). Several multispecies tests have been conducted to determine the effects of cadmium on aquatic communities [*11, 36, 43–48*], and many single-species studies of the effects of cadmium on aquatic organisms have been conducted [*49*]. Taub and coworkers have used gnotobiotic multispecies tests in investigating the effects of mercury, cadmium, toxaphene, dichlorodiphenyltrichloroethane (DDT), and polychlorinated biphenyl (PCB) [*47, 48*], however, these studies were not designed to

compare the sensitivity or efficiency of single- and multispecies tests. Unfortunately only two multispecies tests were designed such that either a MATC or no observable effect level (NOEL) could be determined [*43,50*]. The other multispecies tests were designated to examine different effects such that the absolute sensitivity of the test system to toxicants could not be determined.

The site-specific criteria for cadmium at a total hardness of 70-mg calcium carbonate/L, as determined from acute and chronic single-species toxicity tests, are 0.017-μg cadmium/L for a 24-h average and 2.077-mg cadmium/L for the maximum value, which cannot be exceeded at any time [*49*].

In the multispecies test, based on protozoan colonization [*43*], the NOEL ranged from 0.8-μg cadmium/L to greater than 9.5-μg cadmium/L. The authors indicate that this variability is due to increased stress tolerance in more mature communities. Niederlehner et al [*43*] compared the results of their study to single-species tests. Unfortunately none of the multispecies tests were conducted in such a way that an MATC value could be obtained. Niederlehner et al [*43*] concluded that their multispecies study "compared favorably to single-species toxicity tests in time and cost." Niederlehner et al [*43*] also point out that the protozoan multispecies test "had the advantages of using indigenous species and incorporating emergent properties of communities such as predation competition and succession." Even though more variable, the range of NOELs for the multispecies test was similar to the criterion determined from single-species tests.

Hedtke [*50*] studied copper toxicity in a sediment-water microcosm, which included periphyton, macroinvertebrates, and macrophytes. That study investigated population- and ecosystem-level effects at a sufficient range of concentrations to establish a NOEL. The results of the microcosms study were compared to the U.S. Environmental Protection Agency (EPA) hardness-related criterion. At a hardness comparable to the multispecies test the recommended 30-day average concentration is 20 μg/L, the previously recommended 24-h average concentration was not to exceed 5.6 μg/L [*51*]. Effects of copper were observed in the microcosms at 9.3 μg/L but not at 4.0 μg/L. Therefore, the NOEL was approximately 6.1 (geometric mean), which is in good agreement with that established in the earlier guidelines [*51*]. This indicates that at least for copper the single- and multispecies tests had similar sensitivities.

Maki [*52,53*] compared the effects of 3-trifluromethyl-4-nitrophenol (TFM) in single-species, multispecies, and in-situ tests and concluded that "laboratory toxicity data from studies of the acute effects of TFM in single-species tests can be used to predict community-level effects in laboratory and natural streams."

After reviewing the available literature where single-species and multispecies test results can be compared, I agree with the conclusions of Smies [*54*] that: "Available experimental data do not indicate that microecosystems are superior to classical tests for environmental risk assessment." Smies [*54*] suggested that a standardized microecosystem test should not be included in regular testing procedures.

Multispecies Laboratory Tests-Field Comparisons

The results of laboratory-scale single- or multispecies tests have seldom been compared to field-scale tests. Giddings et al [*55*] studied the effects of a coal-derived oil in aquariums and ponds and found that similar results were obtained in both experiments and the mechanisms for the observed ecosystem-level effects. Predictions of laboratory-derived acute toxicity tests have also been found to be consistent with observations of results under field conditions for copper [*56*]. The results of the chronic effects of copper, as determined by laboratory tests, were not accurate and underestimated the impact of similar copper concentrations on the stream [*56,57*]. The chronic effects of pentachlorophenol observed in laboratory multispecies tests were found to be similar to those observed in outdoor ponds dosed with similar concentrations of PCB [*58*].

Maki [*53*] compared the effects of the lampricide, 3-trifluromethyl-4-nitrophenol (TFM), in a laboratory-scale mircocosm test to a natural stream dosed at the same rate. The conclusions of his study, which investigated the periphyton and macroinvertebrate communities, were that the laboratory-scale multispecies test accurately modeled the natural system.

Conclusions

I understand the desire for standardization of tests by regulatory agencies and industries. However, standardization of protocols will most likely create more artifacts than are presently inherent in single-species tests. If aquatic toxicologists are unable to define all of the parameters that contribute to the variability of standard single-species tests it will be especially difficult to do so for multispecies tests.

If multispecies tests are demonstrated to be more representative of the real world, the effort will be merited. However, this has not yet been established. Until this has been done, I do not feel that a single multispecies test should be adopted and a standardized protocol developed. I have argued against the standardization of multispecies toxicity tests because I think this effort is counterproductive to the effective use of multispecies tests. That does not mean that I feel these types of tests are not useful. It simply means that I feel that they are difficult to interpret and not amenable to standardization; without standardization the tests will not be useful for regulatory screening tests.

In other papers, I have described observations of multispecies tests that could not have been easily predicted from single-species tests conducted under laboratory conditions. However, these results were as much caused by increased size and physical complexity and abiotic realism as they were by

using multispecies simultaneously [*35,59*]. An area of multispecies testing that has merit is that of in-situ multispecies tests. The realism and control of unexplained variability for these systems make them extremely useful research tools.

Multispecies tests can be best used when they are designed to isolate particular mechanisms or species for research in the context of some of their biotic and abiotic interactions. The use of mesocosm-size multispecies systems will allow inclusion of more realistic scaling by including ecologically relevant chemical and physical parameters and enough species to allow the study of a self-sustaining ecosystem [*60*].

A review of the literature has revealed no evidence that multispecies tests are more or less sensitive than a battery of single-species tests. Additional comparisons of the type conducted by Niederlehner et al [*43*] are needed for a number of toxicants so that the relative sensitivity of multispecies tests relative to single-species tests can be established. These toxicants should include both organic and inorganic toxicants with a range of accumulation potentials, modes of toxic action, and persistence.

Recommendations

1. Multispecies toxicity tests, while more complex than single-species tests and displaying ecosystem-level processes, will not necessarily provide more accurate estimates of the fates and effects of xenobiotics in aquatic systems.
2. Few studies have been conducted to compare relative sensitivities and efficiences of single- and multispecies toxicity tests. Studies determining the relative sensitivities, cost, and efficiencies of these two types of tests should be conducted so that an objective evaluation can be made. While a comprehensive test of the hypotheses cannot be made, I suggest that several such tests be made with a variety of toxicants, which will have been selected to represent a wide variety of modes of action.
3. Multispecies tests are most useful when designed to isolate a particular mechanism or ecosystem component, therefore, a standard multispecies protocol will have limited utility.
4. In-situ multispecies tests can be very useful, mostly because of their more ecologically relevant scale and chemical-physical parameters. Additional research in the area of validation of these systems is needed.
5. Because of the diversity of interactions among organisms, their environment and xenobiotics, no single protocol for multispecies testing can be deemed to be most appropriate for all situations. Therefore, no amount of research will allow the adequacy or sufficiency of these systems to be absolutely established. Instead, multispecies tests should be used as flexible tools, which can be designed to investigate specific mechanisms as part of an inductive-deductive research scheme.
6. Multispecies toxicity tests should not be required as an additional requirement for compound registration or effluent permitting until the relative sensitivity and efficiency of the two types of tests have been established.

7. Multispecies toxicity tests will not necessarily increase the predictive capabilities but may greatly increase the subjectivity, difficulty, and cost of laboratory screening tests. A large body of literature is available for single-species tests so that acute to chronic ratios and ranges of sensitivities among organisms can be calculated. Also, a body of literature on structure-activity relationships is being developed for single-species tests. These relationships do not exist for multispecies tests. Because the results of multispecies tests will not be directly comparable to single-species tests it will be difficult to develop the required body of literature such that multispecies tests can be integrated into the existing hazard assessment schemes.
8. More research into the replicability of multispecies tests is not warranted. The determination of adequate precision for a given application is subjective and basically a statistical problem. I have reviewed this topic extensively elsewhere [*61*].
9. The scale of laboratory-scale multispecies tests is such that they cannot readily include large organisms such as fish or even macroinvertebrates. For this reason, it would be necessary to conduct a single-species test for these organisms in addition to the multispecies test. Therefore, the most appropriate multispecies test will most likely be in the size range of those described as mesocosms [*60*].

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