Methods for Assessing the Effects of Mixtures of Chemicals
Edited by V. B. Vouk, G. C. Butler, A. C. Upton, D. V. Parke and S. C. Asher

*7 Methods for Assessing the Effects of Mixtures on Animal and Plant Populations, Communities, and Ecosystems**

7.1 INTRODUCTION

Ecosystems are seldom exposed to single chemicals, and in nearly all cases the stress of pollution on natural ecosystems is attributable to the combined effects of many chemicals. Assessment of the effects of mixtures on plant and animal populations, communities, and ecosystems, in contrast to the usual practice of assessing the effects of single chemicals on individuals, is therefore necessary. Components of mixtures of chemicals will often partition differently into different environmental compartments, such as the soil, water, biota, etc., and studies are customarily conducted in only one of these compartments. Tests at the community and ecosystem levels could therefore provide a more realistic assessment of the effects of a chemical mixture and therefore much of this paper discusses effects at these levels.

The study of the effects of mixtures of chemicals in the environment has several complications: (a) dissimilar partitioning and persistence of components of the mixture after they enter the ecosystem, (b) short-term versus long-term effects on ecosystems, (c) additive versus non-additive effects among components, (d) abiotic features of the ecosystem, and (e) effects of mixtures on interactions between environments, such as in tidal or estuarine ecosystems, interactions between populations, and interactions between populations and their environment.

Methodologies to deal with these problems, and for the evaluation of the effects of mixtures on functions within the ecosystem, are in a comparatively early stage of development. No one kind of study provides the answer regarding the impact of chemical mixtures. A mixed approach is needed: laboratory studies coupled with field studies, short-term and long-term studies, microcosms and environmental modelling, and so on. Specific tests may accurately predict certain responses and fail to predict others.

* This section was prepared by a working group co-chaired by J. Cairns and Ph. Bourdeau. The members were M. A. S. Burton, D. Calamari, R. H. Green, R. R. Lassiter, P. J. Sheehan (Rapporteur), G. W. Suter II, and R. Truhaut.

This section of the Joint Report is divided into two main parts. The first part, entitled Experimental Considerations, includes discussion on points that should be taken into account when studying the effects of mixtures on plant and animal populations, communities, and ecosystems, as well as methods of determining the effects of individual components, and chemical and species interactions. In the second part of this section, the methods currently used to assess the effects of mixtures of chemicals in the environment are outlined and discussed. These latter methods are divided into aquatic studies, terrestrial studies, laboratory studies, the use of microcosms, and environmental models. Finally, the section is completed with a summary of the major conclusions and recommendations of the working group.

7.2 EXPERIMENTAL CONSIDERATIONS

The experimental considerations for both laboratory and field studies designed to test the effects of mixtures on populations, communities, and ecosystems can be divided into five categories. First are considerations of which biological effects, or response variables, should be observed and measured. Then, given the appropriate response variable, are considerations of the design of the study. Particular to mixtures of chemicals are the problems in determining the effects of individual components of a mixture, and problems in determining the importance of component interactions. The final category addresses a problem that is unique to the study of communities and ecosystems which is the effect of mixtures on species interactions. The complexity of these considerations has been discussed by Butler (1984).

7.2.1 Choice of Response Variables

There is a great variety of indigenous species assemblages and ecosystem types which may be affected by mixtures of chemicals. The type of system and its biotic and environmental make-up will shape the response of the ecosystem and the approaches to assessing effects. Data on release, bioactivity, and the environmental partitioning of the potentially toxic components of a mixture provide guidance as to the type of communities and ecological functions most likely to be affected. The appropriate choice of response variables for a quantitative assessment study in the field, in the laboratory, or in the construction of a mathematical model will thus be case-specific and not as readily generalized as, for instance, the use of lethality to quantify response in single species toxicity tests. Since components of the mixture may partition into different parts of the ecosystem, quantitative measurement of several heterotrophic or autotrophic characteristics will often be essential, rather than a single index of community or ecosystem status. The choice of standard response variables for single chemicals may have to be re-examined when dealing with mixtures.

Some general considerations that apply to the choice of response variables in environmental studies at all levels (population, community, ecosystem) include the following:

(1) a measure of biological response which can be generalized to other populations, ecosystems, or communities;
(2) a variable which is directly and robustly linked to the hypothesis being tested;
(3) a variable which is measured easily, quickly, inexpensively, and accurately;
(4) stability of the variable in space and time;
(5) direct economic or aesthetic value if possible; and
(6) a variable selected to suit the site; for instance, primary production is important in lakes but not in streams where leaf decomposition is the primary energy source.

These conditions are difficult to satisfy in any environmental study and are especially difficult when dealing with mixtures of chemicals and with responses at the population, community, and ecosystem levels; the higher the level, the greater the difficulty. Studying the hierarchy of responses to chemical mixtures at different levels is obviously expensive and difficult to complete. At higher levels the responses are longer-term, more complex and stochastic, and more difficult to express. The environment and organisms degrade and modify the chemicals, and at higher levels of organization more of these interactions must be considered.

The choice of response variables can be different at each level of the hierarchy. At the population level there are important variables that do not exist at the level of the individual organism such as genetic variability, heterogeneity of response to genetic variability, and the resulting change in genetic composition over several generations. This is exemplified by the many examples of the development of resistance to pesticides and sterilants. Other response variables examined at the population level include lethality, reproductive success, reproductive strategy, birth rate, age structure, spatial distribution and growth. The response of a single population to chemical stress may or may not be manifest as a general response of the community or ecosystem as a whole, depending on the role the population plays in structuring the community and in controlling functional processes. In some cases the loss of an individual population may have a strong impact, as illustrated by the elimination of keystone predators (Paine, 1974); in other cases, the loss of a population may have a negligible impact due to redundancy in functional pathways in the ecosystem (Sheehan, 1984a).

When testing at higher levels of biological organization the choice of response variables is dependent upon the type of community or ecosystem examined, the conditions specific to the site, as well as the composition of the chemical mixture, if known. When testing at these levels one first examines the system and then determines the important inputs and outputs for that particular system which might be altered by the mixture of chemicals. A variable is then selected which is most likely to reflect the displacement of a function of the system. The variable is therefore more likely to be selected *a posteriori* and be site-specific. For example, a response in primary production in an aquatic system dominated by plankton is expected to be greatly different from that of a terrestrial system dominated by higher plants. Also, differences in community structure will affect the responses of component species (e.g. the amount of cover a canopy provides).

There are obvious problems in defining an adverse impact of chemical exposure at the community and ecosystem level. The question posed is whether transient changes in response variables (reflecting an imbalance in the system) are of ecotoxicological significance, as many of the species which play an important role in primary production and nutrient cycling have a rapid reproductive rate and are capable of adapting to the stress of chemical mixtures. The question of which impacts are critical is highly pertinent, and at the same time extremely difficult to decide. Impacts may be of two basic types: reversible impacts, and persistent impacts. For reversible impacts, the critical variables which must be used to interpret the seriousness of the impact are the maximum tolerable change in response and the maximum delay in recovery to the normal operating range. Various authors have suggested that a delay in recovery of microbial community activity of less than 40 days to as much as 60 days may be tolerable, and a delay longer than this period represents possibly critical ecological consequences (Domsch *et al.*, 1983). Generally, a monitoring period of at least 30 days is necessary to recognize persistent impacts (Stockner and Anita, 1976). The relative change in response has to be quite large to enable recognition of a critical impact even after 90 days of exposure. For example, Domsch *et al.* (1983) suggest that a 60% inhibition after 90 days can be considered critical. Their discussion emphasizes the problems inherent in the interpretation of short-term function tests and points out the need to develop and use long-term testing procedures.

There is no general agreement on the best response variable to choose to measure the impact of chemical mixtures in communities and ecosystems, in spite of decades of efforts to formulate 'indices of community health' (Sheehan *et al.*, 1984). These indices include community productivity, parameters of species abundance and distribution such as diversity indices or biomass, taxonomic and trophic composition, microbial metabolism, and, in the case of ecosystems, transfer and transformation of energy and mineral nutrients. Data from a limited number of controlled field experiments are available on changes in the energy–mass balance of a perturbed system (Bjor and Teigen, 1980; Likens *et al.*, 1970). The use of variables which describe the stability of the community or ecosystem (e.g. the rate of recovery or the resistance to structural changes) (Sheehan, 1984b) has been extremely limited and could be further developed.

The use of indices for response variables may be inadequate because they can be influenced by many factors. For example, moderate pollution can both increase and decrease species diversity (Rosenberg, 1976). However, this approach is attractive in that a single variable can be used to summarize the biological response of a community, and different communities composed of different species can be compared. Another possible approach is to use the abundance of an indicator species as a response variable, if the most appropriate indicator species for the particular chemical impact has been determined. Perhaps laboratory LC_{50} determinations could screen candidate species for this purpose. Again one must be cautious because tolerance or sensitivity to an impact may be related to the particular natural, pre-impact environment (Fisher, 1977).

7.2.2 Experimental Design

Concerning the second main category of statistical considerations in the study of chemical mixtures, the design of the experiment, the following principles apply. These principles are discussed in detail by Green (1979, 1984, and in this volume).

In laboratory studies care should be taken to ensure that the various combinations of concentrations of different chemicals, to which randomly chosen test organisms are exposed, are arranged in a balanced factorial design. This is more difficult to satisfy in field studies, but in an environmental study it is even more important because of the increased number of variables. With careful planning and some preliminary sampling, it is often possible to sample in such a way that the biological response variables are measured at locations and times which yield a roughly balanced design with respect to the sample sizes of different combinations of chemical concentrations. In testing for interaction of different chemicals in producing a biological response, it is critical that the concentrations of different chemicals are not spuriously correlated because of an unbalanced design. Each chemical should be an independent variable.

Determination of exposure time will depend on the objectives of the study, the level of the anticipated biological response, and the composition of the mixture of chemicals.Some components of the mixture will be distributed more quickly throughout the environment than others, and considerable differences in time are needed for different species to reach stable body burdens of each chemical. Description of genetic changes within populations and successional changes in the multispecies community requires observations allocated over a time period covering many generations of the organism or organisms involved. Long exposure times more accurately reflect the situation in nature.

What is a meaningful error term to be used as the null hypothesis variance in statistical tests for long-term studies? This is a basic philosophical question, which determines the meaning of 'significant effects'. If the replicate sample variance is used as the error term, and many replicate samples are taken, then effects too small to be of biological concern may be judged significant. Regarding significance of interactions (e.g. between different chemicals in producing the biological response), Vandermeer (1981) has commented that, 'It is only a question of enough grant money to generate enough degrees of freedom to demonstrate them statistically. What we want to know is whether they are important biologically . . . .' Perhaps significance should be judged relative to natural year-to-year variation in populations and communities, which would mean that impact studies of sufficient duration should be carried out to provide adequate 'among-years' degrees of freedom.

Spatial allocation of samples is usually necessary, if only to sample over a range of chemical concentrations that vary in space. Unfortunately other environmental factors are also likely to vary in space, and they may influence the biological response variable, thus inflating the error variance. This spatial environmental variation should be controlled in some manner, either statistically, by measuring it as a covariate, or more directly, by reducing the natural environmental heterogeneity. Artificial substrate sampling is one way to reduce environmental heterogeneity (Cairns, 1982).

It is not merely an oversight that few data are available that would permit analyses of responses of ecosystems to chemicals. Such data can be obtained only by carefully planned field studies which are extremely costly. Furthermore, they are highly specific and generalizations are apt to be difficult. We are left, therefore, with the necessity for some form of prognostic or diagnostic tool for the majority of assessments such as the use of microcosms or mathematical models (see section 7.3).

7.2.3 Methods of Determining Effects of Individual Components of a Mixture

Most studies of the effects of mixtures of chemicals treat the mixture as a single entity. The mixture (e.g. industrial effluent, river water, etc.) is added to the test system and the result is observed. These studies are valuable; however, little is learned about the roles of the various components of the mixture on the target organisms. As most environmental exposures are to dilute mixtures of numerous potentially toxic compounds, researchers have used various means to associate ecological response with the concentration of various components or fractions of a mixture. One approach is to correlate the response with the components of the mixture in greatest abundance (Sheehan and Knight, 1985), with the sum of concentrations of various individual components similar in type (e.g. 'heavy' metals) (Tyler, 1976), with a specific fraction of mixture such as the soluble fraction of oils (Harty and McLachlan, 1982), or with the known discharge or application rates of the mixture (Herricks *et al.*, 1981). Correlations have also been made between the response and a number of variables including concentrations of a chemical in the biota and the composition or amount of rain (for air pollutants such as acid rain), or correlations have been made indirectly with distance from the source of the effluent. There are recognized limitations in each of these pragmatic approaches to the problem of distinguishing which single chemical or combination of chemicals in the dilute mixtures is most directly related to the adverse response of the system. Limitations include missing association with a highly toxic substance present at very low concentration, and the inability, in most cases, to identify associations between components which contribute to synergism or antagonism in response.

Two major methods for elucidating the role of individual chemicals in the effects caused by a mixture are (a) fractionating the original mixture, testing each fraction, and comparing the effects of the fraction with the effects of the original mixture, and (b) building up submixtures from the components of the mixture to mimic the mixture. Both approaches are prohibitively expensive, particularly with a large number of components. Nevertheless, in certain situations detailed investigation of mixtures is worthwhile. These situations include:

(1) where areas of adverse effects are seen and it is impossible to assign effects to any particular chemical;
(2) when knowledge of which component(s) of a mixture is causing an environmental problem may enable simpler clean-up procedures, or reformulation of the mixture, rather than removing the entire mixture;
(3) if the mixture to be released to the environment is known, knowledge of interactions may enable synergistic reactions to be predicted.

A discussion of these points can be found in Peakall (this volume).

A major problem in determining which component of a mixture is responsible for an environmental effect can be in the identification of the components. Over 800 chemicals have been found in the Great Lakes (IJC, 1983). Once the components of a mixture emitted to the environment have been determined, another complication could be background contamination with ubiquitous chemicals such as polychlorinated biphenyls (PCBs) or the presence of impurities such as dioxins.

A useful approach to the analysis of mixtures has been to examine chemical and ecological variables with a combination of discriminant analysis and other multivariate techniques (see Green, 1979). Such a tool, if applied within a correct and appropriate experimental design, can provide an aid to the interpretation of associations between various chemicals and response variables. In addition, these techniques are often useful in resolving the difficulty of quantifying both material influences and effects due to pollutants. Examples of this type of analysis indicate that measurement of several chemical and response variables may be necessary to establish a statistical link between the mixture and its ecological impact. This type of analysis assists the identification of the specific variables that determine the differences observed between the exposed and unexposed communities and ecosystems, and allows an estimation of the contribution to response of each individual variable, including environmental chemicals. Multivariate approaches are also applicable to the analysis of data obtained with model ecosystems. It should be remembered, however, that the interpretations provided by the discriminant techniques must be based on basic knowledge of the ecology of the system to assure their accuracy.

There are several experimental approaches which, if combined with monitoring programmes, can aid in our interpretation of the influence of individual components of a mixture on the general functional response of the ecosystem. A combination of laboratory bioassays with mixed cultures or with natural communities exposed to single chemicals thought to be the major contributors to the toxicity of the mixture is one approach that has been regularly applied to the productivity of phytoplankton (Bartlett *et al.*, 1974). *In situ* bioassays that test the response to changes in the composition of the chemical mixture may also be useful in clarifying the contribution of various components or fractions to the combined toxicity. Currently, such techniques are used with advantage in monitoring plant yields under the stress of mixtures of gaseous air pollutants (Foster *et al.*, 1983). Open-top chambers, some equipped with filters selective for one chemical or a combination of chemicals in the gaseous mixture, are set up in the experimental area. Response is compared with samples from chambers with filtered air, unfiltered air, and from the unexposed system, and these results are related to the levels of individual components in air for each exposure. Such *in situ* manipulations of a pollutant mixture have been used effectively to distinguish the impact of ozone in mixtures containing moderate levels of sulphur dioxide, nitrogen oxides and ozone (see, for example, Duchelle *et al.*, 1982).

The application of experimental *in situ* modifications of a mixture in aquatic ecosystems is possible but has proved difficult. Enclosures of various size can be used in lakes to isolate the microbial and microinvertebrate communities. Although the limitations of enclosure methods have been widely discussed (Giesy, 1980; NRC, 1981), their use in studies of the effect of complex effluents may be of some value. In some cases, where water is contaminated with a mixture of metals and other compounds, a chelator could be used to significantly alter the composition of potentially toxic pollutants. A reduction in toxicity of waters polluted by metals by adding a chelator has been well demonstrated under laboratory conditions (Payne, 1976), and occasionally with field samples (Sundra, W., personal communication).

In situ modifications of the composition of water contaminated with other types of inorganic material known to be toxic, or with potentially toxic organic materials, are extremely difficult. There are few available techniques for the selective removal of these materials. Wells *et al.* (1982) have suggested the use of non-toxic mineral oil to partition the fractions of oil–dispersant mixtures in laboratory tests. Such a technique has not been fully evaluated in the laboratory and *in situ* studies are rarely attempted in marine environments where oil spills are most likely.

Whole system manipulations have been used in a very few cases to attempt to experimentally reproduce effects observed in long-term monitoring data. The examples of artificial lake and stream acidification and of the liming of previously acidified lakes have provided insight into this complex pollution problem involving reduced pH and consequent chemical changes (Hall and Likens, 1980; Schindler *et al.*, 1980).

The diversion of an effluent from a contaminated ecosystem, or the radical alteration of its composition during a monitoring study, can provide retrospective data on the impact of varying concentrations of a mixture, or a fraction of the mixture, and on the ability of the ecosystem to regain normal functions. The study of the recovery of Lake Washington following diversion of sewage (Edmondson, 1971, 1972) and the recovery of a Norwegian fjord following initiation of industrial and domestic waste treatment (Rosenberg, 1976) are classical examples.

7.2.4 Methods of Determining the Effects of Chemical Interactions

Due to the number of chemicals in the environment, the potential for chemical interactions to influence biological response is great when the chemicals reach the environment together and in high concentrations. Unfortunately, due to the complexity of monitoring the ecological response of natural systems to mixtures of chemicals, chemical interactions have often either been ignored (e.g. Brasfield, 1972), or been cited as a potential source of error (Phillips, 1980). The difficulty and expense of conducting field studies for testing interactions among chemicals are almost prohibitive; the number of possible interactions rises rapidly with the number of potentially interacting chemicals. Transformation products of each component may be more toxic than the original chemical. Rationale for the priority of testing interactions has to be developed and could include:

(1) tests of combinations of chemicals that are to be used in the environment;
(2) tests when additional compound(s) are to be added to a system known to be contaminated with other compounds;
(3) tests of combinations when there is an *a priori* reason to suspect that interaction may occur (see Peakall, this volume).

Areas which have been investigated for chemical interaction include pesticides (Macek, 1975), which often reach the environment in chemical combinations. Heavy metals are frequently found together in the environment and a great deal of work has been done on the effects of combinations of metals, especially on fish (FAO, 1980). Interaction of organochlorine compounds (Dieter and Ludke, 1975; Ludke, 1977) and of detergents (Solon and Nair, 1970) with other chemicals found in the environment has also been examined. Such tests have effectively demonstrated the combined action of several chemicals in inducing an adverse response (see Peakall, this volume, for discussion).

Many experimental designs which suffice for testing the effects of individual chemicals cannot be used for testing chemical interactions. Factorial studies have been used to assess the interaction of metals, and of metals and organic compounds on the primary productivity and heterotrophic activity of natural aquatic microbial communities (Ibragim *et al.*, 1980; Kaitala *et al.*, 1984). The test systems are relatively simple microcosms, are replicable, and can be incorporated into a factorial design. In these studies experimental duration is longer than the generation period of the microbial community and so the terms *inhibition* and *stimulation* refer to changes in the 'state' of the system rather than to actual direct inhibition or stimulation of the organisms. Changes are mediated through nutrient and substrate availability, as well as trophic and competitive interactions. Although somewhat similar test systems are available for soil communities these have not been applied to studies of effects of chemical interactions on soil processes. Long-term interaction studies would necessitate the use of larger, more complex microcosm test chambers within a similar factorial design framework. There are, in general, few procedures for testing the long-term impact of interacting chemicals at the community or ecosystem level (see Sheehan, this volume).

More complex greenhouse and field experiments have been used to assess the response of plant communities to the interaction of a limited number (two or three) of chemicals (Foster *et al.*, 1983). Two obvious and minimal requirements are that (a) two or more chemicals are present over a range of concentrations, and (b) all possible combinations of levels of chemicals that vary in concentration are equally represented (a balanced design). The experimental design should be appropriate for a factorial analysis of variance (ANOVA). Further statistical considerations in the testing of chemical interactions are outlined by Green (this volume).

7.2.5 Methods of Determining Effects of Species Interactions

The interactions between species (e.g. herbivory, predation, parasitism, symbiosis, competition) are the functional aspects of biological communities. They act upon the individual populations to regulate community structure. Species interactions may be involved in ecotoxicology in two ways. First, toxicity-induced reductions in the population size of one species may, through species interactions, indirectly affect other species. Commonly observed examples of this indirect effect include the proliferation of algae following reduction of zooplankton populations (Hurlbert, 1975), and the explosion of insect pests on crops following the elimination of insect predators and parasites by pesticides (Pimentel, 1972). The second class of effects is the direct action of chemicals on the dynamics of species interaction. These direct effects have rarely been demonstrated, but examples include increased susceptibility of oxidant-injured trees to fungal parasites, and the sensitivity of fish infected by parasites to toxic materials (Suter, this volume).

The distinction between direct and indirect effects on species interactions is important in the design of testing and monitoring programmes. Indirect effects can be prevented by protecting the individual species; therefore, single species tests would be adequate. If prediction as well as protection is desired, effects on secondarily affected species could be predicted by relatively simple models or by multispecies tests. Direct effects on species interactions cannot necessarily be predicted from assessments based on single species tests. The subtle behavioural and physiological changes that control the dynamics of many species interactions are not routinely measured in single species tests and are not necessarily correlated with standard response variables such as lethality. Direct effects of chemical mixtures on species interactions can be predicted by multispecies testing, by (in theory) detailed modelling of interactions, or by testing the behavioural and physiological responses of individual species.

The use of species interaction tests is generally not impeded by a lack of potential test systems (Giddings, 1980; Suter, 1980) or by the problems that are unique to mixtures as opposed to single chemicals. Rather it is impeded by a lack of demonstrated instances of direct effects on species interactions, and by a lack of representative tests for examining these interactions (Suter, 1980). Further, in most natural ecosystems there are hundreds, if not thousands, of species interactions which are linked in webs of interactions. This complexity is compounded by the attempt to identify interactions between the components of mixtures of chemicals.

Currently, the solution to these difficulties is to limit species interaction tests to a few important types. These would be interactions involving simple systems such as agricultural or managed forest areas where a limited number of species interactions contribute to the performance of economically significant and relatively uniform systems. Examples include the symbiotic associations between nitrogen-fixing bacteria (*Rhizobia*) and legumes, and between most higher plants and mycorrhizal fungi. The end-point in these cases is the productivity of the higher plant, but the presence of the symbiont increases the explanatory power and realism of the tests.

In the field monitoring of biological effects of chemical mixtures, effects on species interactions are seldom investigated. This is largely due to the difficulty of recognizing such effects in the field. For example, if the population of a species declines in the presence of pollutants, the decline may be attributed to effects on its ability to catch prey, avoid predators, or resist parasites, as well as to direct toxic effects. In such cases, an explanation of the response must be provided by a combination of sophisticated monitoring (e.g. examination of stomach contents, fat content, and parasite burden) and either field experiments or laboratory multispecies experiments. Other cases such as effects of chemical mixtures on the interaction of forest trees with herbivores and parasites permit, and even require, monitoring of the interactions in the field.

In conclusion, a few multispecies tests are appropriate for the testing of the response of species interactions to new chemical mixtures, but their greatest utility is likely to be in helping to explain the causes of changes in populations observed in the field. Combinations of monitoring, experimentation in the field, and explanatory laboratory tests would provide a better understanding of both the mode of action of toxic materials and the role of multispecies processes in ecological toxicology.

7.3 APPROACHES TO ASSESSING THE EFFECTS OF MIXTURES IN POPULATIONS, COMMUNITIES, AND ECOSYSTEMS

There are no universally accepted protocols for testing the effects of mixtures on the structure and function of natural communities and ecosystems. There are some standardized tests generally applied to pure cultures, such as the OECD test of algal growth (OECD, 1981), which provide process-orientated data and may be modified to be a more representative test of the community function. Also available are several experimental techniques which have been used to quantify the effects of chemical mixtures, and to distinguish the influence of chemical interactions on community and ecosystem function under specific microcosm and field conditions. These methods have been developed to meet specific experimental objectives and should not be considered for standardization as a test protocol. However, within general guidelines, methods could be developed which would be applicable to both short- and long-term function tests. Examples of various approaches to test the impact of chemical mixtures on community and ecosystem function are discussed below and by Sheehan (this volume).

7.3.1 Aquatic Studies

Sufficient research has not been conducted to establish a widely applicable, rational, and workable approach for evaluating or predicting the joint action of chemicals in the aquatic environment. Recent reviews (Alabaster and Lloyd, 1982; Calamari and Alabaster, 1980; EIFAC, 1980) have summarized the available research on the effects of chemical mixtures on aquatic organisms and the approaches used in evaluating these effects.

7.3.1.1 Populations

Biological monitoring of aquatic populations has been widely practised in water pollution control activities. This has been useful on a site-specific basis but, because of the lack of definition of the constituent chemicals in the mixture, the rate at which they varied, and the failure to correlate the response with specific components of the mixture, the applicability of the results to other sites is limited and the development of predictive models difficult. The studies were conducted to assess the 'state of health' of the aquatic populations and there are few data on the levels or composition of the chemical mixture. In addition, the problem of prediction is now a key point in scientific and regulatory activities and these studies have generally been performed *a posteriori*.

There are some field studies on fish populations that were reviewed in EIFAC (1980), and were well supported by analytical data. The research started with a comparison between predicted and observed toxicity of effluents containing different chemicals, in laboratory experiments (Lloyd and Jordan, 1963, 1964). The assumption was that toxicities were additive and that the total toxicity could be calculated by adding toxic units (TU). The research project was successfully developed into field studies and Edwards and Brown (1967) found the TU (the sum of concentrations of chemicals expressed as a function of their respective concentration, lethal to 50% of fish after a defined period) above which a fish population would not exist. A further paper (Brown *et al.*, 1970) improved the described framework; however, the information given on the fish population was referred to in a concise way and was not analytically displayed or quantified.

A wide study on fish and fisheries in an area with several lakes polluted by copper and zinc was also performed in Norway (EIFAC, 1977). The sum of the concentrations of copper and zinc slightly exceeded the water-quality criteria proposed by EIFAC (1973, 1976) in Lakes Ringvatnet and Hostovatnet, where fish were abundant. Lake Bjorra, which had copper and zinc concentrations much higher than EIFAC standards, was fishless.

7.3.1.2 Communities and Ecosystems

Perhaps the most widely applied tests for effects of a mixture in aquatic systems are the algal assay procedures which measure short-term algal cell growth or phytoplankton productivity. Algal assays have largely ignored the question of chemical interaction, except in the case of metal–chelator bioassays. The period of exposure for these test systems is generally short, usually less than the 30- to 60-day period necessary to predict a critical ecological impact on microbial function. These tests are, therefore, of little value in making long-term predictions, but are useful in comparing the immediacy and severity of the short-term impact of various chemicals and mixtures of chemicals. The procedures are simple and fairly replicable and, if applied on natural communities (in the laboratory or *in situ*), may provide tests for inhibition of the photosynthetic process. There is evidence that in some cases the inhibitory effect is reversible even if the recovery to control levels of productivity is not re-established during the test period. For example, several laboratory and *in situ* tests, performed in BOD bottles and dialysis membrane bags, of endemic microbial communities exposed to PCBs have shown only transient depressions in autotrophic activity (e.g. Powers *et al.*, 1977). However, in those cases where community structure was also examined, PCBs and many pesticide compounds have longer-term effects on the ecosystem. These effects were mediated through changes in community composition at the producer level, which can have a significant impact on the transfer of energy to consumers (see, for example, O'Connors *et al.*, 1978).

Longer-term studies of the impact of mixtures on the structure and function of aquatic ecosystems have been generally carried out in microcosms and field tests using enclosures, or, in a very few cases, in whole system studies. Experimental designs have generally focused on communities and processes most likely to be affected by the mixture, based on its partitioning within the environment and on what is known of the bioactivity of the specific components or fractions of the mixture. For example, the importance for toxicity tests of knowing the distribution of petroleum hydrocarbons among the oil, water and vapour phases has been emphasized for toxicity tests at all levels (Bobra *et al.*, 1983; Wells *et al.*, 1982).

Results of tests and observations have indicated that, even within a specific environment, stress-induced changes in communities are likely to affect several functional processes. An example is again provided by the examination of longer-term effects of petroleum hydrocarbon fractions in water and sediments. The dissolved fractions of oils have been shown to disrupt the production to respiration ratio of aquatic microbes in microcosm tests (Giddings, 1982). In addition, *in situ* studies of oil fractions in sediments have demonstrated significant alteration of nitrogen fixation rates, carbon dioxide and methane production rates, and glucose uptake and mineralization rates (Griffiths *et al.*, 1981a,b). These examples emphasize the importance of selecting response variables on the basis of the importance and sensitivity of the process within the environmental compartment that is likely to be affected.

Wider application of various experimental methods and several potential approaches were reviewed by Giddings (1980). The testing of mixtures in aquatic ecosystem models will depend on further development and evaluation of these techniques.

7.3.2 Terrestrial Studies

Population changes of terrestrial animals caused by pollutants have been little studied compared with aquatic populations. One reason is that water as a vehicle provides a more uniform distribution of the chemicals to the organism. Only in the case of airborne transportation are toxic materials distributed with some uniformity to terrestrial systems, and even then the movement of the chemical from the first point of contact is difficult to define. Long-range transport of sulphur oxides and nitrogen oxides has been well documented in studies of forest systems, but the effects observed were largely on aquatic systems.

The best studies have been those involving agricultural and forest chemicals, but, even in these cases, although a variety of chemicals may be involved, there has been little progress in dealing with the problem of interaction in mixtures. Potts and Vickerman (1974) have shown changes in species diversity of insects on agricultural crops treated with a variety of fungicides and insecticides. Heavy mortality has been observed in forest song birds following treatment with combinations of pesticides (Pearce and Peakall, 1977) but no long-term population changes were detected. However, in these examples, although involving chemical mixtures, the techniques used were the same as for single chemicals, and it was not possible to determine which chemicals caused the major effect.

Information on the effects of mixtures of pollutants on terrestrial plant communities is very limited and mainly concerned with observations of changes in species composition or numbers of organisms. Natural processes of succession and competition have to be taken into account in interpreting changes in polluted habitats, but for forest trees and for cryptogamic species replacement of sensitive by more tolerant species in the vicinity of smelters has been attributed to emissions of metals and gaseous pollutants.

Changes in any of the constituent plant species can affect the total productivity of the ecosystem. The need to integrate the information on all components of communities to assess the effects of pollutants was emphasized with particular reference to plants by Kickert and Miller (1979). Genetic variability within a population will, however, complicate the interpretation. Elevated peroxidase activity, for example, has been described in conifer needles from polluted sites, but may show considerable variation within populations (Bialobok, 1980).

The evolution of tolerant strains following long-term exposure to single and mixed pollutants also results in difficulties in describing the sensitivity of the species. Data have been obtained chiefly under controlled conditions (Roose *et al.*, 1982) and with an emphasis on agricultural and forestry crops, for which decreases in productivity may have an economically important effect. Reductions in growth ring width, for example, have reflected pollutant emissions in areas where comparisons have been possible among homogeneous populations of conifers (Westman, 1982). Techniques for screening populations of crop plants, involving fumigation with single or combined gaseous pollutants, have been described by Drummond and Pearson (1979). Such methods require a uniform age structure of the population tested, since state of maturity also affects susceptibility to pollutant stress.

There have been numerous studies under controlled conditions for assessing the effects of mixtures of gaseous pollutants, chiefly on single species (Wellburn, this volume). When considering the effects on communities and ecosystems, the size of the system is important and the diversity of species represented in an enclosure may reflect only a small part of a complex ecosystem. In agricultural or forest systems, however, the frequent use of open or closed-top chambers has provided data on these species in the field.

For plants rooted in soil, the mineral composition may also play a role in affecting the susceptibility to atmospheric pollutants or in interacting with pollutants in the soil (e.g. Cowling and Koziol, 1982). The considerable differences between cultivars, as well as between species, in uptake of pollutants are of importance where, for example, sewage sludge applications result in high concentrations of metal mixtures in diverse agricultural soils.

There is a lack of studies on plant populations in ecosystems when compared with data described in this chapter for fish populations. Longer-term studies on plant communities with concurrent measurements of pollutant concentrations are needed for terrestrial systems especially. In aquatic habitats, some data are available for algae, but for other plant groups few investigations have been reported.

Short-term laboratory studies on the effects of chemical mixtures on terrestrial communities or ecosystems have largely focused on the microbial processes in the soil. In terrestrial ecosystems the decomposition of organic material and cycling of mineral nutrients are carried out primarily by the soil biota. The effects of chemicals on soil are best studied in terms of the effects of the entire mixture, not on species but rather on the processes of interest in the whole soil because

(1) individual microbial and invertebrate populations are of no inherent interest;
(2) it is not possible to predict the response of a process from the response of individual populations;
(3) the large number of taxa involved makes single species testing prohibitively expensive;
(4) the physical and chemical characteristics of soil greatly modify the availability and effects of the components of the mixture;
(5) a whole *in situ* soil community is much more easily extracted and maintained than pure microbial cultures; and
(6) the effects of a mixture are not readily predictable from the effects of its components because of the diverse interaction of physical, chemical and biological processes that determine the fate and effects of chemicals in soil.

Because of the high functional redundancy of soil biota, their enzymatic versatility, their rapid reproduction and evolutionary rate, and the ability of soil to sorb or otherwise sequester a diversity of chemicals, soil processes are not generally as sensitive to toxic materials as other biota (Domsch *et al.*, 1983). However, simple tests for the effects on carbon and nitrogen mineralization (carbon dioxide efflux and mineral nitrogen concentrations) are inexpensive to perform and could easily be combined with studies on the fate of the components of mixtures in the soil.

Microcosm, lysimeter, and field studies have been effective in assessing the impact of complex mixtures of air pollutants on soil processes, nutrient dynamics, and primary production in relatively complex plant–soil environments. Microcosm studies could contribute substantially to our understanding of the effects of mixtures and chemical interactions if chemical transformation and partition kinetics are measured simultaneously with structural and functional responses of the system. Lysimeter studies have the advantage of providing data on the input–output of components of the mixture, as well as the storage and leaching of essential plant nutrients. Controlled chamber and field studies provide in general the most accurate estimates of the effects of mixtures on productivity and nutrient cycling in terrestrial systems. Such techniques have been most widely employed in agroecosystems for the study of effects of gaseous air pollutants. Field tests of mixtures of chemicals in environments other than agroecosystems will be necessary to determine other site-specific effects of air pollutants or pesticide–herbicide mixtures and to validate laboratory and microcosm extrapolations.

7.3.3 Laboratory Studies

Inferences can often be made to single species populations in nature from well-designed, replicated individual organism studies in the laboratory if they are conducted on a sample of the natural population rather than on an inbred, laboratory strain as is common practice. If means, variances, and perhaps response distributions can be estimated for concentrations of chemicals in mixtures in relation to such response variables as mortality, fecundity, respiration, and growth rate, then some inferences can be made about how the population is likely to respond in the natural situation.

Experimental laboratory studies have been used to clarify the results of monitoring studies. The laboratory situation allows for analysis of each component of a chemical mixture and could more easily examine chemical interactions detected in the field. For example, Bartlett *et al.* (1974) used laboratory bioassays with algal cultures to explain the depression in primary productivity noted in portions of the Coeur d'Alene River contaminated with heavy metals.

Four conditions appear to be necessary for good correspondence between field and laboratory evidence:

(1) comparisons should be made at similar levels of biological organization (e. g. species/species or community/community);
(2) indigenous species should be used;
(3) environmental quality conditions should be nearly identical; and
(4) pretest environmental conditions should be similar for organisms in laboratory and field.

In addition, an attempt should be made to understand the effects of the stress produced by maintaining a population in a laboratory environment.

7.3.3.1 Microcosms

The use of soil microcosms is essential to the demonstration of more long-term effects of chemical mixtures on microbial processes under conditions which mimic nature better than other types of laboratory studies. A microcosm is not a miniature ecosystem, but may be rather a model of certain attributes of ecosystems (e.g. detritus processing).

Due to time and size constraints, model ecosystem tests under laboratory conditions must primarily focus on the activities of microbes, microinvertebrates and the small or early stages of higher plants. These groups exclude larger producers which dominate terrestrial systems and large consumers which play important roles in the regulation of energy and material movement. However, microbial communities control critical pathways in energy fixation, organic decomposition, biological mobilization, cycling of essential elements, and the modification of chemical mixtures. These processes can provide, therefore, important evidence of disruption in 'normal' system functions. Cairns and Buikema (this volume) found good correspondence between field and laboratory data when using artificial substrates colonized by microorganisms. As with sediment microbial communities, soil microbial response to pollutant mixtures is detectable with generalized metabolic measures such as soil respiration and dehydrogenase activity (e.g. Killham *et al.*, 1983), or with more specific metabolic parameters such as glucose mineralization (e.g. Strayer and Alexander, 1981) or hydrogen oxidation (e.g. Rogers and McFarlane, 1982). Effects on specific nutrient transformations and on nutrient retention are also widely reported.

Some microcosms (see, for example, Cairns and Buikema, this volume) use assemblages of species from natural systems that are brought into the laboratory for testing the effects of mixtures of chemicals. Although the species are accumulated on artificial substrates they are not artificial communities since they closely resemble those found in natural systems. Although the composition of species assemblages are rarely identical, they respond to similar concentrations of particular mixtures of chemicals in quite a predictable fashion. This is probably because, with a large array of species (frequently in excess of 50), the range of sensitivity is usually normally distributed (assuming no prior exposure that would result in selection).

Microcosms may be used for both functional (various rate processes) or structural (trophic balance) measurements. It is worth noting that certain single species toxicity test measurements (e.g. mortality) may also be made in microcosms.

Microcosms could contribute substantially to our understanding of pollutant interactions if chemical transformation and partitioning kinetics were monitored simultaneously with functional responses. For example, Kaufman (1977) used a laboratory-soil microcosm to demonstrate the effect of one pesticide on the degradation of others. However, none of the various microcosm systems for testing the effects of chemicals on soil processes reviewed by Suter (1980) appear to be appropriate for testing the interactions of the numerous combinations of chemical components likely to be found in many complex effluents. As with aquatic ecosystems, tests of multiple interactions between chemicals and soil processes must, for practical reasons, be confined to simple, reproducible experimental techniques (Sheehan, this volume).

7.3.4 Environmental Mathematical Models

An alternative approach to the use of laboratory studies is the use of mathematical models to estimate environmental effects using direct toxicological data, such as LC_{50} values, as input. An example is the use of a log–log linear model to fit data (see section 5). This approach has not been applied to any notable degree to the study of mixtures of chemicals, and an acceptable methodology has not been established.

To develop environmental models that are capable of analysing the effects of mixtures, several problems must be overcome. Two fundamental problems are (a) to select useful ways of modelling ecosystems, and (b) to select the compatible and useful ways of modelling the effects of mixtures. At present, 'selection' will most likely entail the development of new methods and mathematical relationships, particularly for modelling the effects of mixtures (see section 2).

Models of ecosystems and various subcomponents are widely available, although additional work is needed to incorporate expressions for the effects of chemicals. The incorporation of functional redundancy into ecosystem models is a simple matter, but taken to extremes the cost of calculations could become prohibitively high. This aspect of modelling, however, is important because of the role of functional redundancy in the response of ecosystems to perturbations. Generally, the greater the redundancy, the lower is the expected fluctuation.

Models of joint action of mixtures of chemicals exist (Ariens *et al.*, 1957; Gray, 1974; Hewlett and Plackett, 1959, 1964), but have not been formulated at levels suitable for direct application in ecological models. For application in ecological models, one approach is to use uptake and depuration models to provide calculations of tissue concentrations. Models of this sort (Lassiter, this volume) assume that uptake and depuration of mixtures of chemicals are independent processes, i.e. the presence of any chemical does not influence the uptake and depuration of any other. Undoubtedly exceptions will be found to this assumption, but for neutral, non-polar organic chemicals it will be true in general. Uptake and depuration models of mixtures of chemicals serve to calculate the internal concentrations of components of the mixture. This is important because of very different time scales of uptake and depuration rate of different mixture components.

A non-additive response to two or more chemicals (the response to the mixture is different from the sum of the responses to the chemicals applied separately) is an interaction. The possibility of interaction occurring poses in modelling a nearly insurmountable problem. Practically, the only means by which interactions can be identified and quantified is by an experiment with the particular mixture. As discussed in section 7.2.4, the number of combinations necessary for this approach to be useful is astronomical, and therefore impossible. Either a means of effectively reducing the number of experiments or a means of predicting expected activity relationships is necessary if interaction is to be considered in models or otherwise (see sections 2 and 5).

It is also necessary to know the behaviour of the components of the mixture, i.e. whether all chemicals are partitioned and impart their toxicity similarly or in very different ways. In the former case, a single critical level of the mixture at which the response occurs can be used in the model. Concentration addition models have been used successfully for these cases if each chemical is expressed in common toxicity units. Where partitioning and toxicity are expressed differently for each chemical, however, a different critical level could exist for each physiological system, and models for net toxicity would be cumbersome.

One approach, which can be called the test system approach, is to observe a response at one level of biological organization and, using this experimental base (including statistical and mathematical relationships, and intuition), interpret and project the response to another level of organization. Real effects are observed in response to the specific mixtures of chemicals; however, no general theory has been proposed that would permit objective extrapolation to other organizational levels or sampling over a range of chemical concentrations that vary in space.

The weakness of the modelling approach is that it is necessary to use an abstract representation of environmental systems or organisms and, therefore, the projections of the model must be tempered with the knowledge that they are based on a particular abstraction.

It is not expected that definitive results would be obtained by mathematical modelling any more so than by the test system approach. A model should ideally possess generality, realism, and precision; however, any particular approach tends to sacrifice one of these goals (Levins, 1966). Any model simplifies reality, and, 'What really matters is not the degree of perfection, but the adequacy for prescribed purposes' (Skellam, 1969). The adequacy of any fitted model should be assessed by examining bivariate plots and the patterns of the residual errors (Green, 1979).

Currently, ecosystem models are not available for the assessment of the effects of mixtures of chemicals. Their potential for use in a role that is complementary to existing methods makes it necessary for work toward their availability and application to proceed. Models need to be developed using data from laboratory experiments and then tested rigorously with results from field studies. The exploratory and heuristic use of models (as opposed to attempts to predict definitively) is expected to be beneficial for the ecological assessment of chemicals.

7.4 CONCLUSIONS

(1) Components of mixtures of chemicals will often partition into different environmental compartments (e.g. soil, water, air, biota); simple, single species tests are customarily carried out in systems that represent usually only one of these compartments. Tests at the community and ecosystem level could therefore

be a more realistic assessment of the effects of a chemical mixture. Studies of mixtures at these levels have several problems that are unique to this type of investigation. A combined approach, using several integrated methods, is needed to examine these problems.

(2) The response of a population to the stress of a chemical mixture may or may not be manifested as a response at the community or ecosystem level, depending on the role the population plays. Response variables of populations include, for example, growth rate, genetic structure, age distribution, lethality and spatial distribution.

(3) At the community and ecosystem levels, the choice of the response variable for the assessment of effects of mixtures will likely be case-specific, and generalizations to other areas will be difficult. Examples of response variables include community productivity, parameters of species abundance and distribution, transfer and transformation of energy, and measurements of ecological stability.

(4) Good experimental or sampling design is critical for studies on mixtures of chemicals and on the interaction of components. The design should be balanced, and controls should be included. An ecological effect should be determined as a statistic averaged over several systems, with and without the mixture present. Exposure time must be appropriate to the level of the biological response, the objectives of the study, and the components of the mixture of chemicals.

(5) Spatial allocation of samples in the field should be controlled statistically, by sampling in homogeneous systems, or by using artificial substrate sampling.

(6) Studies of mixtures at the community and ecosystem levels are extremely difficult and expensive. Few attempts have been made to develop a method of transferring data obtained at a simple level to a higher level of biological organization.

(7) Most mixtures vary markedly in both the quality and quantity of constituent chemicals. Although expensive, examination of the effects of the components in a mixture can be worthwhile. Several methods are available. The most useful approaches involve the use of multivariate and discriminant analysis techniques, *in situ* and laboratory bioassays, enclosures, or whole system manipulations.

(8) The potential for chemical interactions in the environment is great, but is difficult to assess. Simple microcosms, incorporating a factorial design, have been used to assess interactions of aquatic microbial communities, and greenhouse and field experiments have been used to examine the interaction of two or three chemicals.

(9) Few multispecies tests are appropriate for testing the response of species interactions to new chemical mixtures. Species interactions, however, may help to explain changes in populations observed in the field.

(10) Although there are no universally accepted protocols, several methods

are available to examine the effects of mixtures in aquatic environments. Fewer studies are relevant to terrestrial ecosystems.

(11) Methods used for single chemicals are often appropriate for chemical mixtures. However, species and chemical interactions, and the effects of the components of the mixture, are often undetected.

(12) Short-term effects of mixtures on populations in nature can often be inferred from well-designed, individual organism laboratory studies. Effects on communities in nature can be estimated in the laboratory using microbial, microinvertebrate, and small plant communities.

(13) Microcosms are useful in determining the long-term effects of mixtures on microbial processes.

(14) Ecosystem models are currently not available for the assessment of the effects of mixtures of chemicals although potentially models could play an important role in complementing existing methods, and as exploratory and heuristic tools.

7.5 RECOMMENDATIONS

(1) Links between ecological theory and ecotoxicology are weak and studies should be carried out to reinforce this linkage. In ecotoxicology, knowledge has developed at different rates. Specifically, more is known about the effects at the level of individuals than at the level of populations, and even less is understood at the more complex levels of communities and ecosystems. To overcome this imbalance greater emphasis should be placed on research and applications at more complex levels of organization.

(2) Ecotoxicological practice should be mixed and include integrated tests of very different types such as single species, microcosms, and field studies, and at different levels of biological organization.

(3) In studying the effects of mixtures, toxic unit summations have been shown to be useful for explaining short-term toxicity. However, this does not apply to long-term toxicity. It is recommended, therefore, that the number of studies on long-term effects of mixtures of chemicals at low concentrations be increased.

(4) Due to the complexity of ecosystems, there is a variety of responses to fluctuations of natural conditions. There is a similar variety of responses to the presence of mixtures of contaminants. It is, therefore, important to improve the capability to discriminate between natural variation and change due to the presence of contaminants.

(5) The enormous number of combinations of chemical mixtures and ecosystems precludes a purely experimental approach. There is, therefore, a fundamental need to develop predictive models as an aid in overcoming this difficulty. These models must be validated in regard to both the final output and the assumed causal components.

7.6 REFERENCES

Alabaster, J. S., and Lloyd, R. (Eds.) (1982). *Water Quality Criteria for Freshwater Fish*, 2nd edition. Butterworths, London: 361 pages.

Ariens, E. J., Van Rossum, J. M., and Simonism, A. M. (1957). Affinity, intrinsic activity and drug interaction. *Pharm. Rev.*, **9,** 218–236.

Bartlett, L., Rabe, F. W., and Funk, W. H. (1974). Effects of copper, zinc and cadmium on *Selanastrum capricornutum. Water Res.*, **8,** 179–185.

Bialobok, S. (1980). Forest genetics and air pollutant stress. In *Effects of Air Pollutants on Mediterranean and Temperate Forest Ecosystems, Proceedings of Symposium, 22–27 June 1980, Riverside, California*, pp. 100–102. Pacific South West Forest and Range Experiment Station, Berkeley, California.

Bjor, K., and Teigen, O. (1980). Effects of acid precipitation on soil and forest. 6. Lysimeter experiment in greenhouse. In Drabløs, D., and Tollan, A. (Eds.) *Ecological Impact of Acid Precipitation*, Proceedings of an international conference, Sandefjord, Norway, The SNSF Project, pp. 200–201.

Bobra, A. M., Shiu, W. Y., and Mackay, D. (1983). Acute toxicity of fresh and weathered crude oils on *Daphnia magna. Chemosphere*, **12,** 1137–1149.

Brasfield, H. (1972). Environmental factors correlated with size of bacterial populations in a polluted stream. *Appl. Microbiol.*, **24,** 349–352.

Brown, V. M., Shurben, D. G., and Shaw, D. (1970). Studies on water quality and the absence of fish from some polluted English streams. *Water Res.*, **4,** 363–382.

Butler, G. C. (1984). Developments in ecotoxicology. *Ecol. Bull. (Stockholm)*, **36,** 9–12.

Cairns, J., Jr. (1982). *Artificial Substrates*. Ann Arbor Science, Ann Arbor, Michigan: 279 pages.

Calamari, D., and Alabaster, J. S. (1980). An approach to theoretical models in evaluating the effects of mixtures of toxicants in the aquatic environment. *Chemosphere*, **9,** 533–538.

Cowling, D. J., and Koziol, M. J. (1982). Mineral nutrition and plant response to air pollutants. In Unsworth, M. H., and Ormrod, D. P. (Eds.) *Effects of Gaseous Air Pollution in Agriculture and Horticulture*, pp. 349–375. Butterworths Scientific, London.

Dieter, M. P., and Ludke, J. L. (1975). Studies on combined effects of organophosphates and heavy metals in birds. 1. Plasma and brain cholinesterase in *Coturnix* quail fed methyl mercury and orally dosed with parathion. *Bull. Environ. Contam. Toxicol.*, **13,** 257–262.

Domsch, K. H., Jagnow, G., and Anderson, T.-H. (1983). An ecological concept for the assessment of side-effects of agrochemicals on soil microorganisms. *Residue Rev.*, **86,** 65–105.

Drummond, D. B., and Pearson, R. G. (1979). Development of pollutant screens. In Heck, W. W., Krupa, S. V., and Linzon, S. N. (Eds.) *Handbook of Methodology for the Assessment of Air Pollution Effects on Vegetation*. APCA Specialty Conference Proceedings, Chapter 8. APCA, Pittsburgh, Pennsylvania.

Duchelle, S. F., Skelly, J. M., and Chevone, B. I. (1982). Oxidant effects of forest tree seedling growth in the Appalachian mountains. *Water Air Soil Pollut.*, **18,** 363–374.

Edmondson, W. T. (1971). Fresh water pollution. In Murdoch, W. W. (Ed.) *Environment*, pp. 213–229. Sinauer Associates, Inc., Stanford, Connecticut.

Edmondson, W. T. (1972). Nutrients and phytoplankton in Lake Washington. *Am. Soc. Limnol. Oceanogr., Spec. Symp.*, **1,** 172–193.

Edwards, R. W., and Brown, V. M. (1967). Pollution and fisheries: a progress report. *Water Pollut. Control*, **66,** 63–78.

EIFAC (1973). *Working Party on Water Quality for European Freshwater Fish. Report on Zinc and Freshwater Fish.* EIFAC Technical Paper 21. European Inland Fisheries Advisory Commission, Rome: 22 pages.

EIFAC (1976). *Working Party on Water Quality Criteria for European Freshwater Fish. Report on Copper and Freshwater Fish.* EIFAC Technical Paper 27. European Inland Fisheries Advisory Commission, Rome: 21 pages.

EIFAC (1977). *Working Party on Biological Monitoring. Report on the Effect of Zinc and Copper Pollution on the Salmonid Fisheries in a River and Lake System in Central Norway.* EIFAC Technical Paper 29. European Inland Fisheries Advisory Commission, Rome: 34 pages.

EIFAC (1980). *Working Party on Water Quality Criteria. Report on Combined Effects on Freshwater Fish and Other Aquatic Life of Mixtures of Toxicants in Water.* EIFAC Technical Paper 37. European Inland Fisheries Advisory Commission, Rome: 49 pages.

FAO (1980). Water quality criteria for European freshwater fish. In *Report on Combined Effects on Freshwater Fish and Other Aquatic Life of Mixtures of Toxicants in Water.* EIFAC Technical Paper 37. European Inland Fisheries Advisory Commission, Rome: 49 pages.

Fisher, N. S. (1977). On the differential sensitivity of estuarine and open-ocean diatoms to exotic chemical stress. *Am. Nat.*, **111,** 871–896.

Foster, K. W., Timm, H., Labanauskas, C. K., and Oshima, R. J. (1983). Effects of ozone and sulfur dioxide on tuber yield and quality of potatoes. *J. Environ. Qual.*, **12,** 75–80.

Giddings, J. M. (1980). Laboratory tests for chemical effects on aquatic population interactions and ecosystem properties. In Hammons, A. S. (Ed.) *Methods of Ecological Toxicology: A Critical Review of Laboratory Multispecies Tests.* EPA 560/11-80-026, pp. 23–91. US Environmental Protection Agency, NTIS, Springfield, Virginia.

Giddings, J. M. (1982). Effects of the water-soluble fraction of a coal-derived oil on pond microcosms. *Arch. Environ. Contam. Toxicol.*, **11,** 735–747.

Giesy, J. P., Jr. (Ed.) (1980). *Microcosms in Ecological Research.* Technical Information Center, United States Department of Energy, Washington, DC.

Gray, J. S. (1974). Synergistic effects of three heavy metals on growth rates of a marine ciliate protozoan. In Vernberg, F. J., and Vernberg, W. B. (Eds.) *Pollution and Physiology of Marine Organisms*, pp. 465–485. Academic Press, New York.

Green, R. H. (1979). *Sampling Design and Statistical Methods for Environmental Biologists.* John Wiley & Sons, Chichester, New York: 257 pages.

Green, R. H. (1984). Statistical and non-statistical considerations for environmental monitoring studies. *Environ. Monit. Assess.*, **4,** 293–301.

Griffiths, R. P., McNamara, T. M., Caldwell, B. A., and Morita, R. Y. (1981a). A field study on the acute effects of the dispersant Corexit 9527 on glucose uptake by marine microorganisms. *Mar. Environ. Res.*, **5,** 83–91.

Griffiths, R. P., McNamara, T. M., Caldwell, B. A., and Morita, R. Y. (1981b). Field observations on the acute effects of crude oil on glucose and glutamate uptake in samples collected from arctic and subarctic waters. *Appl. Environ. Microbiol.*, **41,** 1400–1406.

Hall, R. J., and Likens, G. E. (1980). Ecological effects of experimental acidification of stream ecosystems. In Drabløs, D., and Tollan, A. (Eds.) *Ecological Impact of Acid Precipitation.* Proceedings of an international conference, Sandefjord, Norway, The SNSF Project, pp. 375–376.

Harty, B., and McLachlan, A. (1982). Effects of water soluble fractions of crude oil and dispersants on nitrate generation by sandy beach microfauna. *Mar. Pollut. Bull.*, **13,** 287–291.

Herricks, E. E., Sale, M. J., and Smith, E. D. (1981). Environmental impact analysis of aquatic ecosystems using rational threshold value methodology. In Bates, J. M., and Weber, C. I. (Eds.) *Ecological Assessments of Effluent Impacts on Communities of Indigenous Aquatic Organisms*. ASTM STP 730, pp. 14–31. American Society for Testing and Materials.

Hewlett, P. S., and Plackett, R. L. (1959). A unified theory of quantal responses to mixtures of drugs: non-interactive action. *Biometrics*, **15,** 591–610.

Hewlett, P. S., and Plackett, R. L. (1964). A unified theory for quantal responses to mixtures of drugs: competitive action. *Biometrics*, **20,** 566–575.

Hurlbert, S. H. (1975). Secondary effects of pesticides on aquatic ecosystems. *Residue Rev.*, **57,** 81–148.

Ibragim, A. M., Patin, S. A., and Sokolova, S. A. (1980). Combined effects of some metals on primary production in coastal waters of the Red Sea. *Oceanology*, **20,** 300–301.

IJC (1983). *Great Lakes Water Quality Board Report*. International Joint Commission.

Kaitala, S., Kuparinen, J., and Maximor, V. N. (1984). The investigation of heavy metal and pentachlorophenolate stress on heterotrophic activity and primary productivity in a factorial experiment. In *Proceedings of Conference on Bacteria in Toxicity Testing, Burlington, Ontario, Canada*, p. 21.

Kaufman, D. D. (1977). Biodegradation and persistence of several acetamide, acylanilide, azide, carbamate and organophosphate pesticide combinations. *Soil Biol. Biochem.*, **9,** 49–57.

Kickert, R. N., and Miller, P. R. (1979). Responses of ecological systems. In Heck, W. W., Krupa, S. V., and Linzon, S. N. (Eds.) *Handbook of Methodology for the Assessment of Air Pollution Effects on Vegetation*. APCA Specialty Conference Proceedings, Chapter 14. APCA, Pittsburgh, Pennsylvania.

Killham, K., Firestone, M. K., and McColl, J. G. (1983). Acid rain and soil microbial activity: effects and their mechanisms. *J. Environ. Qual.*, **12,** 133–137.

Levins, R. (1966). The strategy of model building in population biology. *Am. Sci.*, **54,** 421–431.

Likens, G. E., Bormann, F. H., Johnson, N. M., Fisher, D. W., and Pierce, R. S. (1970). Effects of forest cutting and herbicide treatment on nutrient budgets in the Hubbard Brook watershed-ecosystem. *Ecol. Monogr.*, **40,** 23–47.

Lloyd, R., and Jordan, D. H. M. (1963). Predicted and observed toxicities of several sewage effluents to the rainbow trout. *J. Proc. Inst. Sewage Purif.*, **1963,** 167–173.

Lloyd, R., and Jordan, D. H. M. (1964). Predicted and observed toxicities of several sewage effluents to rainbow trout: a further study. *J. Proc. Inst. Sewage Purif.*, **1964,** 163–186.

Ludke, J. L. (1977). DDE increases the toxicity of parathion to *Coturnix* quail. *Pestic. Biochem. Physiol.*, **7,** 28–33.

Macek, K. J. (1975). Acute toxicity of pesticide mixtures to bluegills. *Bull. Environ. Contam. Toxicol.*, **14,** 648–652.

NRC (1981). *Committee to Review Methods for Ecotoxicology. Testing for Effects of Chemicals on Ecosystems*. National Academy Press, Washington, DC: 103 pages.

O'Connors, H. B., Jr., Wurster, C. F., Powers, C. D., Briggs, D. C., and Rowland, R. G. (1978). Polychlorinated biphenyls may alter marine trophic pathways by reducing phytoplankton size and production. *Science*, **201,** 737–739.

OECD (1981). *OECD Guidelines for Testing of Chemicals*. Organization for Economic Cooperation and Development, Paris: 700 pages.

Paine, R. T. (1974). Intertidal community structure. Experimental studies on the relationship between a dominant competitor and its principal predator. *Oecologia (Berl.)*, **15,** 93–120.

Payne, A. G. (1976). Application of the algal assay procedure in biostimulation and toxicity testing. In Middlebrooks, E. J., Falkenborg, D. H., and Maloney, T. E. (Eds.) *Biostimulation and Nutrient Assessment*, pp. 3–27. Ann Arbor Science, Ann Arbor, Michigan.

Pearce, P. A., and Peakall, D. B. (1977). The impact of fenitrothion on bird populations in New Brunswick. In Roberts, J. R., Greenhalgh, R., and Marshall, W. K. (Eds.) *Fenitrothion: The Long-term Effects of Its Use in Forest Ecosystems*. ACSCEQ Document NRCC 16073, pp. 299–306. National Research Council of Canada, Ottawa.

Phillips, D. J. H. (1980). *Quantitative Aquatic Biological Indicators. Their Use in Monitoring Trace Metal and Organochlorine Pollution*, Chapter 11, pp. 292–325. Applied Science Publishers, London.

Pimentel, D. (1972). Ecological impact of pesticides. *Environ. Biol.*, **72,** 1–27.

Potts, G. R., and Vickerman, G. P. (1974). Studies on the cereal ecosystem. *Adv. Ecol. Res.*, **8,** 107–197.

Powers, C. D., Rowland, R. G., O'Connors, H. B., Jr., and Wurster, G. F. (1977). Response to polychlorinated biphenyls of marine phytoplankton isolates cultured under natural conditions. *Appl. Environ. Microbiol.*, **34,** 760–764.

Rogers, R. D., and McFarlane, J. C. (1982). Hydrogen oxidation in soils as a possible toxic-effects indicator. *J. Environ. Qual.*, **11,** 364–368.

Roose, M. L., Bradshaw, A. D., and Roberts, T. M. (1982). Evolution of resistance to gaseous air pollutants. In Heck, W. W., Krupa, S. V., and Linzon, S. N. (Eds.) *Handbook of Methodology for the Assessment of Air Pollution Effects on Vegetation*. APCA Specialty Conference Proceedings, pp. 379–409. APCA, Pittsburgh, Pennsylvania.

Rosenberg, R. (1976). Benthic faunal dynamics during succession following pollution abatement in a Swedish estuary. *Oikos*, **27,** 414–427.

Schindler, D. W., Wagemann, R., Cook, R. B., Ruszczynski, T., and Prokopowich, J. (1980). Experimental acidification of Lake 223, Experimental Lakes Area: background data and the first three years of acidification. *Can. J. Fish. Aquat. Sci.*, **37,** 342–354.

Sheehan, P. J. (1984a). Functional changes in the ecosystem. In Sheehan, P. J., Miller, D. R., Butler, G. C., and Bourdeau, Ph. (Eds.) *Effects of Pollutants at the Ecosystem Level*. SCOPE 22, pp. 101–145. John Wiley & Sons, Chichester, New York.

Sheehan, P. J. (1984b). Effects on community and ecosystem structure and dynamics. In Sheehan, P. J., Miller, D. R., Butler, G. C., and Bourdeau, Ph. (Eds.) *Effects of Pollutants at the Ecosystem Level*. SCOPE 22, pp. 51–99. John Wiley & Sons, Chichester, New York.

Sheehan, P. J., and Knight, A. W. (1985). A multilevel approach to the assessment of ecotoxicological effects in a heavy metal polluted stream. *Verh. Int. Ver. Limnol.*, **22,** 2364–2370.

Sheehan, P. J., Miller, D. R., Butler, G. C., and Bourdeau, Ph. (Eds.) (1984). *Effects of Pollutants at the Ecosystem Level*. SCOPE 22. John Wiley & Sons, Chichester, New York: 443 pages.

Skellam, J. G. (1969). Models, inference, and strategy. *Biometrika*, **25,** 457–475.

Solon, J. M., and Nair, J. H. (1970). The effect of a sublethal concentration of LAS on the acute toxicity of various phosphate pesticides to the fathead minnow (*Pimephales promelas* Rafinesque). *Bull. Environ. Contam. Toxicol.*, **5,** 408–413.

Stockner, J. G., and Anita, N. J. (1976). Phytoplankton adaptation to environmental stresses from toxicants, nutrients, and pollutants—a warning. *J. Fish. Res. Board Can.*, **33,** 2089–2096.

Strayer, R. F., and Alexander, M. (1981). Effects of simulated acid rain on glucose mineralization and some physicochemical properties of forest soils. *J. Environ. Qual.*, **10,** 460–465.

Suter, G. W., II (1980). Laboratory tests for chemical effects on terrestrial population interactions and ecosystem properties. In Hammons, A. S. (Ed.) *Methods of Ecological Toxicology: A Critical Review of Laboratory Multispecies Tests*. EPA 560/11-80-026, pp. 93–153. US Environmental Protection Agency, NTIS, Springfield, Virginia.

Tyler, G. (1976). Heavy metal pollution, phosphatase activity and mineralization of organic phosphorus in forest soils. *Soil Biol. Biochem.*, **8,** 327–332.

Vandermeer, J. (1981). A further note on community models. *Am. Nat.*, **117,** 379–380.

Wells, P. G., Abernethy, S., and Mackay, D. (1982). Study of oil–water partitioning of a chemical dispersant using an acute bioassay with marine crustaceans. *Chemosphere*, **11,** 1071–1086.

Westman, L. (1982). *Growth of Coniferous Trees in Monitoring Programmes for Point Sources Emitting Sulphur, Fluorine, and Metal*. Report PM 1063. Statens Naturvardsverk, Solna, Sweden.