Methods for Estimating Risk of Chemical Injury: Human and Non-human Biota and Ecosystems
Edited by V. B. Vouk, G. C. Butler, D. G. Hoel and D. B. Peakall
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# *4 Approaches to Measuring Chemical Injury in Non-human Biota and Ecosystems**

## 4.1 INTRODUCTION

Considering that there are about 0.4 million identified plant species and some 1.2 million known animal species, and that thousands of man-made chemicals are being released or are likely to be released to the environment, the interactions between species and of species and environmental chemicals are often very complex. This gives ecotoxicologists a task of enormous magnitude and intricacy. Only careful selection of chemicals, species and tests will allow the ecotoxicologists to focus on conditions in which chemicals are likely to cause serious ecological damage.

In ecotoxicology the acceptable risks vary greatly. Domestic animals and endangered species are considered at the individual level. The degree of damage acceptable for slow breeding warm blooded animals is much lower than for insects with high reproduction potential. For slow breeding species, the effects on small groups or minor populations are the major concerns; for species with high reproductive potential only effects on communities are likely to be significant. The extent of the loss is also important; for example, mortality of a few fish at the point of discharge of a chemical may be acceptable, whereas widespread mortality in a river is not.

Under natural conditions, animal and plant populations fluctuate, sometimes drastically, but within a limited range. An array of time-dependent changes represents a natural phenomenon in all ecosystems. Ecological damage, as the term is used in this report, means adverse changes in ecosystems frequently reflected in reductions in size of populations to levels below their normal lower limits. In some cases it is more convenient to substantiate ecological damage by observing rates of ecosystem processes, such as fluxes of energy and of essential nutrients, rather than by monitoring the size of populations.

* Prepared by D. B. Peakall (Chairman), D. Calamari, H. Croze, J. W. Huckabee, T. T. Kozlowski, V. Landa, L. Moustafa, P. Sheehan, R. K. Tucker, and M. Waldichuk.

## 4.2 STEPWISE APPROACH TO TESTING

The tools available for appropriate assessment and prediction of adverse effects in ecosystems are limited in number and adequacy. In testing for effects of environmental chemicals, a stepwise approach may provide a conceptual framework for a practical organization of testing. In consecutive steps, the complexity of tests generally increases with the level of ecological organization being studied. The stepwise procedure might have the following structure and include the following types of tests.

*Level 1* encompasses, generally, short-term tests as well as some growth and reproductive studies on microorganisms and microinvertebrates.

*Level 2* includes a limited search for sensitive species and possibly for ecosystem properties and/or processes, such as photosynthesis, likely to be affected by exposure to chemicals. At this level, biochemical mechanisms of action are also studied.

*Level 3* incorporates tests for evidence of cumulative effects.

*Level 4* includes multispecies and microcosm tests examining effects that depend on interactions among populations. Multispecies systems involve interactions of populations, such as predation and competition, as well as ecosystem processes like productivity and nutrient cycling.

The final level (*level 5*) would encompass large-scale field and ecosystem testing to examine changes in structure and functioning of the ecosystem as a whole.

A promising approach to the integration of ecotoxicological data is the use of mathematical models to describe and simulate ecological reactions to chemicals.

Practical considerations generally dictate that all potentially toxic chemicals be tested at the lowest tier. Additional testing a higher levels is advisable if key effects are observed. The intent of the stepwise approach is to minimize the use of available funds and labour while providing a logical set of testing procedures. In this report, testing methods will be reviewed within this conceptual framework.

## 4.3 SINGLE SPECIES TESTS

### 4.3.1 Basic Tests

Level 1 testing is represented by the Organisation for Economic Cooperation and Development minimum premarket tests (OECD, 1979, 1980) or the base-set of tests of the Commission of the European Communities (CEC, 1979). The OECD premarket testing system comprises the 96-hour algal growth test, the 24-hour immobilization test, acute and 14-day oral toxicity tests, reproduction test on *Daphnia* spp. and the 96-hour acute toxicity ($LC_{50}$) test for fish. In addition, tests on rats [acute ($LD_{50}$) and 14- to 28-day repeated dose oral toxicity tests], mutagenicity tests and tests for various physicochemical properties are available. While these tests are generally acute toxicity tests, the algal and *Daphnia* tests include several cell divisions and a nearly complete life cycle, respectively.

Primary producers, in particular planktonic algae, are the basis of food chains in aquatic ecosystems. Algae can be cultured quickly in large quantities in pure culture; clones of uniform genetic composition can be easily maintained and algal assay methods are relatively inexpensive. In assessing the impact of a chemical on an aquatic system, algal assays can provide information about the availability of chemicals for uptake and their stimulatory or inhibitory effects.

In order to assess the hazard of chemicals to the structure and function of the autotrophic component of aquatic ecosystems, the effects of chemicals can be measured in both monoculture and mixed populations. Field and controlled ecosystem studies can also be carried out.

In algal batch assays, there is a time-dependent relationship between exposure and effect, the effect consisting mainly of reduction of growth in terms of both maximum yield and growth rate. Since many individual organisms are involved, this assay may be considered as a population assay. Several generations are exposed for a short period and the rate of cell division is observed. Algal batch assays have been widely used in eutrophication studies and the data obtained have been useful for the overall comprehension of phytoplankton reaction to certain chemicals. The method continues to be employed for identification of available growth limiting nutrients and for evaluation of trophic conditions in order to estimate the permissible load of nutrients (see Calamari *et al.*, this volume).

Algal assays are increasingly used for assessing toxic effects of chemicals in aquatic environments because such assays can generate quantitative data on factors that can influence primary production. Because they are simple, flexible and reliable, algal batch tests are well-adapted for standardized assays. However, there are inherent pitfalls in interpretation, if it is assumed that the results of *in vitro* culture studies can explain the dynamics of phenomena taking place in natural aquatic ecosystems.

Short-term toxicity tests in batch cultures can be carried out by measuring $^{14}C$ uptake during a 3- to 4-hour incubation immediately following addition of the chemical to be tested. Advantages of such tests are reduction in time spent, sensitivity and easy testing of volatile, unstable or reactive chemicals.

Unlike batch cultures, continuous cultures provide a constant environment once they reach steady-state conditions. Continuous cultures eliminate the variation with time found in batch cultures, and better represent the relationship between a constant environment and the effects of chemicals on growth rate over a long period of time. So far, the use of continuous cultures in toxicity studies has been limited, although it has been widely applied in nutrient uptake studies.

The OECD *Daphnia* spp. test comprises two parts: determination of 24-hour $EC_{50}$ (acute immobilization), and a 14-day reproduction test, which gives $LC_{50}$ values at intervals up to 14 days. Information is obtained on time required for the emergence of first brood, as well as on number of eggs and live and dead young.

Concentration–response curves can be prepared. The slope of the time–response curve can give information on the rate and degree of absorption, detoxication and elimination of the chemical. The ratio of the 14-day to 24-hour $LC_{50}$ values provides an indicator of chronic effects (OECD, 1983).

The concentration–response relationship for aquatic organisms exposed to a chemical is usually curvilinear. The response threshold is represented by the point on the concentration axis below which the existing measurement techniques cannot measure the response. This point is sometimes referred to as the 'no-effect' concentration or more appropriately 'no-observed-effect' concentration. The medial lethal concentration, $LC_{50}$, is a standard measure of toxicity of a chemical and occurs along the concentration–response curve beyond the 'no-observed-effect' point. The region in between is regarded as the range of sublethal concentrations of the chemical. Delayed effects of chemicals on aquatic organisms must also be taken into account. For example, for certain organisms exposed to cadmium and some organochlorine compounds there is a latency period before death occurs. In order to detect delayed response, it is useful to continue the exposure for an extended period beyond that normally used in a standard short-term assay.

### 4.3.2 Additional Species and Ecosystem Process Tests

If there is concern either about ecotoxicological data or about physicochemical properties of chemicals (for example, indicating potential for bioaccumulation or resistance to biodegradation) or if the volume of the chemical is large and its use pattern suggests escape to the environment, further testing (level 2) is necessary. The type of systems to be tested will depend on the sensitivity found in the first round of tests and on exposure analysis which would point to sections of the ecosystem most likely to be exposed to the chemical.

Responses of terrestrial plants to chemicals will vary widely depending on species, clones, ecotypes and cultivars; growth patterns and developmental stages; environmental regimes; plant vigour; and species competition (see Kozlowski, this volume).

Methods chosen should recognize that chemicals sequentially influence plants at the cell, organism, species, community and ecosystem level. Because of lack of suitable control plots in the field it is often desirable to first screen effects of chemicals in controlled-environment chambers (phytotrons). In the field, exposure chambers can often be used advantageously to create suitable control plots. Such chambers are advisable for non-woody crop plants and other small plants common to grasslands, tundra, bogs and shrub-dominated communities.

Among the most useful measurements in plants are changes in pigments and rates of photosynthesis. However, alteration of rates of photosynthesis is not always a good measure of eventual biomass change because growth potential is influenced by the seasonal pattern of photosynthesis, partitioning of photosynthate within the plant and the relation of photosynthesis to respiration (Kramer and Kozlowski, 1979).

One of the most useful indices of the impact of chemicals is a change in the growth of ecosystem components. This information can be obtained by comparing the growth of plants in the presence and absence of the chemical. It should be recognized, however, that net changes in biomass are affected by weight losses due to respiration, insect injury, diseases, normal shedding and death of plant parts and harvesting.

Efforts should be made to transfer growth and yield data to a form usable for economic assessment or ecological evaluation (Heck *et al.*, 1979). For most plants the greatest interest is in above-ground parts. However, for root crops the emphasis often is on root growth (Kramer and Kozlowski, 1979).

For small plants (annual plants, crop plants, old fields, grasslands, tundra, bogs, and shrub-dominated communities), sequential harvests should be taken on sample plants (or plants parts) and dry-weight increment determined.

Insects, both as regards the number of species and the number of individuals, represent a major component of the fauna. Insects are adapted to special ecological conditions by their morphology, physiological functions and behaviour. They react in a very sensitive way to specific changes in the environment at the level of individuals, populations and communities. In connection with pesticide production, methods have been developed for testing chemicals both in the laboratory and under field conditions.

The most widely used test involving a terrestrial vertebrate is the rat $LD_{50}$. The data base obtained from rat $LD_{50}$ values has been used to predict lethality in other species. For example, $LD_{50}$ values for the rat and two avian species (starling and red-winged blackbird) were compared by Schafer (1972). For 61 different pesticides and other chemicals both avian species were, on the average, more sensitive than the rat, the starling being roughly five times and the red-winged blackbird ten times more sensitive. Only a small percentage of differences of $LD_{50}$ values, however, fall outside two orders of magnitude. More details on the scope and limitations of this type of extrapolation are considered elsewhere (Peakall and Tucker, this volume).

The comparison of oral $LD_{50}$ and 5-day dietary $LC_{50}$ values (Kenaga, 1974) for five compounds are poorly correlated because of the effects that can be caused by compounds that are readily absorbed and rapidly metabolized, compared to those that are poorly absorbed and persistent. Somewhat better correlations were found using a larger number of compounds (Heath *et al.*, 1972).

Despite the weaknesses of the $LD_{50}$ or $LC_{50}$ tests (see Dobson, this volume), the acute lethality tests give a reasonable first approximation of the number of deaths that can be expected at a given exposure to a chemical, i.e., in spill situations or if deliberately used, for example, as a pesticide. The predictive value of acute lethality tests for effects on populations or ecosystems is likely to be very low. Unless $LD_{50}$ or $LC_{50}$ values correlate well with life-cycle tests or some more limited tests that include reproduction, then acute lethality is unlikely to predict chronic effects. Although a large body of data does not exist at present, it seems unlikely that any strong correlation will be found.

If there is sufficient evidence for ecological concern from data on sensitive species or processes and if the chemical is persistent, thereby maintaining appreciable concentrations in the environment, or is expected to be available through continuous releases, tests for chronic toxicity are recommended.

### 4.3.3 Tests for Reproductive Capacity and Other Long-term Tests

Complex protocols exist for tests for reproductive capacity of both mammals and birds (level 3 testing). The difficulty is in knowing when to use these complex tests. Even with these tests, several environmental problems of the past would have missed using commonly available laboratory animals, for example, the effect of PCBs on mink reproduction (this species is much more sensitive than the rat, Aulerich and Ringer, 1977) and on fish-eating birds (behavioural response that would not show up in captivity, Fox *et al.*, 1978) or DDE-induced eggshell thinning (most commonly used test species are not sensitive, although some orders of birds are highly sensitive, Peakall, 1975). Early-life stage tests might replace full life-cycle studies. The easiest early-life-cycle test that is available for terrestrial vertebrates involves injection of chemicals into the avian egg. This procedure might be worthwhile for reasonably stable compounds judged by the physicochemical data, especially if there is any likelihood of bioaccumulation. However, the predictive value of this test needs to be evaluated.

A variety of behavioural effects has been used to test the toxicity of chemicals. Warner *et al.* (1966) emphasized that such effects integrate various biochemical and physiological changes and are sensitive indicators of sublethal toxicity. There is, however, considerable difficulty in evaluating the significance of induced behavioural abnormalities as regards the survival of individuals and populations in their natural habitats.

For the marine environment, criteria have been developed by the Joint Group of Experts on the Scientific Aspects of Marine Pollution (GESAMP) for the selection of environmental variables for inclusion in water pollution monitoring programmes. As a first step in selection of variables, these criteria might be applied for predictive purposes to numerous techniques now available for measuring effects of chemicals on aquatic animals. The criteria include ecological significance, relevance to other effects, specificity, reversibility, range of taxa affected, quantitative aspects (does the effect bear a quantitative or predictable relationship to the cause?), sensitivity, scope, response rate, signal/noise ratio, precision, cost and range of application (see Waldichuk, this volume).

Using a system of ranking biological variables that can be measured for an estimate of pollution effects, GESAMP (1980) arrived at a series of measurements out of 36 considered, which could be recommended for immediate use in monitoring programmes in any region. These included feeding rate, body condition index, scope for growth, oxygen/nitrogen ratio, lysosomal stability, taurine/glycine ratio, liver as fraction of body weight, and incidence of ulcers and fin erosion.

It can be argued that most methods recommended by GESAMP are too complex for use by the average regulatory biological laboratories, especially if they lack research capability. In many cases, it may still be necessary to resort to the standard acute assay, preferably with a flow-through system, and allow for a longer exposure than the usual 48 or 96 hours. Animals with short-generation time can be used for full life-cycle testing. A high-calibre chemical analytical capability is essential in order to measure accurately the concentrations of chemicals, salinity, dissolved oxygen, pH and water hardness.

A question is always raised about the applicability of laboratory tests to field situations. Coupled with laboratory experiments, it is sometimes prudent to carry out simple field tests. If chemicals are already being released to the aquatic environment, caged animals can be used to measure effects in the field. It is possible to measure in this way acute toxicity and such effects as behavioural changes.

## 4.4 MULTISPECIES TESTS

Multispecies tests represent level 4 testing. Tests at the lower levels of the step approach are largely conducted on individuals and single isolated populations. Although single species tests are very useful and at present are the major and only accepted means of estimating the impact of chemicals on individuals and populations as well as on higher levels of ecological organization, it is not known how reliable these tests are for predicting effects on communities and ecosystems. Succeeding levels of ecological organization have unique properties; therefore, reaction of ecosystems to stress should not be expected to be merely a summation of the reactions of individual component species. There is evidence that some chemicals can jeopardize a population, community or ecosystem but these effects are not predictable from toxicity tests on single species (e.g. Fisher *et al.*, 1974; Van Voris *et al.*, 1980). Such effects depend on interactions of populations, and can be as significant to the functioning of an ecosystem as are the more easily measured direct effects. For these reasons, suitable laboratory tests should be developed to predict the intergrated reactions to chemical stress of biological communities and the ecosystem as a whole.

Tests on multispecies systems, which can often be done as microcosm studies, are called for if there is evidence of chronic effects in single species tests or the ability of the chemical to accumulate in the organisms. Changes in behaviour that could lead to alterations in survival and thus in community structure, changes in availability of one component of the ecosystem or the ability of a species to significantly alter the structure of the chemical can also indicate a need for multispecies tests.

Methods that examine competitive, predatory and grazing relationships and mutualistic interactions, such as those between plants and mycorrhiza, are feasible within a laboratory or in limited scale field studies. For example, many algae are more sensitive to toxic chemicals when competitors are present than they are in pure culture (Mosser *et al.*, 1972, Fielding and Russell, 1976). PCBs at concentrations as low as 0.1 g/l inhibited nutrient uptake of the marine diatom, *Thalassiosira pseudonana*, when algal competitors for limiting nutrients were present but not in pure culture (Fisher *et al.*, 1974). In an ecological context, such tests are more useful for predicting changes in phytoplankton composition and the structure and dynamics of a natural autotrophic community than are many individual toxicity tests (Calamari *et al.*, this volume).

Although multispecies laboratory tests are potentially valuable research tools, they have some limitations as regards changes in ecosystems. Most of these tests are in the initial stages of development. A basic stumbling block to their refinement is incomplete understanding of the significance of disruption of specific interaction phenomena for the overall effectiveness of an ecosystem to function adequately. In addition, very little work has been done on the sensitivity of these tests for distinguishing real changes induced by chemical stress from fluctuations inherent in natural and model ecosystems. Reproducibility of test results and their applicability in predicting changes in various habitats or ecological situations will require extensive further studies.

A recent review commissioned by the US Environmental Protection Agency (Hammons *et al.*, 1981) pointed out the need to better define:

(1) the limits to which a biological system can be stressed before its recovery to the initial state is no longer possible,
(2) measurable properties indicating adverse community and ecosystem effects, and
(3) cross-system generality of such properties.

Multispecies laboratory tests of potential utility in risk assessment have been extensively reviewed by Witt *et al.* (1979), Giesy (1980), Harris (1980), Hammons (1981) and Hammons *et al.* (1981). Hence, only a few select methods will be discussed in this section to illustrate the range of tests being developed.

### 4.4.1 Potential Tests for Competition

At the level of the primary producers, various methods for examining competitive interactions for essential nutrients have been developed for algae in continuous culture (see Fisher *et al.*, 1974; Fielding and Russell, 1976), and for pasture grasses and legumes in greenhouse and small field plot studies (see

Bennett and Runeckles, 1977; Kochhar *et al.*, 1980). Other candidates for plant competition tests could be representatives of different life forms, taxonomic groups or community types. Because autotrophs lack a diverse resource base and the variety of behavioural responses of a heterotrophic community, competition is intense and its mechanisms are limited. Shifts in population dominance of plant species competing under the stress of a toxic chemical are of ecological importance to the consumer community because, for grazers, the choice and nutritional value of food change with population shifts. There is also obvious commercial importance in unwanted shifts associated with stress spurred by competitive changes in pasture and crop lands. Further assessment of the significance of altered plant structure to ecosystem function (for example, primary and secondary production) is essential for validating the predictive character of these simple, sensitive and reproducible test systems.

Various competition tests to examine the interaction of zooplankton species in aquatic systems (see for example, Marshall, 1969; Goulden and Hornig, 1980) and arthropod species in terrestrial systems have been developed but have not been used to any great extent in assessing the effects of chemicals. Use of competing *Drosophila* populations in studies of genetic fitness is particularly highly developed (see Hammons *et al.*, 1981) as are the studies with *Tribolium* beetles (Park, 1948; Mertz *et al.*, 1976). These interactions show a fine competitive balance, may be highly sensitive to chemicals, and could be therefore important in developing theory and models of competition in the toxicology of ecosystems. However, they do not appear to be particularly representative of important consumer-level competitive interactions in the field, and therefore lack ecological realism essential for predicting community level changes.

Structural changes in the community of soil arthropods could be important in decomposition and nutrient cycling processes, but these litter communities display high species diversity and low feeding specificity, thus impeding the development of competitive test systems. Competitive interactions of longer-lived species have generally not been considered as an appropriate focus for ecotoxicological tests because of the time required for manifestation of significant changes (several generations may be required).

### 4.4.2 Potential Predator–Prey Test Systems

For aquatic ecosystems, predator–prey tests incorporating fish as the predator and zooplankton, shrimp or fish as the prey, and tests examining zooplankton–zooplankton feeding have potential use in hazard assessment. Most investigations of effects of chemicals on predator–prey interactions have been behavioural studies. Behavioural responses to chemicals are generally quite sensitive, and natural predators can often discern small anomalies in prey behaviour. Both observations suggest potential inclusion of predator–prey interactions in ecotoxicological testing programmes. However, there are problems with such tests, particularly in regard to fish. Generalizations about reactions of a specific predator–prey combination (generally based on prey exposure) are different. As summarized by Giddings (1980), an effect observed in one situation can only indicate potential effects on other predator–prey interactions but the magnitude, direction or even occurrence of effects in other species cannot be accurately predicted. In addition, factors other than predation often have greater influence on population abundance and distribution, and, therefore, detailed information on population dynamics and trophic structure would be essential in choosing appropriate test combinations.

Obligatory fish predation on zooplankton and zooplankton predation on zooplankton may provide test combinations with fewer factors confounding their predictive utility. In these test systems, exposure of the predators to chemicals may provide a logical experimental approach. Both mechanismic approaches such as reactive distance and capture success (Gerritsen and Strickler, 1977) and population approaches are feasible (see Drenner *et al.*, 1978). Tests are relatively simple, rapid and reproducible. Predation-induced changes in zooplankton communities are an important ecological phenomenon with significant impact on the diet of commercial and game fish and on the composition of the phytoplankton community. These test systems have not been adequately studied under chemical stress.

In terrestrial ecosystems, the relatively high sensitivity of insect predators to pesticides suggests that these species may be matched with appropriate prey (pest species) to make up a sensitive test system. Much of the laboratory research in this area has focused on predator effectiveness in biological control of prey populations and has not dealt specifically with chemical stress, although this would appear to be feasible. Most studies have examined parasitoid relationship. Searching capacity has been cited as the most important indicator of a parasitoid's ability to act as a biological control factor (Huffaker *et al.*, 1963) and thus provides an indicator of predator–prey interaction. However, use of such criteria should be supported with population data.

The best studied non-parasitoid feeding interactions are between ladybird beetles and aphids. This pairing has undergone considerable laboratory (Murdoch and Marks, 1973) and field (Hagen *et al.*, 1971) investigations. The response data for a predator–aphid system would be generally relevant to many agricultural and home garden systems.

Other invertebrate predator–prey test systems are in various stages of development. By far the most advanced involves predatory mites. Mite test systems are highly sensitive to species choice, physical conditions, host characteristics and input ratio of predator to prey (Huffaker *et al.*, 1963). Systems such as those reviewed by Suter (1980) which are finely balanced should be highly sensitive to chemicals and have a short response time (less than a month). Transposition of results from mite tests to other species is questionable.

Although laboratory studies using vertebrate predators have been successful (see Holling, 1959 for deer mice and sawflies), vertebrate predators generally have complex feeding strategies (switching) and often require an extensive test area, thereby complicating the development of a vertebrate–arthropod or vertebrate–vertebrate test system.

### 4.4.3 Grazing Tests

Grazing of phytoplankton by zooplankton is an important pathway for the movement of energy and nutrients in aquatic systems and, in addition, it is a possible determinant of phytoplankton community composition. Major problems in developing grazing test procedures are poorly developed laboratory and *in situ* techniques for measuring grazing rates.

Herbivore grazing on flowering terrestrial plants is of particular interest in relation to those interactions that diminish commercial crops. Insect–plant pairs such as aphid–alfalfa, white fly–plant and scale insects–plant have been closely examined in greenhouse and field studies (although not as regards response to chemical stress). The plant and grazer interactions in these systems are easily established and changes in net plant production can be achieved in some cases in a relatively short period. More extensive studies may be necessary to account for reproductive capacity of both plants and insects. Questions remain as to the relevance of herbivore insect–domestic plant interactions to the more highly evolved and perhaps more sensitive insect–plant grazing interactions in natural communities. These candidate test systems have potential utility but need further development and validation before they would be readily accepted as test systems in the toxicology ecosystems.

### 4.4.4 Mutualistic Interactions

Legume–rhizobia and mycorrhiza–plant interactions provide an ecologically relevant basis for tests of symbiotic relationships, because primary productivity can be ultimately affected. Test development should include examination of the influence of soil type and legume–rhizobium or plant–fungus species differences. The obvious benefit of this association can be measured in the quantity and quality of plant production. Time course studies have shown that sensitivity of the system may diminish with time owing to degradation of the chemical or selection of a resistant symbiont strain. Short-term tests may, therefore, effectively predict potential interference problems.

### 4.4.5 Ecosystem Test Models

Two general approaches have emerged in the course of development of laboratory ecosystem test models. One, the generic approach, is based on the concept of a test system which exhibits universal ecosystem properties (for example, primary productivity, organic decomposition, nutrient cycling) but is not expected to mimic a particular ecosystem. The other approach involves a realistic simulation of a specific ecosystem.

#### *4.4.5.1 Types of Test Models*

Mixed flask cultures, such as those of Beyers (1963) and more recently of Leffler (1977), of bacteria, algae and microinvertebrates have been suggested as the ecosystem-level 'white rat'. They are useful at the higher levels of chemical screening, as they are small and replicable and could provide data on system-level processes or changes in homoeostasis of the model system. Generic test sensitivity to chemicals, as well as evidence of consistent reproducibility and 'ecological realism' of response, remain to be validated.

More realistic ecosystem models are being developed for several aquatic habitats such as ponds (Giddings and Eddlemon, 1978), lakes (Harte *et al.*, 1980), streams (Rodgers *et al.*, 1980) and coastal marine systems (Oviatt *et al.*, 1977). Only a limited number of reports of effects of chemicals on such test systems exist, but there are indications that metabolic reactions to perturbation (for example, primary productivity response) may be assessed more easily than nutrient related properties. Several problems have become apparent in developing these tests. Many aquatic ecosystems are inherently variable and unpredictable and model systems are no less so. Macroconsumers are difficult to maintain in small microcosms, and if included, often exert an inordinate influence on system structure and function. The shallow depth and small size of test systems exaggerate the influence of sediment and restrict the behaviour of some microinvertebrates. The large surface-to-volume ratio of test systems enhances 'wall effects', primarily expressed as growth of attached algae. Of the various microcosm types, the pond simulations are structurally and functionally closest to mimicing the natural water bodies and have been included at least in one chemical manufacturer's assessment programme.

Sediment and soil cores (Porcella *et al.*, 1976; Jackson and Hall, 1978) have been used as homogenized or intact systems to examine microbial processes. Carbon dioxide evolution, mineralization of nitrogen and leaching of nutrients have been examined as measures of decomposition and nutrient cycling processes. Such test systems are thought to be representative only of site-specific properties. The sensitivity of soil processes to chemical stress is well-documented only for certain metals, and there is some evidence indicating that soil microbes and microbial processes may be less sensitive to organic compounds than are individual macrospecies.

Questions remain about validity of generalizing from test data obtained with microcosm models of specific aquatic or terrestrial systems. Although the value of these tests would increase if data could predict 'typical' effects in a number of similar ecosystems, the available information is insufficient to calibrate laboratory systems for general predictions of safe exposure levels in nature.

### *4.4.5.2 The Microcosms as a Testing Tool in Ecosystem Toxicology*

Many multispecies test systems that are being developed can be classified as microcosms. A brief review of microcosms as a toxicity testing tool thus provides a possibility to summarize the status of multispecies test systems in general.

Because of the very wide range of possible microcosm designs, it has been suggested that microcosms could occupy a primary role in the toxicology of ecosystems. However, this technique is still too limited to permit a recommendation for routine use. Selection of the experimental paradigm for design of a microcosm test will perforce be a function of the specific test. Thus, it is unrealistic to speak of the standardization of microcosms, except in considering a specific goal.

Six major documents dealing with the use of microcosms in ecotoxicology have been published recently (Gillett and Witt, 1979; Witt *et al.*, 1979; Giesy, 1980; Hammons, 1981; NRC, 1981; Levin *et al.*, 1984). Several authors contributed to more than one of these reviews, and the total number of contributing scientists was several score. Even though the objectives of each document were different, there were no major disagreements about the use of microcosms in ecotoxicology. The main conclusions agreed upon include the following.

(1) It has yet to be determined which ecosystem variables are most suitable for specific microcosm application.

(2) Microcosms are limited by scale relationships (size dependency of organisms and area/volume ratios) and are unstable in time.

(3) Microcosm design requires an ecosystem perspective.

(4) As complexity of microcosm design increases, realism and cost tend to increase and precision tends to decrease.

(5) There are few *a priori* rules for inclusion of common factors in microcosm design; generic design microcosms have a very limited use.

(6) Microcosms occupy a place in a tiered scheme of environmental hazard assessment of chemicals.

(7) Microcosm experiments are of limited use until they are validated by field experiments.

(8) Mathematical models must play a central role in all environmental hazard assessments because of the inherent limitations of empirical data.

(9) Microcosms provide information at the process level; they are subsets of ecosystems, not miniature ecosystems. Processes include energy relationships, nutrient cycling, chemical transformations and organism responses at population or community level.

(10) Both 'synthetic' (gnotobiotic) and 'natural' (excised or reconstructed) microcosms have potential use in hazard assessment.

(11) For assessing environmental hazards, population- or community-level metabolic processes are more appropriate than organism-level processes.

(12) Criteria for using results from microcosm tests in the regulatory process have not been developed.

(13) The usefulness of microcosms for routine screening of potentially hazardous substances is not clear.

(14) No laboratory system or microcosm is currently ready for use as a test protocol or for prediction of environmental hazards of chemicals.

## 4.5 TESTS AT THE ECOSYSTEM LEVEL

This is the last level of testing (level 5). Tests conducted in natural ecosystems may in some cases be the only way to determine the effects on ecosystem processes, because of complex interconnected abiotic and biotic phenomena associated with specific compounds and the exposed ecosystem. For instance, Coles and Ramspott (1982) demonstrated that laboratory experiments failed to predict the migration of radionuclides in groundwater, possibly owing to differences in chemical speciation of ruthenium under field and laboratory conditions. Numerous studies have also shown that species at the top of a trophic web are often sensitive to certain compounds and can exert regulatory influences on ecosystem homoeostasis, but macroconsumer interactions cannot be easily incorporated into laboratory or small-scale field studies. The impact on ecosystem function of the elimination of larger consumers is also difficult to demonstrate in the laboratory.

Large-scale field tests can be used also to validate results from multispecies laboratory test systems which are not self-validating. Tests at this level are the most realistic and should provide the best assessment of changes in ecosystem dynamics. However, technical and economic problems associated with experiments at this level prohibit extensive use of ecosystem-scale studies. Considerable attention must be given to the proper choice of experimental design, site and pollutants to maximize information and to provide bench-marks for cross-site and cross-chemical comparisons.

Ecosystem-level testing should incorporate monitoring of the toxic chemical in the environment and biota and determination of biological effects at several levels including changes in:

(1) population dynamics,
(2) community structure, and
(3) ecosystem function.

The ultimate focus should be at the level of ecosystem processes (such as productivity, organic decomposition, element cycling) and on the dynamics (homoeostasis) of dominant communities. A process-oriented approach best addresses the question of how seriously the ecosystem function is disrupted by experimental exposure. Mechanisms of compensation inherent in the ecosystem (for example, ability of tolerant populations to increase the use of resources that become available when sensitive populations are lost) are accounted for with this choice of endpoint, whereas they are not obvious in strictly structure-oriented approach.

The time between initial exposure and expected reaction in experiments at this level is often relatively long (several months to years) depending on the severity of exposure and type of effects examined. For instance, reductions in the rate of photosynthesis may be shown in the short term. However, reductions in primary productivity may not be obvious until completion of the growth period and significant changes may not be demonstrated for several years if the dominant plant species have a normal slow growth rate (mature trees).

The fate and effects of chemical pollutants have been examined only in a limited number of whole-system experiments. The number of such experiments is large if other dramatic perturbations (such as clear-cut logging and nutrient additions to aquatic systems) are considered .

Schindler and his colleagues (1973) examined whole-lake effects of nutrient addition in the Experimental Lakes Area (ELA) of the Canadian Shield. In the latter half of the 1970s, Schindler *et al.* (1980) applied the whole-lake approach to examine acidification of a poorly buffered lake in this area (ELA Lake 223). These studies were considered as an experimental acceleration of acidification processes occurring over certain regions of eastern North America. Acidification over a 3-year period from 1976 to 1978 decreased pH from 6.7–7.0 to 5.7–5.9. However, sulphuric acid addition did not decrease pH as expected owing to complex interactions of the iron mobilization and the sulphate reduction cycles in buffering acid additions. Soluble concentrations of elements such as zinc and aluminium did increase substantially in agreement with both laboratory and monitoring observations. There was, however, no evidence to support the Grahn and Hultberg (1974) self-accelerating oligotrophic hypothesis, because no changes in chlorophyll or primary productivity were evident (Schindler, 1980). This study emphasized several important aspects of these type of tests:

(1) the importance of considering and examining microbial processes,
(2) the problem of experimentally mimicking exposure observed in contaminated systems, and
(3) the uniqueness of site-specific properties.

Hall and Likens (1980) and Hall *et al.* (1980) described experimental acidification of Norris Brook in the Hubbard Brook Experimental Forest. With increased acidity of stream water, the concentrations of aluminium, calcium, magnesium and potassium increased significantly. Emergence of adult mayflies, stoneflies and true flies decreased at low pH (4). Experimental acidification decreased macroinvertebrate diversity and complexity of the food chain. Periphyton biomass increased, and this was partially attributed to reduction in numbers of scraping and collecting insects which generally feed on periphyton. This observation illustrated the importance of using experimental techniques which allow examination of consumer interaction in the control of producer biomass and productivity, phenomena generally difficult to account for in multispecies laboratory systems.

The controlled dosing of a small Ohio stream, Shayer Run, with copper by the US Environmental Protection Agency (Geckler *et al.*, 1976) is a good example of a test for examining stream ecosystems. This exercise was undertaken specifically to validate results of laboratory testing. The results obtained substantiated the importance of considering avoidance behaviour in fish and directing the assessment towards changes in less mobile communities (macroinvertebrates) and the ecosystem as an entity. Studies by Winner *et al.* (1975, 1980) on the Shayler Run macroinvertebrate community following 2 years of experimental exposure to copper at 20–120 $\mu$g/l demonstrated that changes in community structure during this ecologically short period were in agreement with findings resulting from long-term exposure to metals of stream communities (Sheehan and Winner, 1984).

Suter (1982) described several large-scale field techniques for examining perturbations in terrestrial systems. The scale of experiments in terrestrial systems is not as well-defined as that for the aquatic studies previously described. The study area may be a part of an agricultural field, grassland or forest, or it may be as large as an entire watershed, an approach successfully used by Bormann and Likens (1979) and others in the Hubbard Brook Experimental Forest. The scale of such studies depends on community diversity, productivity and patchiness; with ecosystems of low productivity, or high productivity and high patchiness, larger plots are required (Suter, 1982). The size of the perturbed area limits the range of properties that can be monitored. Suter (1980) indicated that short-term effects such as leaf chlorosis, changes in gas exchange rates and behaviour should be monitored and related to medium- and long-term changes in populations (reproduction success), communities (dominance shifts) and ecosystems (primary productivity).

Pesticides and herbicides have been tested for adverse effects in controlled field experiments (Barrett, 1968; Moulding, 1976), and so have various air pollutants (Irving and Miller, 1977; Van Voris and Tolle, 1979).

Several limitations have been noted for tests at the ecosystem level. Reproducibility and precision are difficult to assess for large-scale experiments. A recent discussion of this problem (Levin *et al.*, 1984) provides some insight into the spatial generality of such experimental extrapolations, although there is little specific information in relation to chemical perturbations. Nutrient losses associated with clear cutting in the Hubbard Brook Forest were consistent with the effects of similar logging techniques applied to other forested land in the region but did not quantitatively predict nutrient losses in Oregon forests. In addition, labour and financial expenditures associated with large-scale field tests or ecosystem studies can also be substantial.

In summary, ecosystem level tests are essential and necessary to demonstrate certain long-term effects, to validate laboratory data and to provide appropriate information for predictive test models. Testing at this level is complex, costly and difficult, if not impossible, to replicate.

## 4.6 ECOTOXICOLOGICAL MODELLING

The development and use of mathematical models in ecotoxicology can provide a means for integrating test and monitoring data as well as simulating population and ecosystem responses to chemical perturbations. In a given case, if one knows the quantity of the toxicant in question in each compartment of the ecosystem (such as, water, soil, plants and animals), the size of each compartment and the rates of transfer between the compartments, one can calculate the quantity and therefore the exposure to the toxicant that the target organism will experience. If the damage function is known (i.e. the exposure to the toxicant that will produce an effect), then an evaluation of the toxicant in the environment can be made. Two recent examples of the success of this approach are the Ottawa River Project (Miller, 1977) and the Integrated Lake Watershed Project (Chen, 1982).

This ecosystem approach requires further development of appropriate models. Model calculations should identify the most critical areas to be examined in specific pollution cases. Once the objectives are so identified, the methods with which to conduct the work can be selected (bioassays, microcosms, field studies). Hopefully this process will enable the selection of a small number of situations to be studied in detail.

## 4.7 CONCLUSIONS

(1) Ecosystems vary greatly in their complexity ranging, for example, from a field of corn to a tropical forest. There has been some progress in acquiring the ability to measure the impact of pollutants on ecosystems but the state-of-the-art varies greatly from ecosystem to ecosystem. For complex ecosystems most studies of the effects of pollutants end with effects on populations but concerted efforts should be made to advance towards the effects on whole ecosystems.

(2) Under environmental conditions only in few cases is there exposure to single chemicals. The usual situation is that a wide variety of chemicals are present in varying concentrations under varying conditions. It is important to bear in mind the interactions between chemicals and physical conditions.

(3) Considerable progress has been made to improve the predictive capacity of tests. Assessment of the value and limitations of a single method, while certainly relevant for the method itself, has limited importance in a wider perspective on

environmental pollution. For correct evaluation of methods capable of predicting chemical injury to ecosystems, only integrated information is of real help. The assessment of the impact of a chemical substance on ecosystems has to be made by integrating very different kinds of information including physical and chemical properties of molecules, biotic and abiotic degradability, effects on other organisms at different trophic levels, mode of action of the chemical, and so on. Microcosms can be used for ecotoxicological studies to bridge the gap between simple bioassays and complex field studies.

(4) There is at present a limited capacity to evaluate quantitatively the impact of chemicals on some of the simpler ecosystems. In most cases, we are more at a stage of ranking hazards rather than of quantitative evaluation of each hazard. There is a need to use the available data and focus the direction of research so that we can move towards the goal of risk assessment at the ecosystem level. Unfortunately the available data on the effects of chemicals on ecosystems obtained from reliable long-term studies are extremely limited. Ecological models, based on high quality field data, should greatly assist in advancing our predictive capability.

## 4.8 RECOMMENDATIONS

(1) *Baseline studies*. Evaluation of chemical hazards in the environment depends on the existence of information on the properties of a given chemical and other factors that determine its environmental behaviour. In most site-specific cases this basic information is not available. It is, therefore, recommended that baseline studies be conducted in areas where chemical effluents are expected to increase. These studies should be designed to examine changes in groups of organisms as well as in the physical environment. Such studies are of necessity long term, but the expense and difficulty of this work should not be permitted to influence the priority for carrying out the necessary observations.

(2) *Model development and validation*. The development of appropriate models will produce a framework for identifying and guiding the necessary research on a given chemical hazard, and will thus provide an integration of experimental work so as to yield the most realistic and broadly applicable results. Such models must be validated by field data. Validation is an essential test of assumptions made during model development and, therefore, of the realism of the model. A critical review of the existing models would be useful.

(3) *Selection of test methods*. It would be inappropriate to suggest specific tests for the evaluation of all chemical hazards. Rather, test methods should be selected from the battery of techniques available for obtaining the required information about effects at the appropriate level of ecological organization. In some cases, simultaneous tests will be needed on the effects of chemicals on organisms in the laboratory and on the same organisms in the field. This should provide a measure of the effects of uncontrolled environmental variables in the

field and how these variables might influence the application of laboratory data to the ecosystem.

(4) *Ecologically relevant insect tests.* Because of diversity and adaptation to special ecological conditions, insects provide sensitive systems for testing environmental chemicals. To use this possibility, methods applied in testing pesticides should be adapted, new ecologically relevant species for testing should be identified, and methods for testing of long-term effects of chemicals on insects at the level of individuals, populations, and communities should be developed.

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